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16th CONGRESS
JUNE 9-12, 2011
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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 16th Congress of EHA.

The Scientific Program Committee has compiled an interesting program of Simultaneous Sessions and Poster Sessions from almost 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 11.

On behalf of the EHA Board, the committees and all the people involved in this year's EHA congress, we thank you for coming to London and hope that this Abstract Book will provide you with an important reference source of recent advances in hematology research.

Ivo Touw
Chair Scientific Program Committee

Irene Roberts
Congress President 16th Congress of EHA

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EUROPEAN HEMATOLOGY ASSOCIATION

EHA FUTURE CONGRESSES

17th CONGRESS of the European Hematology Association

Amsterdam, The Netherlands
June 14-17 2012

18th CONGRESS of the European Hematology Association

Stockholm, Sweden
June 13-16 2013

19th CONGRESS of the European Hematology Association

Milan, Italy
June 12-15 2014

16th Congress of the European Hematology Association
London, United Kingdom, June 9 - 12, 2011

POSTER SESSION I

Acute lymphoblastic leukemia - Biology

0001

RESEQUENING OF 97 ONCOGENES AND CANDIDATE ONCOGENES IDENTIFIES TYK2 MUTATIONS IN T-ALL

V Gianfelici,[1] K De Keersmaecker,[1] Z Kalender,[2] E Geerdens,[1] R Vandepoel,[1] M Porcu,[3] A Uyttebroeck,[4] J Cloos,[5] P Vandenberghe,[6] S Aerts,[6] J Cools[1]

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Background. T-cell acute lymphoblastic leukemia (T-ALL) is a malignancy of T-cell precursors that mainly occurs in children and adolescents. Optimization of chemotherapy regimens has resulted in improved responses with cure rates up to 75% in children. However, the current therapies are very toxic and cure rates rapidly decline in older patients, indicating the need for better and less toxic therapies. *Aims.* To discover new mutations that are implicated in the pathogenesis of T-ALL, we sequenced the coding regions of 97 genes in T-ALL cell lines and T-ALL samples. *Methods.* We captured all exons of 97 genes (known and candidate oncogenes/tumor suppressor genes) and subsequently sequenced these exons using 454 sequencing technology. In addition, all exons of the TYK2 gene were sequenced according to the Sanger method in diagnostic samples of 96 T-ALL patients; 54 acute myeloid leukemia (AML) and 53 B-ALL patients. Identified point mutations were cloned and introduced into Ba/F3 (IL3 dependent B-cells) and MOHITO (IL7 dependent T-cells) cells for assessment of transforming capacity, autophosphorylation and activation of downstream signaling pathways. *Results.* Resequencing of these 97 genes in T-ALL cell lines and primary T-ALL cases identified mutations in known T-ALL associated oncogenes such as NOTCH1, FBXW7, PHF6, HRAS/NRAS/KRAS, PTEN, TP53, NF1, as well as novel mutations in genes encoding tyrosine kinases (IGF1R, TYK2) and negative regulators of signaling such as tyrosine phosphatases (PTPRC) and sprouty related proteins (SPREDs). Remarkably, T-ALL cell lines were characterized by frequent mutation of TYK2 (6 of 18 T-ALL cell lines), a JAK kinase family member. While previous studies have described JAK1, JAK2, and JAK3 mutations in ALL patients, TYK2 mutations were previously not described in leukemia. Sequence analysis of TYK2 in primary patient samples identified TYK2 mutations in 2 of 97 T-ALL cases and 1 of 54 AML, but none of 53 B-ALL. Expression of TYK2 mutants in Ba/F3 and MOHITO cells conferred factor-independent growth, with some mutations being significantly more transforming than wild type TYK2, which by itself also transformed these lymphoid cell lines. Western blot analysis showed constitutive phosphorylation of TYK2, and also of JAK1 and STAT3, indicating that TYK2 mutants signal through JAK1 and STAT3. Overall, mutations in JAK1, JAK2, JAK3 and TYK2 were identified in approximately 15% of T-ALL cases. *Conclusion.* In addition to JAK1, JAK2 and JAK3 mutations, our data show that activating TYK2 mutations can also occur in acute leukemia. TYK2 mutations result in constitutive activation of the TYK2/JAK1/STAT3 pathway and represent an attractive new molecular target for JAK kinase inhibitors. These data show that the entire JAK kinase family can be mutated in acute leukemia, and warrant further clinical trials with JAK inhibitors for treatment of patients with JAK1, JAK2, JAK3 or TYK2 mutation.

0002

PTEN/AKT PATHWAY MUTATIONS ARE RECIPROCAL TO NOTCH1-ACTIVATING MUTATIONS IN PEDIATRIC T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL)

L Zuurbier,[1] M Vuerhard,[1] C Kooi,[1] W Smits,[1] J Buijs-Gladdines,[1] M Horstmann,[2] V De Haas,[3] R Pieters,[1] J Meijerink[1]

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PTEN-inactivating mutations that affect the PI3K/AKT pathway are often found in T-cell Acute Lymphoblastic Leukemia (T-ALL). To investigate the incidence of the PTEN/AKT pathway aberrations in pediatric T-ALL, we retrospectively determined the incidence of PTEN, PI3K and AKT mutations in 142 pediatric T-ALL patients treated according to the Dutch DCOG or German COALL protocols. Using PCR-sequencing and array-CGH techniques, PTEN mutations/deletions were detected in 13% of the patients. In addition, we found three patients, of which one also carried a PTEN mutation, having aberrant PTEN splice products. Another 2% of the patients carried an AKT mutation, whereas no PI3K mutations were identified. Using reverse-phase protein microarray analysis (RPMA), we demonstrated that PTEN protein expression was absent in PTEN-mutated or PTEN-deleted patients or patients with aberrant splicing, with the exception of one patient that carried a PTEN missense mutation. We also identified two T-ALL cases that lacked PTEN protein expression in the absence of PTEN mutations, defective splicing or for differential PTEN promoter hypermethylation in these patients. Therefore, 18% of the patients had PTEN or AKT aberrations in total. PTEN or AKT mutations (PTEN/AKT) were especially observed in TAL or LMO¬-rearranged T-ALL patients (p=0.007), but not in HOX-rearranged cases (p=0.003). PTEN/AKT mutations followed a pattern that seems reciprocal to the presence of NOTCH-activating mutations; they hardly co-express NOTCH1-activating mutations and PTEN/AKT-mutated cells express low protein levels of activated NOTCH1, cMYC and MUSASHI. This indicates that PTEN/AKT mutations are not simply secondary events following NOTCH1/FBXW7 mutations such as initially reported1. This also explains γ-secretase inhibitor insensitivity of PTEN-mutated cell lines, rather than acquired resistance as consequence of oncogenic addiction switch1. Survival rates for PTEN/AKT patients seemed comparable to wild-type patients (5-yrs DFS DCOG WT 71±6% vs PTEN/AKT 60±16%, COALL WT 78±6% vs PTEN/AKT 57±15%). However, when NOTCH1/FBXW7-mutated patients (associated with poor survival rates in DCOG and COALL cohorts) were separated from the wild-type group, the presence for NOTCH1/FBXW7 or PTEN/AKT mutations was associated with poor outcome, whereas the disease-free survival rate for wild-type patients was nearly 100% (5-yrs DFS DCOG+COALL WT 92±6% vs PTEN/AKT/NOTCH1 65±5%, p=0.005).

0003

FLOW CYTOMETRY AND IG/TCR QUANTITATIVE PCR FOR MINIMAL RESIDUAL DISEASE QUANTITATION IN ACUTE LYMPHOBLASTIC LEUKEMIA: A FRENCH MULTICENTER PROSPECTIVE STUDY

R Garand,[1] K Beldjord,[2] H Cave,[3] C Fossat,[4] I Arnoux,[4] V Asnafi,[2] Y Bertrand,[5] ML Boulland,[6] C Brouzes,[2] E Clappier,[3] E Delabesse,[6] H Dombret,[7] T Fest,[6] F Garnache-Ottou,[6] F Huguet,[6] MC Jacob,[6] E Kuhlein,[6] S Marty,[6] A Plesa,[8] N Robillard,[1] M Roussel,[6] J Tkaczuk,[6] E Macyntyre,[2] MC Bene,[6] N Ifrah,[6] A Baruchel[3]

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Background. Being a strong prognostic factor in acute lymphoblastic leukaemia (ALL), minimal residual disease (MRD) evaluation is now used for treatment stratification. Two methods are currently used: quantitation of Ig/TCR clonal rearrangements using allele-specific quantitative PCR (IG/TCR-QPCR) and multidimensional flow cytometry (FC). Ig/TCR-QPCR is now standardised and quality control rounds organised by the EuroMRD consortium ensure that results are comparable between participating labs. Such a standardisation process must be set up for FC. Moreover, whether FC can reach Ig/TCR-QPCR performance in routine ALL follow-up monitoring is still questionable. *Methods.* In the multicenter STIC 2006 program, MRD measurements were performed prospectively using IG/TCR-QPCR (4 EuroMRD labs) and FC (8 labs) in 598 follow up samples from 238 patients treated for a BCR-ABL negative ALL (136 adults ALL and 102 high-risk childhood ALL); 153 had BCP-ALL and 85 had T-ALL. Both techniques were performed on the same bone marrow sample. Ig/TCR-QPCR was performed following EuroMRD guidelines. FC was performed on fresh cells. Similar antibody panels, antibody clones and gating strategies were used with 4-color (39%) or 5/7-color (61%). FC-MRD positivity was defined as a cluster of more than 10 events displaying the leukaemia-associated immunophenotype (LAP) among at least 105 total events to reach a 10^{-4} (0.01%) sensitivity. Ig/TCR and LAP markers were considered suitable when allowing MRD detection with a sensitivity of at least 10^{-4}. *Results.* Six FC quality controls were run by sending mock MRD samples (fresh ALL cells spiked into normal bone marrow cells) to all FC laboratories. Among 45 measurements by the 8 labs, false positivity was observed in 2 cases and negativity in none. Ninety percent of positive results were clustered within half-a-log. In addition, a subset of FC data files (n=141) was reviewed collectively. The consensus conclusion (< or ≥10^{-4}) differed from the local centre result in 19/141 (13%) cases and were kept for further analyses. At least one suitable Ig/TCR marker was identified at diagnosis in 214/238 (90%) patients and a suitable LAP in 223/238 (94%) patients. All patients could be monitored using at least one method. Tandem MRD measurement by QPCR and FC was evaluable in 518/598 (87%) samples. The MRD conclusion (< or ≥ 10^{-4}) was identical in 495/518 samples (96%). However, in 73/518 (14%) samples, MRD was detectable with QPCR but not with FC. In 63 of these 73, PCR-MRD was positive not quantifiable (<10^{-4}). When positive ≥10^{-4}, FC and PCR MRD results were correlated (n=109; Spearman coefficient, r2: 0.87, p<0.0001) but clustered within half-a-log in only 69% of cases. In particular, QPCR provided significantly higher MRD results than FC in the 38 adult post-induction samples (median QPCR/FC-MRD ratio=2.0, p<0.03, Mann-Whitney test). *Discussion/Conclusion.* This study shows that multicenter MRD study by FC is feasible. Standardisation has, however, to be improved, in particular for homogenous data interpretation. Results obtained using QPCR and FC are generally similar, but the impact of discrepant positive results, which are apparently not random, needs to be elucidated and, although complementary, these approaches cannot yet be considered interchangeable.

0004

IDENTIFICATION OF NEW PROGNOSTIC FACTORS PREDICTIVE OF EXTRAMEDULLARY RELAPSE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Extramedullary (EM) involvement is defined as leukemic infiltration in EM sites like the central nervous system (CNS), testis, gut or skin. EM involvement at diagnosis is rare (1-4%) in childhood acute lymphoblastic leukemia (ALL) patients, but is more frequent at relapse (~40%). We hypothesized that: 1. migration of ALL cells to EM locations is an active process mediated by molecules present on or in the ALL cells, and 2. at diagnosis a (sub)population of ALL cells with migratory capacity is already present in the bone marrow (BM) and/or peripheral blood (PB). *Aim.* The aim of this study is to find cellular and molecular targets that allow the early identification of ALL patients at risk of an EM relapse. *Methods.* RNA was extracted from ALL cells derived from cerebrospinal fluid (CSF; n=5) or testis (n=8) or isolated from BM after FACS sorting on B-cell precursor (BCP)-ALL cells (>95% pure; n=24). Gene expression profiling was performed using Affymetrix protocols. Protein expression was determined by flowcytometry and ELISA. *Results.* Using Principal Component Analysis or hierarchical clustering, a clear separation between BM-derived and CSF- or testis-derived ALL samples was found. 1238 and 3501 genes were differentially expressed between CSF- and BM-derived BCP-ALL cells and testis- and BM-derived BCP-ALL cells, respectively (FDR≤0.01; fold change (FC)>1.5). Top lists of genes were generated based on differential expression and biological relevance (genes involved in cell adhesion, chemotaxis, or migration). Two of these genes were already analyzed in more detail: 1. SPP1 (mean FC=69, p=5E-10), known to be involved in cell migration and adhesion, and 2. LEPROTL1 (mean FC=5.9, p=3E-5), with unknown function. Increased mRNA expression of both genes was confirmed by RQ-PCR. SPP1 protein expression was not increased in CSF-derived BCP-ALL cells (n=4) compared to BM-derived BCP-ALL cells (n=28). The absence of intracellular SPP1 may partly be explained by secretion, since significantly increased soluble SPP1 levels were found in ALL-positive compared to ALL-negative CSF samples. Protein expression of LEPROTL1 and other top-ranked genes is currently being evaluated on CSF- and testis-derived ALL cells. Diagnostic BM cells from four BCP-ALL patients with isolated CNS relapse were analyzed for (sub)populations of ALL cells with increased SPP1 and/or LEPROTL1 expression. Clear subpopulations of ALL cells (~0.2%) with high LEPROTL1 or SPP1 expression were present in 2/4 and 0/4 patients, respectively, suggesting that LEPROTL1, but not SPP1 may be of prognostic value for the prediction of an (isolated) CNS relapse. *Summary/Conclusions.* EM-ALL cells and BM-derived BCP-ALL cells show distinct gene expression profiles. The presence of a small subpopulation of ALL cells with an EM signature at diagnosis may predict EM relapse. Prospective flowcytometric analysis of LEPROTL1 and other top-ranked genes in BM cells from newly diagnosed ALL patients, combined with a long-term follow-up, is ongoing to evaluate the prognostic significance of these markers. In future, the identification of proteins involved in CNS localization may contribute to the design of targeted therapies for both prophylactic treatment in patients at high risk of EM-ALL and therapeutic intervention in case of CNS relapse.

0005

THE RS564398, A POLYMORPHISM IN 'THE ANTISENSE NON-CODING RNA IN THE INK4 LOCUS', ANRIL, IS ASSOCIATED TO PHILADELPHIA POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) SUSCEPTIBILITY

A Ferrari,[1] I Iacobucci,[1] M Sazzini,[2] A Lonetti,[1] A Boattini,[2] C Papayannidis,[1] P Garagnani,[2] MC Abbenante,[1] V Mantovani,[3] F Cattina,[1] E Marasco,[3] E Ottaviani,[1] S Paolini,[1] D Girelli,[4] M Vignetti,[5] F Pane,[6] S Soverini,[1] M Baccarani,[1] G Martinelli[1]

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Introduction. Little is known about alterations of cyclin dependent kinase inhibitors p15INK4B, p16INK4A and p14ARF due to single nucleotide polymorphisms (SNPs) located within the CDKN2A/B genes and/or neighbouring loci. In order to investigate the potential involvement of such common DNA sequence variants in leukemia susceptibility, an association study was performed. *Methods.* 23 SNPs spanning the MTAP, CDKN2A/B and CDKN2BAS loci, as well as relative intergenic regions were genotyped in a case-control cohort made up of 149 leukemia patients, including Philadelphia positive (Ph+) ALL and acute myeloid leukemia (AML) samples, and 183 healthy controls. 6 SNPs were selected on the basis of their previous association with several diseases, such as coronary artery disease (rs2891168, rs518394, rs564398, rs10757278), type 2 diabetes mellitus (rs564398), frailty (rs2811712). The remaining 17 SNPs were selected to deepen the SNPs coverage for the examined region. Genotyping was performed using

iPLEX Gold technology and MassARRAY high-throughput DNA analysis with Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Sequenom, Inc., San Diego, CA). *Results.* A total of 17 SNPs, spanning the 9p genomic interval that encompasses the MTAP, CDKN2A/B and CDKN2BAS loci, were successfully genotyped and used for investigating their potential associations with the leukemia phenotypes. Five SNPs (rs1012713, rs10965179, rs34011899, rs3731232, rs3218010) with MAF <0.05 in cases and controls, as well as one SNP (rs3931609) showing >20% missing call rates, were instead excluded from the association analysis and potential population stratification affecting the control sample was ruled out as its genotypes distribution satisfies the Hardy-Weinberg equilibrium criterion. Among the 17 SNPs, rs564398, mapping to the CDKN2BAS locus (exon 2) that encodes for ANRIL antisense non-coding RNA, showed a statistically significant correlation with the ALL phenotype, with a risk pattern that was compatible with an overdominant model of disease susceptibility and a OR of 2 (95% CI, 1.20 to 3.33; p= 7.1 x 10-3). *Conclusions*. Since a co-ordinated regulation of ANRIL and p14/ARF, p16/CDKN2A, p15/CDKN2B transcription has been already observed in both physiologic and pathologic conditions, we hypothesized that rs564398 association reflects a condition of high linkage disequilibrium between such polymorphism and a causative variant that is able to alter CDKN2A/B expression profiles by changing ANRIL dosage, thus leading to abnormal proliferative boosts and consequent increased ALL susceptibility. Recently, the rs564398 has been demonstrated by Cunnington M.S. *et al*. (2010) to alter ANRIL expression leading in turn to a deregulation of CDKN2A/B genes. Supported by European LeukemiaNet, AIL, AIRC, FIRB 2006, Ateneo RFO grants, Project of integrated program (PIO), PRIN 2008, Programma di Ricerca Regione - Università 2007 - 2009.

0006

GENOME-WIDE SCREENING FOR TEL-AML1 (ETV6-RUNX1) TARGET GENES

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Background and Aims. The reciprocal translocation t(12;21) (p13;q22) is the most common structural chromosomal alteration in pediatric cancer as it occurs in approximately 25% of B-cell precursor acute lymphoblastic leukaemias (ALL). The resulting chimeric transcription factor TEL-AML1 is expected to function in a dominant-negative fashion. Here, we aimed to identify direct target genes of TEL-AML1 using an inducible TEL-AML1 system in combination with a novel TEL-AML1 antibody recognising the fusion site between the two proteins. *Methods.* We applied several state-of-the-art genome-wide screening methods, namely ChIP-on-chip, gene expression arrays and stable isotope labeling by amino acids in cell culture (SILAC). The large, highly complex data sets were inter-correlated in order to unveil global biological processes affected by TEL-AML1 expression. We used an inducible murine pro B-cell system developed from the BA/F3 cell line (provided by A. M. Ford & Mel Greaves, London) expressing TEL-AML1 in presence of mifepristone. Furthermore, we performed gene expression analysis using mouse genome arrays and protein profiling with SILAC followed by mass spectrometrical identification of expressed peptids. *Results.* Matching results from ChIP and gene expression studies revealed 202 putative target genes directly regulated by TEL-AML1 (125 upregulated, 77 downregulated). From these direct DNA and mRNA targets, eight were subsequently also detected in the SILAC experiments (two upregulated, six downregulated): RIKEN cDNA 9030617O03 gene, *klc4*, *pdlim5*, *rsl1d1*, *gnb2l1*, *hist1h4h*, *ints2*, *metap2*. The clustering of affected biological processes regarding GO terms disclosed several distinct pathways like signal transduction, protein modification and localisation as well as processes involved in regulation of transcription and RNA metabolism. As 67% of the genes corresponding to 'regulation of transcription' (e.g. *snip1*, *rai1*) were found to be downregulated and 83% of those targets corresponding to 'negative regulation of transcription' (e. g. *hdac5*, *zfhx3*, *foxp1*) showed an upregulated expression, this observation comes up to the expectation that TEL-AML1 mainly acts as a transcriptional repressor. Just as most of the genes (78%) involved in 'RNA metabolic processes' (e. g. *ftsj3*, *elac2*), target genes playing a role in 'proteolysis' (e. g. *metap2*, *bace1*, *senp3*) or 'intracellular signaling pathways' (e. g. *gnb2l1*, *rab7l1*) were mainly found to be downregulated as a consequence of TEL-AML1 expression by 75% and 67%, respectively. By contrast, 67% of the demonstrated target genes involved in 'protein modification processes' (e. g. *dusp7*, *prkd2*, *snrk*) showed an upregulation. *Summary and Conclusions.* Our data show that TEL-AML1 expression has an effect on many different biological processes, mostly represented by both up- and downregulated genes. Altogether, our findings show that TEL-AML1 acts as a repressor of transcription and that it also contributes to altered protein localisation and signal transduction. As no target gene commonly known for its oncogenic potential was found to be a direct target of TEL-AML1 in our experiments, one could assume that the observed alterations do not promote onset of overt leukaemia in affected cells by themselves. The altered expression of the eight genes found by ChIP-on-chip, gene expression and protein profiling will be subjected to further investigations in primary material from TEL-AML1-positive patients.

0007

FITNESS OF T(17;19) CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN IMMUNODEFICIENT MICE

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Background. Survival rates in childhood ALL are now in the region of 90%, however treatment outcomes in certain cytogenetic subgroups remain poor. The translocation, t(17;19)(q22;p13), is a rare cytogenetic abnormality, which occurs in less than 1% of childhood acute lymphoblastic leukemia (ALL) and is associated with a very poor prognosis and chemotherapy resistance. Understanding the mechanisms of resistance in these patients is therefore a priority. *Aims.* It has been assumed that following relapse, ALL cells will divide more rapidly than at presentation. In order to test this hypothesis, we have developed an animal model of competitive repopulation using paired samples from presentation and relapse from a case of t(17;19) positive ALL. *Methods.* Leukemic cells from a child with t(17;19) positive ALL obtained at presentation and at relapse were analysed for copy number variations using the Affymetrix Genome-Wide Human SNP Array 6.0 (SNP6.0) platform. Deletion of the NR3C1 gene (glucocorticoid receptor) on chromosome 5 was detected at relapse but not at presentation. This was confirmed by fluorescence *in situ* hybridisation (FISH). Cells were separately transplanted via intrafemoral injection into immunocompromised NOD/scid IL2Rγnull (NSG) female mice. Human blasts recovered from primary transplantations were subsequently used for secondary transplantation in mixed populations with ratios of presentation to relapse cells of 10:0, 9:1, 7:3, 5:5, 3:7, 1:9 and 0:10. Engraftment and progression of disease were assessed by flow cytometry at the terminal timepoint. The percentages of presentation and relapse cells which engrafted in these mice were determined using FISH for *NR3C1* deletion. The expression levels of glucocorticoid receptor (NR3C1) in the harvested leukemic cells were determined by real-time quantitative PCR. *Results.* Microarray analysis identified a deleted region of chromosome 5 involving the NR3C1 gene in cells obtained at relapse but not at presentation. Without treatment, those mice transplanted with relapse cells survived significantly longer than those transplanted with presentation cells (p=0.005), while the survival rates of mixed cells fell between the two curves. All engrafted mice presented with an enlarged spleen indicating leukemic infiltration. This was confirmed by flow cytometry, which identified t(17;19) cells with CD19+/CD10+/CD34- immunophenotype among those isolated from bone marrow (85-92%) and spleen (64-90%). FISH analysis showed that the percentages of presentation cells which engrafted in the mice with mixed populations were always higher than the ratios initially transplanted. Real-time PCR analysis confirmed that the levels of NR3C1 from all ratios of mixed cells represented the level of the presentation cells only. Repopulation experiments are currently being repeated with mice treated with Dexamethasone. *Conclusions.* In this study we observed that in t(17;19) positive ALL, without treatment, presentation cells outgrew cells obtained at relapse regardless of the ratio of cells initially transplanted. It is anticipated that, on treatment, relapse cells will outgrow those obtained at presentation, due to deletion of the glucocorticoid receptor, which would render cells insensitive to steroid treatment.

0008

SEQUENCE OF MUTATIONAL EVENTS AND CLONAL ARCHITECTURE IN BCR-ABL POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background. Intra-clonal genetic diversity is a hallmark feature of cancer and provides the substrate for sub-clonal selection, progression and therapeutic resistance. We recently demonstrated a variegated genetic architecture in the blast cells and leukaemia-propagating ('stem') cells of childhood *ETV6-RUNX1*-positive acute lymphoblastic leukaemia (ALL by single cell analysis with multi-colour FISH probes (Anderson *et al*., Nature 469: 356-361, 2011). *Aims.* In the present study, we aimed to determine whether this genetic diversity also applies to high risk ALL with *BCR-ABL* fusion. *Methods.*We used multiplexed FISH analysis of single cells using probes labelled with five distinct fluorochromes. We screened diagnostic blast cells samples from 8 *BCR-ABL*-positive cases for deletion of *IKAROS* (IKZF1), *CDKN2A/p16* and *PAX5*. The application of multiple FISH probes allowed us to score all cells (200 per patient sample) simultaneously for the *BCR-ABL* fusion (and multiple copies of the fusion), and one or two copy deletions of *IKZF1*, *PAX5* and *CDKN2A*. *Results.* The genetic classification of individual cells allowed a designation of sub-clones and the assembly of putative ancestral trees. Four of the eight cases screened had concurrent *IKZF1*, *PAX5* and *CDKN2A* deletions and in two of these we could distinguish the order acquisition of the deletions. In one case the *IKZF1* and *CDKN2A* and *PAX5* deletions arose independently in different sub-clones; i.e. *IKZF1* was deleted first in one subclone and *CDKN2A* first in another sub-clone. In the second case the *CDKN2A* deletion arose subclonally to both the Ikaros and *PAX5* deletions. We also studied one patient with matched diagnosis and relapse samples available. Comparison of SNP array data at diagnosis versus relapse revealed that different (or reiterated) *CDKN2A*, *PAX5* and *IKZF1* deletions were involved. We developed probes specific for the different genomic regions involved in the two distinctive *IKZF1* deletions (diagnosis versus relapse). FISH analysis then allowed us to identify clonal substructure at diagnosis, including a minor sub-clone (1%) with the genotype of the dominant clone seen in relapse. *Summary/Conclusions.* These results indicate that the sub-clonal architecture in BCR-ABL positive ALL is genetically diverse. The common CNA are acquired in no preferential order, and CNA involving the same gene can arise independently in different sub-clones. An important prediction derived from this pattern of sub-clonal architecture is that the leukaemia propagating cells in *BCR-ABL* ALL should themselves be genetically diverse. We plan to perform *in vivo* transplantation experiments in NOD/SCID/γ^{null} mice with leukaemic cells from patients for whom we find clonal heterogeneity. Regenerated leukaemias will be re-transplanted and the subsequent leukaemias analysed by both SNP arrays and multi-colour FISH to compare genetic diversity of stem cell output as previously with *ETV6-RUNX1*+ cases. We anticipate that these data will allow us to conclude that genetic diversity of leukemic stem cells is a consistent feature of both low and high risk ALL.

0009

MTORC2 PLAYS A DISTINCT ROLE IN MEDIATING ANTI-PROLIFERATIVE AND PRO-APOPTOTIC EFFECTS OF INHIBITORS OF THE PI3K/AKT/MTOR CASCADE IN LONG-TERM CULTURED PRIMARY ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) CELLS

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Background. The PI3K/AKT/mTOR pathway is considered as a downstream signaling pathway of the bcr abl oncogene that is the hallmark of Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (Ph+ ALL) and of CML. Activation of the PI3K signaling pathway has been suggested to play a role in resistance of Ph+ ALL to tyrosine kinase inhibitors (TKI) that target BCR ABL kinase activity. Its role in Ph neg. ALL has not been clearly established. mTOR is a kinase and catalytic subunit of the two complexes mTORC1 and mTORC2. Whereas mTORC1 predominantly controls cell growth, mTORC2 appears to mediate cell proliferation and cell survival. mTORC1 phosphorylates the translational regulators S6K1 and 4E-BP1. The central role of mTORC1 regulation in oncogenic PI3K signaling provides strong rationale for targeting mTORC1 in cancer, but mTORC1-dependent negative feedback loops mitigate the effectiveness. mTORC2 phosphorylates AKT on a regulatory site required for maximal AKT kinase activity. This prompted rationale to develop mTOR inhibitors that target both complexes. *Aims.* To establish the role of components of the PI3K/AKT/mTOR signaling pathway in different subtypes of ALL to overcome resistance to TKI. *Methods.* The effects of selective inhibitors of PI3K (NVP-BKM120) and mTORC1 (RAD001) and of dual PI3K/mTORC1/C2 inhibitors (NVP-BEZ235 & NVP-BGT226) on long-term serum-free cultures of primary human Ph+ (n=6) and Ph neg. (n=6) B-precursor ALL cells were analysed. All inhibitors were kindly provided by Novartis, Basel, Switzerland. Cell proliferation and apoptosis were monitored by XTT-assays and FACS analysis using annexin V/propidium iodide. Phosphorylation of the proteins 4E-BP1 (Thr37/46) & S6 Ribosomal Protein (Ser235/236) downstream of mTORC1 was assessed by Western Blotting. *Results.* The dual PI3K/mTORC1/2 inhibitors (NVP-BEZ235 & NVP-BGT226) were more potent in inhibition of cell proliferation and induction of apoptosis in Ph+ and Ph neg. ALL than the selective PI3K and mTORC1 inhibitors alone. The anti-proliferative and pro-apoptotic effects of these inhibitors were independent of bcr-abl and partial resistance to 1st and 2nd generation TKI. Comparison of the effect of selective PI3K and mTOR inhibitors on mTOR signaling revealed differential regulation of S6 and 4E-BP1. Whereas selective inhibition of PI3K and mTORC1 by BKM120 and RAD001, respectively, resulted in dephosphorylation only of the S6 protein, combined inhibition of PI3K and mTORC1 was associated primarily with a decrease of S6 phosphorylation and only minor dephosphorylation of 4E-BP1. Conversely, exposure to the dual PI3K/mTORC1/C2 inhibitors resulted in nearly complete dephosphorylation of both S6 and 4E-BP1. *Summary/Conclusions.* Our observation that compounds inhibiting PI3K/mTORC1/2 have significantly greater growth inhibitory and pro-apoptotic effects than selective inhibition of PI3K and mTORC1 support a functional role of mTORC2 in survival and growth of B-precursor ALL cells. Our data indicate that mTORC2 contributes substantially to regulation of the downstream target 4E-BP1 by mTORC1 in ALL. Combined targeting of these complexes may provide a novel therapeutic approach for both Ph+ ALL resistant to ABL TKI and Ph neg. ALL.

0010

PRECLINICAL ACTIVITY OF LBH589 ALONE OR IN COMBINATION WITH CHEMOTHERAPY IN A XENOGENEIC MOUSE MODEL OF HUMAN ACUTE LYMPHOBLASTIC LEUKEMIA

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Histone deacetylases (HDACs) have been identified as therapeutic targets due to their regulatory function in chromatin structure and organization. Here we analyzed the therapeutic effect of LBH589, a class I-II HDAC inhibitor, in acute lymphoblastic leukemia (ALL). *In vitro*, LBH589 induced dose-dependent antiproliferative and apoptotic effects, which were associated with increased H3 and H4 histone acetylation. Intravenous (i.v.) administration of LBH589 in immunodeficient BALB/c-RAG2-/-γc-/- mice in which human-derived T and B-ALL cell lines were injected induced a significant reduction in tumor growth. Using primary ALL cells, a xenograft model of human leukemia in BALB/c-RAG2-/-γc-/- mice was established, allowing continuous passages of transplanted cells to several mouse generations. Treatment of mice engrafted with T or B-ALL cells with LBH589 induced an *in vivo* increase in the acetylation of H3 and H4, which was accompanied with prolongued survival of LBH589-treated mice in comparison with those receiving Vincristine and Dexametasone. Notably, the therapeutic efficacy of LBH589 was significantly enhanced in combination with Vincristine and Dexametasone. Our results demonstrate the therapeutic activity of LBH589 in combination with standard chemotherapy in pre-clinical models of ALL and suggest that this combination may be of clinical value in the treatment of patients with ALL.

0011

THE INSULIN RECEPTOR SUBSTRATE 4 GENE IS MUTATED IN PAEDIATRIC T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. The cytogenetic hallmark of T-cell acute lymphoblastic leukaemia (T-ALL) is the presence of rearrangements involving the T-cell receptor (TCR) loci, mainly *TRA@/TRD@* at 14q11 and *TRB@* at 7q34, that dysregulate a variety of genes with oncogenetic potential. Several genes involved in TCR translocations have also been shown to be targeted by alternative mechanisms. *NOTCH1* is the most pertinent example of this. This gene was initially shown to be rearranged by the rare t(7;9)(q34;q34), but is now known to harbour activating mutations in the majority of T-ALL cases. Thus, mutation analysis of an infrequent TCR target may well reveal that it plays a greater role in T-ALL development than surmised based on the incidence of the translocation alone. *Aims.* We recently reported a novel translocation - t(X;7)(q22;q34) - in a paediatric T-ALL and showed that it results in overexpression of the insulin receptor substrate 4 (*IRS4*) gene. We speculated that this gene could be mutated in additional cases, akin to what has been reported for *NOTCH1*. In the present study, we have therefore sequenced IRS4 in paediatric T-ALL samples. *Methods.* The patients (n = 21) with T-ALL represents 78% (21/27) of all T-ALL (<18 years) diagnosed in Lund and Linköping 1990-2007 and comprises all cases from which DNA from diagnostic samples was available. The IRS4 gene at Xq22.3 consists of only one exon (3,881 bp; NM_003604.2), which was amplified by nested extra long polymerase chain reaction (Applied Biosystems, Foster City, CA, USA). Sequencing of the antisense strand of *IRS4* was carried out by the GATC sequencing service (Konstanz, Germany). The sequences were analysed using the Mutation Surveyor software (Softgenetics LLC, State College, PA, USA). *Results.* The entire *IRS4* gene was successfully sequenced in all 21 cases, of which 19 displayed a sequence matching the reference (NM_003604.2). Two cases had sequences that deviated from the reference but which have not been reported as polymorphisms, suggesting that they were mutations. Case 3 displayed an in-frame 594 bp deletion - c.105_698del (NM_003604.2); p.24_221del (NP_003595.1) indicated in figure 1A with a dotted line. Unfortunately, no remission sample was available to ascertain whether this deletion was acquired or constitutional. The found deletion results in the removal of the pleckstrin homology domain represented by a black box in figure 1B whereas the blue box indicates the segment reported as a copy number variation in Yoruba Nigerians (http://projects.tcag.ca/variation/). The pleckstrin homology domain is functionally important and well conserved among the IRS proteins. Case 8 harboured a missense mutation - c.670C>A (NM_003604.2); p.Pro213Thr (NP_003595.1), the position is marked by a red asterisk in figure 1B and 1C. The mutation was not found in either a remission or a relapse sample. Threonine residues have occasionally been reported to be phosphorylated in IRS proteins resulting in inhibition of the IRS activity. *Summary.* We have for the first time identified *IRS4* mutations in T-ALL. Whether such mutations confer a specific biological or clinical impact remain to be investigated in larger patient cohorts.

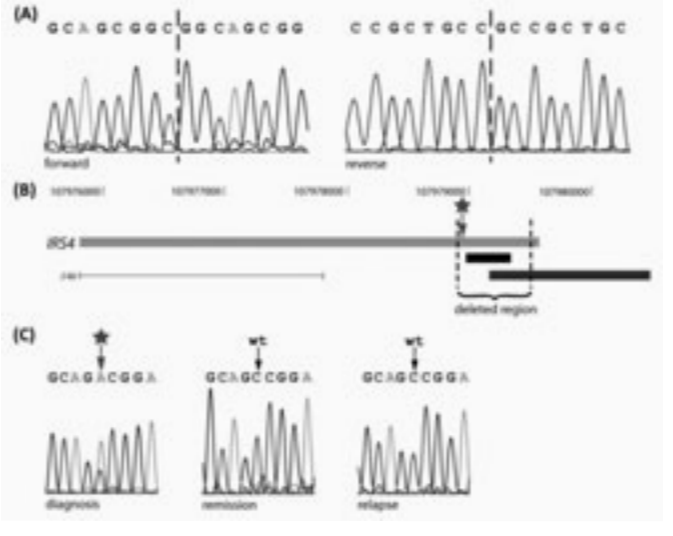

Figure 1.

0012

DISTINCT ACCESSIBILITY OF TLX1 AND LMO2 BREAKPOINT SITES MODULATES INVOLVEMENT IN T-CELL RECEPTOR (TCR)-ASSOCIATED TRANSLOCATION FORMATION

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Background. T-cell receptor (TCR) translocations are a genetic hallmark of T-cell acute lymphoblastic leukemia (T-ALL). Two well known TCR translocation partners, *LMO2* and *TLX1*, have shown to be recurrently involved in these translocations, and are therefore considered to be more prone to the induction of DNA double-strand breaks (DSB) at time of V(D)J recombination. LMO2 and TLX1 are respectively regarded as "Type 1"and "Type 2"translocation models. *LMO2* translocations mostly involve RAG mediated DSB inductions due to the presence of a cryptic recombination signal sequence (cRSS), while the cause of *TLX1* DSB induction is unknown. We hypothesize that chromatin modulation during thymocyte development renders DNA vulnerable to DSB induction. This vulnerability is thought to facilitate both "Type 1 and "Type 2"translocations during V(D)J recombination. *Aims.* We aimed to determine the accessibility of *LMO2* and *TLX1* at breakpoint sites (BPS) during thymocyte development. We did this in order to assess whether there is a correlation between accessibility of BPS during thymocyte development and the involvement of these sites in translocations. *Methods.* We isolated DNA of thymocyte subsets by FAIRE (Formaldehyde Assisted Isolation of Regulatory Elements) to quantitatively determine nucleosome occupancy throughout thymocyte development at TCR-associated BPS. Furthermore, we isolated thymocyte DNA to determine the methylation status of these BPS by means of bisulfite sequencing. Obtained data were correlated to the thymocyte developmental stages at which TCR loci are rearranged and to the occurrence of involvement of the BPS in translocations. *Results.* Our findings show some level of nucleosome depletion at oncogene BPS, with no clear increase in accessibility at a particular thymocyte developmental stage. Nucleosome enrichment levels correlate to the methylation status of the BPS within the *LMO2* locus. However, the *LMO2* translocation hotspot, at which a functional cRSS is located, is hardly nucleosome depleted and is mostly hypermethylated. In contrast, all *TLX1* analyzed BPS were mostly hypomethylated, with only the *TLX1* translocation hotspot showing high levels of nucleosome depletion. *Conclusions.* Nucleosome depletion combined with a hypomethylated status seems to renders BPS within the *TLX1* translocation hotspot more prone to involvement in TCR-associated translocations. The *LMO2* translocation hotspot is hardly accessible during thymocyte development, perhaps an indication that another type of accessibility is needed for RAG targeting. No clear chromatin modulation at the *LMO2* and *TLX1* locus is seen during thymocyte development. This implies that other conditions are imperative in driving the occurrence of T-ALL related translocations in specific thymocyte stages.

0013

IS IMATINIB-RESISTANCE IN BCR-ABL1-POSITIVE LEUKEMIA DUE TO LOSS OF PTEN AND/OR ACTIVATING PIK3CA MUTATIONS?

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Background. The *BCR-ABL1* translocation occurs in 95% of cases with chronic myeloid leukemia (CML) and in 25% of cases with acute lymphoblastic leukemia (ALL). Tyrosine kinase inhibitors (TKI) - routinely administered in CML - are not equally effective for treating ALL. Causes for imatinib-resistance are: (i) the development of clones carrying mutations in the kinase domain of *BCR-ABL1*, (ii) low intracellular drug levels caused by disordered influx and efflux transport, (iii) overexpression of *BCR-ABL1*, and (iv) activation of alternative signalling pathways by oncogenic enzymes like *SRC* kinases. Many studies performed to elucidate imatinib-resistance make use of cells ectopically expressing *BCR-ABL1* or of cell lines which gained resistance after chronic exposure to rising drug concentrations. *Aims.* To find models for TKI-resistance, we analyzed nineteen *BCR-ABL1*-positive cell lines and found that five cell lines (KCL-22, MHH-TALL1, NALM-1, SD-1, SUP-B15) were resistant to imatinib and nilotinib. We set out to investigate whether these cell lines displayed the known molecular causes for TKI-resistance. *Methods.* TKI-reistance was determined by [3H]-thymidine uptake and annexin-V binding assays. Activity of signal transduction pathways was tested by Western blot analysis

using antibodies against phosphorylated *BCR-ABL1* targets. Mutational analysis of the *BCR-ABL1* kinase domain, of *PIK3CA* and of *PTEN* were performed by DNA sequencing. *Results.* None of the resistant cell lines carried mutations in the kinase domain of *BCR-ABL1* or other molecular aberrations previously mentioned in the context of TKI-resistance. The cell lines dephosphorylate the BCR-ABL1 downstream targets ERK1/2 and STAT5 after treatment with imatinib, while PI3K/AKT/mTOR activity remains unaffected. Treatment with AKT1 inhibitors promoted dephosphorylation of the mTOR target RPS6 and induced apoptosis. Cell line KCL-22 carried a PI3Kalpha E545G mutation, a site critical for the constitutive activation of the enzyme, indicating that mutations in the *PI3K* itself may be responsible for imatinib-resistance. Not only oncogenes, but also loss of tumor suppressor genes can activate oncogenic pathways. Accordingly, the T-ALL cell line MHH-TALL1 was found to carry a missense mutation in the PI3K-counteracting phosphatase *PTEN* leading to truncation of the protein after amino acid 241. Quantitative genomic PCR analysis revealed that the second *PTEN* allele was deleted in this cell line. *Summary/Conclusions.* 5/19 *BCR-ABL1*-positive cell lines tested were TKI-resistant. All imatinib-resistant cell lines were responsive to TKI regarding the *BCR-ABL1*-downstream targets STAT-5 and ERK1/2, but resistant with respect to the PI3K/AKT1 pathway. One of the resistant cell lines (KCL-22) carried a PI3Kalpha E545G mutation, a second (MHH-TALL1) did not express the full-length version of the PI3K-counteracting tumor suppressor gene *PTEN*. Either aberration might be responsible for the constitutive PI3K activity, and thus also be responsible for TKI-resistance. Our results highlight the importance of the PI3K in imatinib-resistance.

0014

FREQUENT METHYLATION AND DECREASED EXPRESSION OF THE RIZ1 GENE 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 and is considered to be a tumor suppressor gene. The *RIZ1* gene is inactivated in various tumors by hypermethylation, and epigenetic silencing is the common process behind RIZ1 inactivation. In hematological malignancies, *RIZ1* expression was found to be significantly decreased in leukemia cell lines, primary samples from acute myeloid leukemia and chronic myelogenous leukemia during the transformation from chronic phase to blastic crisis. However, the role of the *RIZ1* gene has not been well examined in adult acute lymphoblastic leukemia (ALL). *Aims.* The aims of this study are to assess the *RIZ1* expression and altered methylation status in adult ALL and to determine the association between these features and clinical characteristics of the patients. *Methods.* We examined expression of the *RIZ1* gene by quantitative real-time reverse transcription-polymerase chain reaction (PCR) analysis and performed methylation-specific PCR on the *RIZ1* gene in newly diagnosed 73 adult ALL patients (62 B-ALL and 11 T-ALL). Normal bone marrow cells (n=10), normal lymphocytes (n=3) and lymphoid cell lines (n=4) were also examined. Characteristics of the patients including age, sex, phenotype of ALL, WBC counts, LDH at the diagnosis, karyotype and response to induction chemotherapies were also investigated. MOLT-4 cells that have the *RIZ1* methylation were treated with DNA methyltransferase inhibitor, 5-Aza-dC. *Results. RIZ1* expressions of 67 ALL (mean 1.043) were decreased compared with those of normal bone marrow mononuclear cells (mean 1.471) (P = 0.030). The *RIZ1* expressions in the T-ALL patients (mean 0.606) were lower than those in the B-ALL patients (mean 1.145) (P = 0.045), although the expressions in normal T-cells (mean 6.933) were higher than those in normal B-cells (mean 3.229). The expression was not associated with other clinical characteristics. Methylation of the *RIZ1* promoter was detected in 11 of the 71 patients (15.5%) while it was absent in healthy controls. The *RIZ1* methylation was accompanied with decrease of *RIZ1* mRNA levels among 9 of the 11 methylation-positive patients (81.8%), although there was no difference in the expressions between methylation-positive (mean 0.818) and -negative patients (mean 1.114) (P = 0.151). *RIZ1* methylation was more frequent in T-ALL (63.6%) than in B-ALL (5.0%) (P < 0.0001), and more frequent in Philadelphia chromosome (Ph)-negative ALL (23.3%) than in Ph-positive ALL (3.7%) (P = 0.027). We found no correlations between the methylation status and other clinical characteristics. 5-Aza-dC treatment of MOLT-4 cells induced demethylation of the *RIZ1* promoter and suppression of cell proliferation and viability in a dose- and time-dependent manner. Restoration of the *RIZ1* expression was induced in the cells treated with a high concentration of 5-Aza-dC. *Summary/Conclusions. RIZ1* expression is decreased in adult ALL. Decreased expression and methylation of the *RIZ1* gene are frequent in T-ALL. 5-Aza-dC treatment of MOLT-4 cells induced demethylation of the *RIZ1* promoter and restoration of the *RIZ1* expression. These results suggest that *RIZ1* is inactivated in adult ALL and this inactivation is associated with methylation in T cell phenotype.

0015

GERMLINE GENOMIC VARIATIONS AT IKZF1, ARID5B, AND CEBPE GENES FOR THE PREDICTION OF DEVELOPING CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN KOREA

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Background. Recent western studies have showed the implication of the germline genomic variations in IKZF1 gene at 7p12.2, ARID5B gene at 10q21.2, and CEBPE gene at 14q11.2 on the risk of childhood acute lymphoblastic leukemia (ALL); the most significant association was observed in the single nucleotide polymorphism (SNP) rs4132601 which located at 3' region of the IKZF1. IKZF1 plays an important role in lymphocyte differentiation, proliferation and function, ARID5B in embryogenesis and growth regulation, and CEBPE in regulation of myelopoiesis. Genomic variants in these genes are therefore considered to be involved in transcriptional regulation and differentiation of B cell progenitors. However, there have been no reports on the role of germline variations in leukemogenesis of childhood ALL in Asian countries. *Aims.* The aim of this study is to show the impact of these genetic variants on childhood ALL in Korea. *Methods.* To examine the association between genetic variations (IKZF1 rs4132601, ARID5B rs7089424, and CEBPE rs2239633) and the risk of childhood ALL, we here analyzed 228 children with ALL and 508 healthy individuals in Korea. *Results.* In ARID5B rs7089424, TG and GG genotypes were significantly associated with a risk for ALL (odds ratio [OR], 1.63; 95% confidential interval [CI], 1.07-2.48; P=0.02 for TG genotype, OR, 2.69; 95% CI, 1.42-5.07; P=0.002 for GG genotype). The allele incidence of ARID5B rs7089424 was also significantly associated with a risk for ALL (OR, 1.66; 95% CI, 1.24-2.22; P=0.0006). CEBPE rs2239633 TT genotype showed a significant association with a decreased risk for ALL (OR, 0.54; 95% CI, 0.33-0.90; P=0.02 for TT genotype). The allele incidence of CEBPE rs2239633 was also associated with a decreased risk for ALL (OR, 0.77; 95% CI, 0.61-0.97; P=0.02). There was no significant association between IKZF1 rs4132601 polymorphism and a risk for ALL in this study. *Conclusions.* These results suggest that genomic variations of ARID5B and CEBPE may play an important role in the risk for childhood ALL in Korea, compared with findings from western countries showing a significant relation between IKZF1 and childhood ALL. Several factors should be considered to explain a discrepancy between our results and the previous studies, which include different genotype frequencies in polymorphisms and varied susceptibility to ALL in different ethnic groups. Further studies incorporating larger number of cases and analyzing other SNPs or other Asian countries are warranted in childhood ALL.

0016

PARALLEL SAMPLING OF BONE MARROW AND PERIPHERAL BLOOD IS ADVISED FOR THE MOLECULAR EVALUATION OF MINIMAL RESIDUAL DISEASE IN BOTH B AND T LINEAGE ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. An accurate detection of minimal residual disease has become a crucial tool to evaluate prognosis and therapeutic strategies both in childhood and adult patients with acute lymphoblastic leukaemia (ALL). The need of repeated (BM) sampling during the clinical course can be problematic for many patients. Preliminary results obtained in children showed that BM sampling can not be replaced by PB in B precursor ALL, but could be considered for T-ALL. Here we report our comparative analysis performed in a large prospective study performed in adult B and T ALL. *Material and Methods.* One hundred and seven adult patients (73 B-precursor ALL and 34 T lineage ALL) enrolled 2 consecutive clinical trials launched by NILG, were monitored for molecular MRD in BM and PB. Two informative Ig or TCR derived molecular probes (with a sensitivity ranging from 10-3 to 10-5) were used in 77 patients (51 B-precursor and 26 T-ALL) while only one probe was available for 30 (22 B-precursor and 8 T-ALL). A paired BM/PB analysis was conducted on 721 paired samples (500 from B and 221 from T-ALL) by real-time quantitative PCR (RQ-PCR). *Results.* In T-ALL 132 out of 221 paired samples obtained during follow-up proved negative both in the BM and PB whereas a positive MRD was found in at least one sample of the remaining 89 paired analyses. Among these latter samples, a positive MRD was detected in both BM and PB with comparable levels of residual disease in 47 paired samples (53%), while the amount of detectable disease was remarkable different (from 1 to 2 log) in 23 pairs (26%). In 8 paired samples (9%) MRD proved positive only in the BM while in 11 (12%) only in the PB. In B precursor ALL 200 out of 500 paired samples showed a measurable MRD level in the BM and/or PB (figure 1 panel A). In 46 paired samples (23%) the amount of MRD was similar in BM and PB while in 72 (36%) MRD was significantly higher (up to 3 log difference) in the BM compared to PB. In only one case (0.8%) a higher MRD level was detected in PB. In 66 paired samples (33%) MRD was detectable only in the BM and in several of these cases the amount of residual disease could be as high as 10-2. In 16 pairs (8%) only the PB proved positive but at very low levels (usually ≤ 10-4) (figure 1 panel B). *Conclusion.* Although MRD detection on BM samples is more sensitive compared to PB, in both B and T lineage ALL. However, in some T-ALL cases, the PB may provide discordant and informative results. All in all, our results suggest that either in B precursor ALL as well as in T-ALL, an MRD evaluation must be always performed on BM but preferably, PB samples should be tested in parallel.

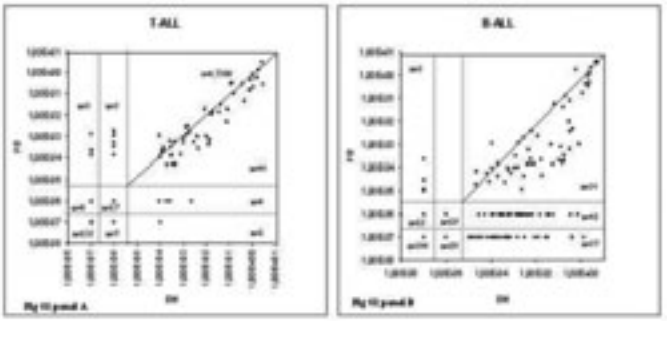

Figure 1. MRD levels in paired BM and PB samples.

0017

GENE MICRODELETIONS IN PHILADELPHIA NEGATIVE ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (PH- ALL)

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Background. Except for the known adverse prognosis of Ph+ ALL, few other cytogenetic and molecular markers are used in the clinical practice for adult ALL. Microdeletions of genes involved in B lymphopoiesis and cell-cycle regulation, such as *CDKN2A*, *PAX5*, *IKZF1*, *ETV6*, *RB1* and *EBF1*, have been reported as a highly frequent event in pediatric ALL, but the frequency of this findings and its prognostic implication in adulthood are still uncertain. *Aims.* To identify the frequency of deletions in these genes and their relationship with clinical data and prognosis in an adult cohort of Ph- ALL patients. *Methods.* We studied 67 patients diagnosed with Ph- ALL with available DNA sample collected at diagnosis. Median age was 38 years (range 15 - 85) and leukocyte count 16.8 x 10^9/L (range 1-371). Nucleic acids were extracted from bone marrow aspirate using standard procedures. We performed Multiplex Ligation Probe Amplification (MLPA), a multiplex PCR based technique that allows comparative quantification of multiple sites, using the SALSA MLPA kit P335-A1 (MRC-Holland, Amsterdam, The Netherlands), that contains, among others, probes for *CDKN2A*, *PAX5*, *IKZF1*, *ETV6*, *RB1* and *EBF1*. The PCR products were separated by capillary electrophoresis on an ABIPRISM 310 DNA Analyzer (Applied Biosystems). Data were analyzed using GeneMapper software v3.2 (Applied Biosystems) and relative copy number was obtained after normalization of peaks against controls. Values below 0.7 indicated loss and over 1.3 indicated gain. *Results.* Forty-one (61%) patients showed abnormalities in at least one of these genes. The most common deletions were found in *CDKN2A* (33%) and *IKZF1* (22%), but were also frequent in *ETV6* (16%), *PAX5* (14%), *RB1* (14%), *EBF1* (11%) and *BTG1* (6%). Deletion of *CDKN2A* were related to leukocyte count >50 x 10^9/L (P = .042) and *ETV6* deletion (P <.001). No other gene microdeletions were significantly associated with any clinical or biological characteristic. Multivariate analysis for overall survival including all significant variables in the univariate analysis (leukocyte count, age, karyotype, *CDKN2A*, *ETV6* and *PAX5* deletions) showed as independent variables: age >50 years [HR 4.3 (CI 95% 1.7 - 10.6); P = .001], *PAX5* deletions [HR 3.5 (CI 95% 1.0 - 11.8); P = .043] and leukocyte count >50 x 109/L [HR 3.1 (CI 95% 1.1 - 9); P = .035]. Similarly, multivariate analyses for relapse free survival showed *ETV6* deletions as the only independent risk factor for relapse [HR 5.2 (CI 95% 1.7 - 16.2); P = .004]. We could not confirm a negative effect of *IKZF1* deletions in our series. *Conclusions.* This study shows the high incidence of deletions in genes of cell-cycle and B-lymphopoiesis in adult Ph- ALL. In particular, deletions of *PAX5* and *ETV6* could be of interest for risk assessment.

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0018

DASATINIB SHOWS ANTI-LEUKEMIC ACTIVITY IN E2A-PBX1-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Chromosomal translocations that activate specific genes are a defining characteristic of human leukemias and of acute lymphoblastic leukemia in particular. The t(1;19) (q23;p12.3) chromosomal translocation, leading to the production of the E2A/PBX1 fusion transcript, is a rare but still one of the most recurring translocations in pe-

diatric ALL, with an overall frequency of 5% to 6%. The rearrangement fuses 5' exons encoding the transactivation domain of the E2A transcription factor gene to 3' exons ecoding the putative DNA-binding region of the homeobox gene, PBX1. The role of E2A-PBX1 in the development of acute leukemia has not been established. *Aims.* The aim of this study is to investigate potential anti-leukemic activity of the tyrosine kinase inhibitor (TKI) dasatinib in long-term cultured primary human E2A-PBX1-positive ALL cells and in E2A-PBX1-positive cell lines. Dasatinib is a multikinase inhibitor with potent activity against BCR-ABL (IC50<1 nM) and Src Family Kinases (IC50 of 0.2 and 1.1 nM) that has been approved for the treatment of patients with CML and Philadelphia-chromosome-positive acute lymphoblastic leukemia. *Methods.* To investigate the anti-leukemic activity of dasatinib, we screened E2A-PBX1-positive cell lines as well as long-term cultured human acute lymphoblastic leukemia cells established recently (Nijmeijer *et al*. 2009). The long-term *in vitro* proliferating cells phenotypically strongly resemble the initial cell population and have a largely identical karyotype. The effect of TKIs on proliferation of ALL cells was determined by the XTT-assay according to standard protocol. Cells incubated without drugs are used as controls and dasatinib is added at serial dilutions to allow IC50-calculations. The effect of TKI treatment on induction of apoptosis was determined by annexin V/propidium iodide staining and analysed by FACS. cDNAs encoding pE2A-PBX1 for expression in Ba/F3 cells were cloned from E2A-PBX1-positive long-term cultured ALL cells by RT-PCR and confirmed by sequencing. The construct were subcloned into pENTR.1A ("Gateway"recombination system-Invitrogen) for further transfer into expression vectors. For retroviral transduction we used retroviral vectors based on the PINCO vector, which harbours the green fluorescence protein (GFP) as reporter. *Results.* Dasatinib strongly (80%) inhibited cell proliferation of all E2A-PBX1-positive ALL cells tested in this study. The anti-proliferative effect was observed at concentrations up to 1µM with IC50-concentrations ranging from 25-50 nM. In contrast, IC50-concentrations were below 5 nM in BCR-ABL-positive ALL cells. Treatment with the kinase inhibitor imatinib and the second-generation BCR-ABL inhibitor nilotinib did not inhibit the proliferation of E2A-PBX1-positive cells. Exposure of E2A-PBX1-positive cells to dasatinib up to a concentration of 1 µM for 72 hours did not led to a significant induction of apoptosis (<20%) compared to a 20-70% apoptosis rate in BCR-ABL-positive ALL cells. Analysis of the anti-leukemic effect of dasatinib on Ba/F3 cells transfected with a plasmid that confers expression of E2A-PBX1 is ongoing. *Conclusion.* Exploration of the tyrosin kinase inhibitor dasatinib as a novel therapeutic approach to E2A-PBX1-positive ALL appears warranted based on its consistent profound growth inhibitory effect on E2A-PBX1 expressing cells.

0019

DNA DAMAGE-INDUCED POSTTRANSLATIONAL MODIFICATIONS OF P53 DECREASED UPON ACTIVATION OF CAMP SIGNALING IN PRE-B ALL CELLS

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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 evaluate the effect of activation of cAMP signaling system on post-translational modifications of p53 in pre-B ALL cells upon exposure to chemotherapeutic drugs. *Methods.* Cultured pre-B ALL NALM-6 cells were exposed to doxorubicin in the presence or absence of cAMP-increasing agents forskolin/IBMX for 24h and then cells were subjected to apoptosis analysis by flow cytometry. Western blot method was used to analyze phosphorylation and acetylation state of p53 protein, total p53, and the levels of other proteins which were involved in doxorubicin-induced apoptosis. Real-time PCR was performed to analyze the expression levels of p53 and p53 target genes. *Results.* These results indicate that elevation of cAMP in B cell precursor acute lymphoblastic leukemia (BCP-ALL) cells harboring wild-type p53 profoundly inhibit the apoptotic response to doxorubicin. We further demonstrate that elevated cAMP levels repressed DNA damage-induced p53 protein phosphorylation, acetylation, and accumulation 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. Whereas elevated cAMP was able to decrease doxorubicin-induced gene expression levels of p53 target genes, it had no effect on p53 mRNA steady-state levels. *Summary/Conclusions.* In conclusion, recognition that elevated cAMP levels may abrogate p53-dependent apoptosis carries clinical implications for patients with wild-type p53 leukemia cells undergoing curative antineoplastic therapy, because a large number of common agents modulate cAMP metabolism.

0020

B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA NEGATIVE FOR CD38: A SUBSET OF PATIENTS WITH DISTINCT BIOLOGICAL AND PROGNOSTIC FEATURES?

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Background. It is known that CD38 expression is associated with poor prognosis in patients with chronic lymphocytic leukaemia; however, its significance is unclear in other lymphoid malignancies. Although some authors have suggested that the negativity of the blasts for CD38 is correlated with a worse prognosis in patients with B-cell acute lymphoblastic leukaemia (B-ALL), it has yet to be clarified. *Aims.* The purpose of this study was to evaluate the main characteristics of patients with B-ALL according to CD38 expression. *Methods.*We analyzed the features at diagnosis and the evolution of 44 patients with B ALL treated at two hospitals in our community over the past ten years. The group consisted of 17 pediatric patients (age below 18 years) and 27 adults, with a median age of 26.5 years (range 2-75) and women 54%. Recorded data included morphology, laboratory findings, immunophenotype, cytogenetics and FISH, clinical features, treatment approach, follow-up and survival. Statistic analysis was performed using SPSS software, and relations between variables were studied using the Fischer exact test (categorical) and the Mann Whitney U test (continuous). *Results.* In 33 patients lymphoblasts were positive for CD38, while eleven did not express this cell surface marker. When compared with CD38 positive B-ALL group, the blasts of patients negative for CD38 were more frequently CD34, KOR-SA and CD13 positive (p=0.05, p=0.013 and p=0.049, respectively), and CD20 and CD22 negative (p=0.014 and p=0.05). Of note, 86% of patients lacking CD38 presented with BCR-ABL1 fusion gene, and only 14% in the CD38 positive group (p=0.001). Although overall survivals were similar, mortality tended to be higher among the CD38 negative B-ALL cases (p=0.058). Finally, there was not correlation between CD38 expression and age, sex, extramedullary involvement, lymphadenopathy, hepatosplenomegaly, blood cell counts, serum lactate dehydrogenase, morphology of lymphoblasts, bone marrow aspirate findings, other recurrent genetic abnormalities different to BCR-ABL1, minimal residual disease status evaluated by flow cytometry, chemotherapy regimens, hematopoietic stem cell transplantation, disease-free survival and overall survival. *Conclusions.* Although further studies involving a larger number of cases are certainly needed, these preliminary results show that: 1) Phenotypically, the absence of CD38 is associated with positive expression for CD34, KOR-SA and CD13, and negative for CD20 and CD22. 2) CD38 negative B-ALL seems to correlate strongly with the presence of BCR-ABL1. 3) Mortality tends to be higher among this subgroup of patients. These findings lead us to consider the possibility that CD38 negative B-ALL patients constitute a subgroup of B-ALL with different biological characteristics and prognosis.

0021

WHOLE GENOME DNA MICROARRAY ANALYSIS OF ADOLESCENT/YOUNG ADULT T-ALL

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Background. Outcomes for adolescent/young adults (AYAs) with ALL have improved in recent years with incorporation of this age group into paediatric protocols. However, event free survival for those aged over ten years remains inferior to their younger counterparts and the biological basis behind this difference is poorly understood. Possible explanations include a higher proportion of poor risk cytogenetic abnormalities in the AYA group, poor compliance with treatment or novel genetic aberrations specific to this adolescent group. This is par-

ticularly relevant to T-ALL where up to 40% of patients have no detectable cytogenetic abnormality. In recent years whole genome microarray analysis has improved our understanding of T-ALL pathogenesis with the identification of multiple novel copy number alterations (CNA). We applied this technology to testing the hypothesis that AYA ALL has a unique genetic profile. *Aims.* Our aim was to perform whole genome copy number analysis in a cohort of AYA T-ALL patients to determine the frequency of known genetic aberrations in our cohort compared to previous paediatric studies and to determine the presence of any novel copy number alterations (defined as recurrent CNA present in two or more samples in our cohort and not described in previous series of T-ALL). *Methods.* Diagnostic DNA from 25 AYA T-ALL patients (age 10 - 25 years) was subjected to copy number analysis using the Affymetrix SNP Array 6.0 platform, and CNAG 3.3.0.0 software for data analysis. Each CNA was classified as a passenger or driver: CNAs were classified as passengers if they were a known variant present in healthy controls or if they had no known/postulated role in oncogenesis. CNAs were classified as drivers if they were recurrent changes or if they had a known/postulated role (e.g known oncogene from another malignancy) in leukaemogenesis. *Results.* The mean number of drivers was 3.8 and the median was 3 (range 1 - 9). Frequency of known CNA in AYA T-ALL in our series was similar to paediatric studies with deletion of p16 again playing a pivotal role. Frequencies of deletion of 1p33, 11p13, 9q34.11-34.13 (leading to upregulation of TAL1, LMO2 and HOXA respectively) and deletion/gain of LEF1, MYB and PTEN were similar to previous paediatric references (see Table 1). A number of individual samples had copy number changes in genes usually associated with other haematological malignancies including loss of ARHGAP26 (JMML) and loss of DLEU2 (CLL). Potential novel areas of recurrent CNA identified included gain of 1p22.3 and deletion of 3q12.2. *Conclusions.* Copy number 'drivers' in our AYA T-ALL cohort were surprisingly similar to the paediatric population. However, the hypothesis that AYA T-ALL represents an independent group at a molecular genetic level cannot be rejected without expansion of this approach to sequence based mutations and cryptic translocations (undetectable by microarray). Gain of 1p22.3 in 12% samples implicated CLCA4, a chloride channel as warranting further study, and deletion of 3q12.2 in 8% of the samples is of interest as this corresponds to a partial deletion of the TFG gene previously implicated in oncogenic rearrangements in anaplastic lymphoma.

Table 1.

Frequency of Known Copy Number Alterations in AYA T-ALL Cohort

Cytoband	Gain/Loss	Gene (s)	AYA T-ALL % Frequency	Historical % Frequency in Paediatric Population (Reference)
9p21.3	Loss	CDKN2A/B	72 %	70% (Van Vlierberghe et al, 2008)
1p33	Loss	SIL, TAL1	24 %	25% (Van der Burg et al, 2002)
10q23.3	Loss	PTEN	12 %	8.5% (Gutierrez et al, 2009)
6q22 - q23	Gain	MYB	12 %	8% (Remke et al, 2009)
9q34.11 - 34.13	Loss	SET-NUP214	4 %	3% (Van Vlierberghe et al, 2008)
4q23 - 4q25	Loss	LEF1	4 %	11% (Gutierrez et al, 2010)
11p13	Loss	LMO2	4 %	3% (Van Vlierberghe et al, 2008)
9q34	Gain	ABL1	0 %	5% (Hagemeijer and Graux, 2010)
17q11.2	Loss	NF1	0 %	2.9% (Balgobind et al, 2008)
Xq26.3	Loss	PHF6	0 %	3% (Van Vlierberghe et al, 2010)

0022

CLINICAL IMPACT OF NOTCH1 AND FBXW7 MUTATIONS AND FLASH DELETION IN PEDIATRIC T LYMPHOBLASTIC LYMPHOMA

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Background. Lymphoblastic T-cell lymphoma (T-LBL) and T-cell acute lymphoblastic leukemia (T-ALL) are lymphoid neoplasms characterized by the proliferation of malignant T lymphoblasts arrested at early stages of maturation. They differ by the extent of bone marrow involvement, which is <25% in T-LBL. Since they share several biological characteristics, including cytology and immunophenotype they tend to both be treated on T-ALL protocols. Relapsed patients with either disease do badly, but the time to relapse tends to be shorter in T-LBL than in T-ALL and the sites of relapses differ. Identification of biological criteria associated with high risk disease is therefore important. In pediatric T-ALL protocols, the measurement of the kinetics of leukemia clearance during early induction is widely used for individual risk based stratification. This approach cannot be extrapolated easily to T-LBL, although detection of submicroscopic bone-marrow involvement (also called minimal disseminate disease or MDD) by flow cytometry or molecular quantification of TCR rearrangements has been associated with unfavourable EFS. NOTCH1 and FBXW7 (N/F) mutations occur in >50% of all childhood T-ALL patients and have previously been associated with a favorable prognostic in several protocols but not all. To date, few data are available on the frequency and the prognostic value associated with N/F mutational status in T-LBL. Chromosome 6q deletion (del6q) has been suggested by Burkhardt *et al.* to identify a poor prognostic group of T-LBL. *Aims.* Monitoring of MDD remains a late criteria for therapeutic adjustment, justifying the identification of molecular poor prognostic markers at diagnosis which would facilitate early therapeutic stratification. In this study, we determined the frequency and the prognostic value of N/F mutations and FLASH deletion at 6q in a homogenous cohort of 47 pediatric T-LBL. *Methods.* tumoral samples were obtained at diagnosis from lymph nodes (n=8), pleural effusions (n=30), mediatinum (n=7) or tissue biopsies (n=2). N/F mutations were identified by direct sequencing and allelic dosage was used to detect FLASH deletions. *Results.* NOTCH1 and/or FBXW7 mutations (N/F^{mut})were found in 24 of 47 T-LBL, when they identified a group with superior prognosis considering the EFS (p=0.01). NOTCH1 mutations were found in 45% (21/47) and FBXW7 mutations in 13% (6/45) of T-LBL. FLASH monoallelic deletions were observed in 20% (9/46) of T-LBL, similar to the previously published incidence of del6q (19%). We observed no biallelic FLASH deletions. The majority of patients with FLASH deletion were not mutated for NOTCH1 or FBXW7 (N/F^{wt})(7/9) but this was not statistically significant. To date, 9 patients from the cohort had a clinical event: 1 with NOTCH1 mutation without FLASH deletion, 4 with FLASH deletion without N/F^{mut} and 4 with neither FLASH deletion nor N/F^{mut}. *Conclusion.* FLASH genetic dosage represent a simple alternative to LOH or CGH to identify T-LBL with del6q. FLASH deletions do not appear to accentuate the bad prognosis of N/F^{wt} T-LBL. Given that FLASH deletion is found preferentially in N/F^{wt} cases it is possible that its poor prognosis reflects the absence of N/F^{mut}, rather than being directly related to the del6q.

0023

IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA THE RISK EFFECT OF NQO1 C609T POLYMORPHISM AND THE PROTECTIVE EFFECT OF MTHFR A1298C POLYMORPHISM NEUTRALIZE EACH OTHER

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Background. Xenobiotic-metabolizing enzymes constitute an important line of defense against a variety of carcinogens. Many are polymorphic, constituting the basis for the wide inter-individual variation in metabolic capacity and possibly a source of variation in the susceptibility to chemical-induced carcinogenesis. *Aims.* The aim of this study was to determine the existence of any association between the genetic polymorphisms of NQO1 C609T & MTHFR C677T & A1298C, and altered risk for pediatric ALL. *Methods.* A total of 91 patients and 311 controls were genotyped by means PCR-RFLP-based assays. Mutated alleles comprising NQO1 C609T & MTHFR C677T & A1298C were analyzed along with the wild-type alleles. *Results.* The frequency of NQO1 609CT heterozygous genotype was 42(46.7 %) among patients compared to 87 (30.2%) among controls; the difference was found to be statistically significant (P. value = 0.003, O.R=2.214 & 95% C.I =1.343 - 3.649). The frequency of the NQO1 609TT homozygous genotype was 8 (8.6 %) among patients compared to 13 (4.5 %) among controls; the difference was found to be statistically insignificant (P. value = 0.04, O.R=2.822& 95% C.I =1.099 - 7.248). this results pointed to 2.2 folds and 2.8 fold increased risk of ALL for both heterozygous and homozygous respectively. MTHFR A1298C heterozygous genotype was significant higher in the control group 140 (45%) however in ALL group 22 (25%) (P = 0.001, OR =0.382 and95% C.I =0.222 - 0.658. this results pointed to 2.6 folds of protective effect among the control group. The frequency of the MTHFR1298AC heterozygous and NQO1 609CT heterozygous, i.e. (ACCT) genotype was 8 (9.5 %) among patients compared to 40 (14.0 %) among controls; the difference was found to be statistically insignificant (P. value = 0.280). also the frequency of combined genotype MTHFR1298AC heterozygous and NQO1 609TT homozygous (ACTT) was 2 (2.4 %) among patients compared to 7 (2.5 %) among controls; the 2 figures are comparable (P. value = 0.969) (Table 50). The protective effect of MTHFR 1298AC allele and the risk effect of NQO1 C609T neutralize each other. *Summary*

and Conclusion. on a separate analysis the NQO1 609Ct&TT associated with increased risk of pediatric ALL while MTHFR 1298AC associated with protective effect. On combined analysis, both effete neutralize each other.

0024

MUTATION OF THE RECEPTOR TYROSINE PHOSPHATASE PTPRC (CD45) IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Deregulated tyrosine kinase signaling is an important factor that contributes to the enhanced proliferation and survival of leukemic cells. In T-cell acute lymphoblastic leukemia (T-ALL), activation of tyrosine kinases (ABL1, JAK1, LCK, FLT3) has been reported in approximately 20% of patients. Additionally, our group recently reported deletions of the tyrosine phosphatase PTPN2 in 6% of T-ALL cases. Loss of PTPN2 was shown to potentiate oncogenic kinases as well as influence response to kinase inhibitor treatment. *Aims.* It can be expected that additional tyrosine kinases and phosphatases remain to be discovered that play a prominent role in the pathogenesis of T-ALL. To identify novel therapeutic tyrosine kinase targets in T-ALL, we screened a panel of 10 T-ALL cell lines with an siRNA library targeting the human tyrosine kinome. *Results.* Our siRNA screen successfully identified known critical tyrosine kinases in three control leukemia cell lines (NUP214-ABL1 in ALL-SIL cells, FIP1L1-PDGFRA in EOL-1 cells, and LCK in HSB-2 cells), establishing it as a reliable tool for target identification. Furthermore, our screen identified several critical tyrosine kinases in other T-ALL cell lines, including LCK (in three cell lines), NTRK2, and JAK1 (each in one cell line). We identified and confirmed JAK1 to be an essential kinase for the proliferation of DND41 cells. The JAK1 gene itself was not mutated in DND41, but we identified a heterozygous nonsense mutation in the PTPRC gene. PTPRC encodes the tyrosine phosphatase CD45, a known negative regulator of JAK kinases. In functional assays in T-cell lines, siRNA mediated knockdown of CD45 indeed caused increased JAK/STAT signaling downstream of cytokine receptors. Sequence analysis of PTPRC subsequently identified missense and nonsense mutations in 3 of 15 T-ALL cell lines and 5 of 65 primary T-ALL cases at diagnosis. *Conclusion.* Our data demonstrate that siRNA screens can identify tyrosine kinases that are essential for the proliferation of T-ALL cells. Based on these results, we identified loss-of-function mutations in the receptor tyrosine phosphatase CD45 (PTPRC). Our data provide genetic and functional evidence for a tumor suppressor function for CD45.

0025

C-JUN PROMOTES BCR-ABL INDUCED LYMPHOID LEUKEMIA BY INHIBITING METHYLATION OF THE 5' REGION OF CDK6

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Background. The transcription factor c-JUN and its upstream kinase JNK1 have been implicated in BCR-ABL induced leukemogenesis. JNK1 has been shown to regulate BCL2 expression thereby altering leukemogenesis, but the impact of c-JUN remained unclear. *Aims.* In this study we show that JNK1 and c-JUN promote leukemogenesis via separate pathways, since lack of c-JUN impairs proliferation of p185BCR-ABL transformed cells without affecting viability. *Methods/Results.* The decreased proliferation of c-Jun cells is associated with the loss of cyclin dependent kinase 6 (CDK6) expression. In c-Jun cells CDK6 expression becomes down-regulated upon BCR-ABL induced transformation which correlates with CpG island methylation within the 5' region of Cdk6. We verified the impact of Cdk6 deficiency by using Cdk6-/- mice that developed BCR-ABL induced B-lymphoid leukemia with significantly increased latency and an attenuated disease phenotype. In addition we show that re-expression of CDK6 in BCR-ABL transformed c-Jun cells reconstitutes proliferation and tumor formation in Nu/Nu mice. *Summary.* In summary, our study reveals a novel function for the AP-1 transcription factor c-JUN in leukemogenesis by antagonizing promoter methylation. Moreover, we identify CDK6 as relevant and critical target of AP-1 regulated DNA methylation upon BCR-ABL induced transformation, thereby accelerating leukemogenesis.

Acute myeloid leukemia - Biology 1

0026

SMALL SOMATIC MUTATIONS IN ACUTE PROMYELOCYTIC LEUKEMIA (APL) IDENTIFIED BY EXOME SEQUENCING

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The t(15;17) translocation which results in the PML/RARA fusion is the disease defining lesion in nearly all cases of acute promyelocytic leukemia (APL). Despite the importance of the PML/RARA fusion for the pathogenesis of APL it is most likely not sufficient to cause leukemia alone. For example, collaborating mutations affecting the FLT3 receptor tyrosine kinase are found in about 20-30% of APL patients. To screen systematically for additional mutations, we performed whole exome sequencing of 3 APL patients. Thereby, we generated at least 5 Gbp of exome sequence for each of the APL samples and for each of the corresponding remission samples. This allowed us to cover at least 80% of RefSeq coding exon positions with a minimum read depth of 10 and at least 75% of RefSeq coding exon positions with minimum read depth of 20. By comparing the APL exome sequence with the remission exome sequence, we screened for small APL-specific genetic variants. We were able to confirm 3 to 5 somatic mutations per patient by Sanger sequencing. These APL specific mutations affected not only known mutational targets in leukemia, such as WT1 and KRAS, but also genes with potential implications in leukomogenesis, such as LYN, encoding a kinase which acts downstream of FLT3, and a novel homeobox gene. Our findings demonstrate that exome sequencing is an efficient method to screen for leukemia specific point mutations and small indels that may collaborate with the PML/RARA fusion during the onset and progression of APL.

0027

HIGHLY EXPRESSED MIR-125B AND LET-7C FORM ONE POTENTIAL MECHANISM TO EVADE THE GROWTH INHIBITION OF TGF? IN ACUTE MEGAKARYOBLASTIC LEUKEMIA OF DOWN SYNDROME PATIENTS

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Background. TGFβ has been shown to play a crucial role in cancer development through its function as a tumor suppressor. Via the SMAD-signaling pathway it regulates apopotosis as well as differentiation. In hematopoiesis TGFβ is mainly known through its inhibitory effects on proliferation. Children and neonates with Down syndrome (DS; i.e. trisomy 21) are highly predisposed to develop acute megakaryoblastic leukemia (ML-DS) and the antecedent transient leukemia (TL-DS). Significantly increased levels of TGFβ in the amniotic fluid of DS-pregnancies and the secretion of TGFβ through the leukemic megakaryoblasts are suggestive for a pivotal escape mechanism of the leukemic cells to evade the growth inhibition of TGFβ. The molecular mechanism of this disruption of the TGFβ pathway have still to be elucidated in ML-DS. Especially the role of the highly expressed chromosome 21 (hsa21)-encoded miRNAs miR125b-2, miR99a and let-7c in this TGFβ escape mechanism of ML-DS cells remains unknown. *Aims.* Here, we studied the function of hsa21-encoded miRNAs miR-125b-2, miR-99a and let-7c in TGFβ signaling. We hypothesized a putative role of these miRNAs in DS leukemogensis by suppressing its inhibitory effects allowing ML-DS-blasts to grow under high TGFβ conditions. *Methods.* We employed pathway-specific in silico target prediction (Diana mirPath and miRGen), followed by *in vitro* studies to assess the response of various cell lines to TGFβ and by functional validation in lentivirally transduced cell lines. *Results.* Two independent target prediction algorithms showed in silico enrichement of TGFβ pathway components in the potential targetome of the Hsa21 encoded miRNAs, especially miR-125b-2 and let-7c. We addressed our attention to identify the key factors of the disruption of the TGFβ signaling, which seems to be negatively regulated through the miRNAs. Consistent with these data we found decreased mRNA levels for SMAD3 (~10fold in ML-DS cell lines compared to K562 cells exhibiting low endogenous hsa21 miRNA expression), for TGFBR1(~ 5fold compared to K562) and for SMAD2 and SMAD4. Next, we assessed the viability of amongst others leukemic cell lines with known TGFβ-escape mechanisms, ML-DS and non DS-AMKL cell lines with various levels of miRNA expression under a wide range of TGFβ concentrations . These studies gave high indices that the TGFβ resistance is caused by miR-125b-2 or let-7c. Thus, we focussed on two TGFβ-responder cell lines, NB4 (FAB-classification M3) and MV4:11 (FAB-classification M5). Overexpression in NB4 and MV4:11 with decreased basic levels of these essential miRNAs conferred a stronger survival (up to 1.4fold increase of viable cells after 5 days of TGFβ-incubation relative to controls) under high TGFβ concentration. *Summary.* Our data revealed the connection between the phenotypic non-responder morphology of the ML-DS blasts and miRNA expression levels. We could demonstrate the interplay of miR-125b-2, let-7c and TGFβ signaling showing the morphological change of high sensitive cell lines into more resistant cell lines through overexpression of miR-125b-2 in MV4:11 and through overexpression of miR-125b-2 and let-7c in NB4 cells.

0028

HIGH-THROUGHPUT CLONAL SEQUENCING OF SMALL RNA TRANSCRIPTOME IN ACUTE MYELOID LEUKAEMIA CHARACTERISED HUNDRED OF NOVEL SMALL RNA MOLECULES

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Background. Despite many years of clinical research into the treatment of acute myeloid leukaemia (AML), there is still a real need for new therapeutic targets. Because of their regulatory role in gene expression, microRNAs (miRNAs) and other small non-coding (snc)RNAs could potentially be exploited in clinical treatment regimes. However, a deeper understanding of their role in AML needs to enable this. It was recently established by real-time PCR that the expression level of miRNAs provides molecular signatures characteristic of the major translocation-mediated gene fusion events in AML. However the results were restricted to a fraction of known miRNAs. *Aims*. This study aims to systematically characterise the whole small RNA transcriptome in AML. This includes the quantification and the discovery of novel tumour associated miRNAs and sncRNA species, including edited molecules, relevant to the occurrence, development and management of the disease. *Methods.* We report the results of high-throughput clonal sequencing (Solexa-Illumina) of 38 libraries of size-fractionated RNA obtained from 36 cytogenetically and clinically distinct cases of AML and 2 normal bone marrow from healthy donors. Sequences were aligned to the genome of reference (NCBI36/hg18) and to miRNA and sncRNA databases (MiRBase and fRNAdb) using Novoalign. A location was recorded where >=2 reads mapped in any sample. The distribution of sncRNAs and miRNAs was determined for each sample. MiRDeep and snoSeeker were used to identify potential novel miRNA and small nucleolar RNA (snoRNA) candidates, respectively. *Results.* We detected the expression of 765 known miRNAs and 684 uniquely located species of sncRNAs, including small nuclear (sn)RNAs, piwiRNAs, Y-RNAs, and small nucleolar (sno)RNAs. MiRNAs and other sncRNAs accounted for 53.8% and 21% of total reads, respectively. Alignment of the sequence to the hairpin miRNA database confirmed the presence of 144 miRNA star (*) not yet reported in the miRBase database. Approximately 3.7% of total reads were from unknown tags and were investigated for novel miRNA and sncRNA species. A total of 575 potential miRNA candidate hairpins were identified. Of these, 126 were located in exonic regions of the genome. We also identified 37 C/D Box and 51 H/ACA Box, potential novel snoRNAs. In general, the novel sncRNAs were expressed at a low level compared with the already known molecules. ANOVA test applied to the 38 AML samples in order to find miRNAs with statistically significant differences in expression level among the major cytogenetic groups identified 177 miRNAs passing a 0.5% false discovery rate (FDR) filter. These included 70% of miRNAs at 14q32 imprinted region in t(15;17) AMLs. *Summary/Conclusions.* High-throughput sequencing revealed a complex distribution of small non-coding RNAs, including the detection of new hairpin molecules, in cancer cells. Also it provided the small RNA expression pattern in AML. Further investigations, especially in the role of snoRNAs, may uncover novel aspects of the disease aetiology.

0029

TET2 AND IDH1/2 MUTATIONS IN SECONDARY ACUTE MYELOID LEUKEMIAS: A FRENCH RETROSPECTIVE STUDY

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Background. TET2 mutations were recently involved in 20-26% of MDS, in 12% of MPN and in 10-17% of AML. The impact of TET2 mutations is not fully examined in secondary AML (SA) which encompass myelodysplasia-related changes (MRC) AML and therapy-related (TR) AML according to the WHO classification *Methods.* In this study, we have collected bone marrow samples from 247 patients at diagnosis identified as SA. We have determined the status of TET2 gene together with other genes frequently mutated in AML (IDH1, IDH2, NPM1, FLT-3, N and K-RAS, c-KIT). *Results.* The cohort of 247 samples was subdivided in 201 MRC AML and in 46 TR AML. A normal karyotype (NK) was found in 70 patients (39.5%). 69 abnormalities of the TET2 sequence were found and dispatched among 49 patients corresponding to 19.8% of patients with at least one TET2 mutation. TET2 mutations are significantly more frequent in MRC AML (22.3%) than in TR AML (8.7%) (P=0.03). The SA patients harbouring a TET2 mutation are significantly older and present higher levels of hemoglobin and leukocyte, involving monocytes, than unmutated patients. They also exhibit a significant lower MCV and platelets count. Percentage of blasts in the bone marrow is similar in the two groups. These particular modifications of the biological parameters are observed independently of the presence of a normal karyotype. A NK is present in 51% of TET2 mutated patients and in 23% of unmutated patients, indicating that TET2 mutations are strongly associated with NK (P<0.001). By contrast, 57% of unmutated patients versus 29% of TET2 mutated patients have a complex karyotype. IDH mutations (~14% of the whole cohort) are not mutually exclusive of TET2 mutations. No statistical association can be found between TET2 mutations and NPM1, FLT-3, N and K-RAS mutations. There is a slight increase of c-KIT mutations in the TET2 mutated group as described in mastocytosis. In 158 patients receiving intensive chemotherapy at diagnosis, the complete remission rate and the overall survival are identical in the TET2 mutated and unmutated groups. *Conclusion.* TET2 mutated SA at diagnosis present particular clinical and biological characteristics but no association with markers of poor prognosis as complex karyotype or FLT-3 mutations. TET2 mutations have no impact on survival.

0030

DELETION OF THE TUMOR SUPPRESSOR GENE NF1 OCCURS IN 5% OF MYELOID MALIGNANCIES AND IS ACCOMPANIED BY A MUTATION IN THE REMAINING ALLELE IN 56% OF CASES

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Background. Alterations of the RAS pathway play an important role in the pathogenesis of myeloid malignancies and occur either by activating mutations in RAS itself or by mutations in genes involved in RAS-dependent pathways such as FLT3, KIT or CBL. The NF1 gene encodes neurofibromin 1 and is a negative regulator of RAS signaling. *Aims/Methods.* In order to analyze the role of NF1 in adult AML, we first evaluated NF1 gene expression in 272 AML using microarrays (HG-U133 Plus 2.0, Affymetrix, Santa Clara, USA). These included cases with t(15;17) (n=15), t(8;21) (n=16), inv(16) (n=7), t(11q23)/MLL-rearrangement (n=10), inv(3)/t(3;3) (n=3), complex karyotype (n=47), normal karyotype (n=97), and with various other genetic abnormalities (n=77). *Results.* The median NF1 expression intensity was 131.6. 68 cases showed an expression intensity of NF1 below 98.6 (first quartile, low expression group). In this cohort cases with t(8;21) (n=10) or complex karyotype (n=18) were over-represented (Chi-square: p<0.0001 and p=0.021, respectively), while cases with normal karyotype (n=16) were under-represented (Chi-square: p=0.016). Low NF1 expression could be due to a deletion of one NF1 gene copy. Therefore, we performed FISH analysis using a NF1-probe in 54/68 of cases with low NF1 expression with available material. In 11/54 of these cases (20.4%) a NF1 deletion was identified by FISH. Chromosome banding analysis in the 11 cases with NF1 deletion revealed a complex karyotype (n=7, one of theses cases with inv(3)), a normal karyotype (n=2), an inv(3) (n=1), and a 5q-deletion accompanied by +21 (n=1), respectively. To further investigate the incidence of NF1 deletion in myeloid malignancies 889 additional patients were analyzed by FISH for NF1 deletion. A heterozygous NF1 deletion was observed in 46/889 (5.2%) patients. In detail, 23/315 (7.3%) de novo AML, 3/72 (4.2%) s-AML, 4/25 (16%) t-AML, 7/176 (4.0%) CMML, 2/165 (1.2%) MDS, and 7/136 (5.1%) MPN showed NF1 deletions. Chromosome banding analysis in the NF1-deleted cases revealed a normal karyotype (6/509; 1.2%), an inv(16)/t(16;16) (6/37; 16.2%), an inv(3)/t(3;3) (3/27; 11.1%), a complex karyotype (n=19/62; 30.6%) or other abnormalities (12/162; 7.4%). The frequency of NF1 deletions was remarkably high in patients with inv(16)/t(16;16) and inv(3)/t(3;3) (6/37 cases (16.2%) and 3/27 cases (11.1%), respectively), both subgroups are known to be associated with NRAS mutations. The frequency of NF1 deletions in patients with complex karyotype was 30.6% (19/62) and in patients with normal karyotype 1.2% (6/509). Further, next-generation deep-sequencing (454 Life Sciences, Branford, CT) was used to study molecular NF1-mutations in 32 patients with NF1 deletions. In 18 cases (56.3%) a NF1 mutation was detected. *Conclusions.* NF1 deletions occur in 7.3% of de novo AML, 4% of CMML, 1.2% of MDS and 5.1% of MPN and therefore are a frequent and important alternative genetic mechanism for activating the RAS pathway in adult myeloid malignancies. Furthermore, in 58% of cases with NF1 deletion as detected by FISH a NF1 mutation was observed in the remaining allele. Future studies are necessary to determine the prognostic impact of NF1 deficiency either caused by downregulation, deletion and/or mutation in the various subtypes of myeloid malignancies.

0031

NPM1 DELETION/HAPLOINSUFFICIENCY IS A FEATURE OF MYELOID LEUKEMIAS WITH HIGH RISK CYTOGENETICS

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Background. We previously identified heterozygous *NPM1* exon 12 somatic mutations in approximately 60% of adult acute myeloid leukemia (AML) with normal karyotype (NEJM 2005). The unmutated allele was never deleted in AML mutated cases. *NPM1* haplo-insufficiency leads to genomic instability and causes bone marrow dysplasia in mice (Nature 2005). *NPM1* maps at human chromosome 5q35. *Aims.* To investigate *NPM1* haploinsufficiency in human myeloid leukemias with complete or partial loss of chromosome 5 (-5/5q-). *Methods.* A total of 213 bone marrow or peripheral blood samples were studied from 120 females and 93 males (age range 9-94) with myeloid leukemias and -5/5q- or not. Findings from 145 cases have already been published (PLoS One 2010). The other 68 cases [39 myelodysplastic syndrome (MDS), 27 AML, and 2 primary myelofibrosis (PMF)] belong to a consecutive prospective series of cases that were analyzed in our laboratory from January 1st 2007 to January 31st 2011. Cytogenetically, 65/213 cases had isolated 5q-; 127 cases had a -5/5q- plus one or more changes; 21 cases had a complex karyotype without -5/5q-. To investigate *NPM1*/5q35 we used interphase FISH with clone RP11-117L6 encompassing the entire gene. *TP53*, *ATM*, and *XRCC2* deletions were investigated by FISH with RP11-199F11 clone, LSI p53/LSI ATM (Vysis, Abbott), and CTD-2326K14. In 31 retrospective cases with *NPM1* deletion exon 12 mutations of the residual allele were investigated by direct sequencing. *Results. NPM1* heterozygous deletion was found in 58 cases: 56/127 (44%) with non isolated -5/5q- and 2/65 (3%) with isolated 5q- (P=0.000). According to *NPM1* status, cases with non-iso-

lated -5/5q- were grouped as NPM1+/- and NPM1+/+. A diploid karyotype was present in 2/56 NPM1+/- (3.5%) and in 23/71 NPM1+/+ (32.3%) (P<0.001). Significantly more monosomies were found in the NPM1+/- subgroup (median: 3, range: 0-9 vs median: 2, range:0-7) (P=0.009). Gross chromosomal changes, i.e. markers, rings, and double minutes, were found in 43/56 NPM1+/- (76.7%) and in 33/71 NPM1+/+ (46.4%) (P=0.0005). Distribution of events involving *TP53*/17p13, *ATM*/11q23 or *XRCC2*/7q36 was not significantly different in NPM1+/+ and NPM1+/- subgroups. No cryptic deletion emerged in cases with complex karyotype without -5/5q-. *Summary/Conclusions. NPM1* deletion/haploinsufficiency clearly emerged as a feature of over 40% of human myeloid leukemias associated with high risk cytogenetics including -5/5q-., with the incidence reaching 41.9% in the prospective series of 68 cases. NPM1+/- cases showed a significantly higher prevalence of monosomies and gross chromosomal changes, suggesting that in humans, as in mice, *NPM1* haploinsufficiency was related to genomic instability independently of TP53. AML/MDS associated with *NPM1* deletion/haploinsufficiency are strikingly different from the karyotypically stable, low-risk AML associated with *NPM1* heterozygous mutations. Accordingly *NPM1* sequence mutation and deletion are mutually exclusive.

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0032

REACTIVATING PP2A BY FTY720 AS A NOVEL THERAPY FOR AML WITH C-KIT TYROSINE KINASE DOMAIN MUTATION

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Background. C-KIT is a type 3 receptor tyrosine kinase. Its tyrosine kinase domain (TKD) mutations are associated with poor prognosis in acute myeloid leukemia (AML). The most frequently occurred C-KIT/TKD mutation in AML is D816V. Protein phosphatase 2A (PP2A) is a human tumor suppressor and its dysfunction might contribute to malignant cell behavior. As far as we know, PP2A activity status in AML subgroups harboring C-KIT/TKD mutation has not been demonstrated. FTY720 shows promising preclinical activity in patients with refractory CML, while its role in C-KIT/TKD AML remains to be elucidated. *Aims.* To investigate PP2A statues in C-KIT/TKD AML and develop FTY720 as a novel therapy for C-KIT/TKD AML. *Methods.* Eight AML patients harboring C-KIT/D816V and twelve AML patients harboring wide-type C-KIT (C-KIT/WT) were recruited and informed consent at Union hospital of Wuhan in this study. Real-time quantitative PCR was used to identify C-KIT/WT and C-KIT/D816V. PP2A activity in the peripheral blood mononuclear cell of patients was assayed with a PP2A Immunoprecipitation Phosphatase Assay Kit. Furthermore, primary AML cells and cell lines were treated with FTY720 alone or combined with specific PP2A inhibitor okadaic acid (OA) in culture. Cell growth was assessed using the CCK8 Kit. Cell apoptosis was analyzed by flow cytometry after dual staining with annexin V/ propidium iodide. Western blotting was used to analyze the protein changes. All experiments were repeated three times. Statistical analysis was performed using SPSS 17.0. *Results.* PP2A activity was significantly decreased in AML subgroups harboring C-KIT/D816V mutation compared with C-KIT/WT AML (P = 0.043). In addition, FTY720 induced toxicity in all AML cells, including Kasumi-1, HL60 harboring C-KIT/WT and primary cells from AML patients in a time- and dose-dependent manner. PP2A inhibitor OA rescued these cells from FTY720-induced apoptosis. Furthermore, Increased PP2A activity was detected after FTY720 treatment. When cells were pretreated with OA, PP2A activity was partially rescued. Finally, PP2A expression remained unchanged after FTY720 treatment. Our results strongly suggested that FTY720-induced cytotoxicity was mediated by PP2A activation without altering its expression. Interestingly, it was observed that cells harboring C-KIT/TKD mutation were more sensitive to this agent (IC50: 13.5 μM VS 20 μM, P< 0.05). *Conclusions.* From the standpoint of prevalence, PP2A activity is decreased in patients with C-KIT/TKD AML. Our study also provides evidence for the PP2A-dependent toxicity of FTY720 in AML cells, and these effects of FTY720 appear to be most marked in C-KIT/TKD AML where conventional chemotherapeutic approaches are most likely to fail. The significant preclinical efficacy of FTY720 indicates that it may be valuable therapeutic agents for refractory C-KIT/TKD AML.

0033

GLOBAL DNA METHYLATION ANALYSIS OF ACUTE MYELOID LEUKAEMIA REVEALS LEUKAEMIA-SPECIFIC AND SUBTYPE-SPECIFIC PATTERNS ASSOCIATED WITH GENE PROMOTERS, CPG ISLANDS AND REPEAT ELEMENTS

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Methylated DNA immunoprecipitation followed by high-throughput sequencing (MeDIP-seq) of 12 acute myeloid leukaemia and 4 normal bone marrows has been performed. An ethical approval has been obtained for this study. This revealed 18,081 regions of the genome that exhibit differential levels of methylation between leukaemia and normal bone marrow ($p < 0.05$). Such differentially methylated regions (DMRs) included not only certain gene promoters, gene bodies, CpG islands, CpG island shores but also members of the LINE and SINE repeat families. The majority of regions exhibited hypermethylation in leukaemia, with a smaller number exhibiting leukaemia associated demethylation. The gene promoters methylated in leukaemia included previously identified genes e.g. MYOD1, ID4, DCC but also revealed potentially important novel methylation targets e.g. DPP6, CASP7, TERT. The following karyotypic AML subtypes were included in the analysis t(8;21), t(15;17), +8 and normal karyotype. It was also possible to identify regions exhibiting strong subtype specificity, including certain gene promoters, gene bodies, CpG islands, CpG island shores especially in t(8;21) subtype. Additionally clear subtype specificity was found in the methylation of members of the LINE and SINE repeats families. Confirmation of these data was obtained by pyrosequencing analysis of chosen regions in 63 additional AML samples. Gene expression profiles were available for certain of the AMLs analysed by MeDIP-seq. We were therefore able to demonstrate negative correlation between gene expression and corresponding methylation of promoter, CpG islands and CpG island shores (1-3KB from transcriptional start site) in a direct manner ($p < 0.0001$). However, gene body methylation was insignificant to corresponding gene expression. Furthermore, we were able to demonstrate significantly different promoter methylation levels for genes up and down regulated in t(8;21) and normal karyotype AMLs ($p = 0.005$, $p = 0.044$ respectively). Interestingly, however, there was no significant difference in methylation levels for such genes in our t(15;17) AMLs.

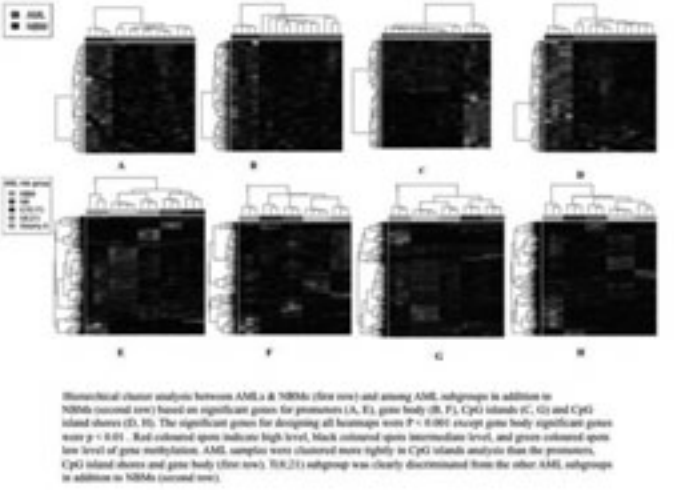

Figure 1.

0034

THERAPY-INDUCED SELECTIVE ELIMINATION OF LEUKEMIA INITIATING CELLS IN XENOTRANSPLANTATION MODEL OF AML

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Background. Acute Myeloid Leukemia (AML) is a heterogeneous disease associated with various genetic and epigenetic abnormalities. It is characterized by the uncontrolled proliferation of myeloid blast cells blocked in their differentiation. Epigenetic abnormalities are reversible in contrast to genetic abnormalities, which are irreversible. Agreeing on these concepts it was argued that epigenetic targeting possibly would bring back the malignancy to a more normal state. 5-azacytidine (5AZA, DNA methyl transferases inhibitor) and valproic acid (VPA, Histone deacetylases inhibitor) are among the various pharmacological drugs used as epigenetic modifiers in clinics to treat AML. Based on these reports, we hypothesized that combining these drugs along with a differentiating and apoptotic agent ATRA may have good therapeutic effects in all AML subtypes including ATRA resistant AML. *Aims.* Establishment of a preclinical model for the validation of drugs in targeted therapy of AML. *Methods.* Blood and bone marrow samples from patients were collected after informed consent was obtained. CD3 negative cells were transplanted through retro-orbital sinus into male NOD/shi-SCID IL2Rg-/- (NOG) mice. AZA and VPA was injected daily for 7days and on day 8 ATRA pellet was implanted and monitored foe 21days after that mice were sacrificed. The cells were collected from bone marrow and spleen along with blood from untreated and treated mice for various assays (QRTPCR, MGG staining, immunophenotyping, FISH, culture in semisolid medium and secondary transplantation). *Results.* Treatment of xenotransplanted mice (FAB: M2, M3 and M5) with the combination AZA, VPA and ATRA, results in the progressive elimination of leukemic cells from the mice environment although. This effect is related to the differentiation of abnormal leukemic human cells, as the remaining human cells after the treatment were mature and differentiated. In case of Acute promyelocytic leukemia (APL) there was decrease in the PML-RAR transcript after treatment, clearly indicated the decrease in the leukemic blast in the mice. In addition, the treatment also leads to the elimination of leukemic stem cells as no secondary engraftment was obtained whereas the hCD45 cells from the untreated mice were successfully grafted in the 2nd generation of mice. Interestingly, with the decrease in human cells in the treated mice, there was a restoration of the mouse hematopoiesis suggesting that there is no toxic effect of the drug combination on normal hematopoiesis and the hematopoietic microenvironment. *Conclusion.* This is a useful tool to validate the real effectiveness and the presence of leukemic initiating cells after treatment. These results also show that as in a human setting an efficient therapeutic effect of this drug combination may be obtained in xenotransplanted mice. Thus provides an appropriate system to study and optimize drugs before entering to clinics. Email: patelsatyananda@gmail.com

0035

DNMT3A MUTATIONS IN INTERMEDIATE-RISK ACUTE MYELOID LEUKEMIA

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Background and Aims. Recently, mutations in *DNMT3A* gene were found to cause worse survival of patients with AML. We attempted to test their impact in our patient cohort. *Patients and methods.* 163 AML patients with an intermediate-risk cytogenetic profile, diagnosed between years 1999 and 2008, were screened for the presence of mutations in *DNMT3A* gene. The male/female ratio was 74/89. The median age at diagnosis was 56.1 (18.2-81.7) years and the initial median WBC count was 23.5 x 10^9/L (0.7-483.7). RT-PCR followed by direct sequencing (exons 11-26) was used to test the presence of *DNMT3A* mutations. *Results.* 44 patients out of 163 (27.0%) carried a mutation in *DNMT3A* gene. 28 of them had a single nucleotide change at codon Arg882 (21 had Arg882His, 6 Arg882Cys and 1 had Arg882Ser). 11 patients had another missense mutation while the remaining 5 carried various frameshift mutations. The incidence of mutations was slightly higher among female patients (30.3% vs. 23.0%) and was independent of the age of patients. According to the FAB classification, *DNMT3A* mutations were most frequently present at subtypes M1 (14/37; 37.8%) and M4 (11/35; 31.4%), on the other hand, none of 9 patients with M5 subtype harbored the mutation. Occurrence of *DNMT3A* mutations was associated with the presence of *FLT3*/ITD (P=0.012). Patients with *DNMT3A* mutation had significantly higher initial WBC counts than those without it (47.5 vs. 14.6 x 10^9/L; P=0.005). This was even more evident in patients with single nucleotide change at codon Arg882 (78.7 vs. 14.6 x 10^9/L; P=0.0004). There was no difference between mutated and unmutated group in reaching complete remission (CR) (52.3% vs. 54.6%; P=0.395). Patients with any change of codon Arg882 more easily reached CR than those with any other *DNMT3A* mutation (57.1% vs. 43.8%; P=0.196). The CR rate within the *DNMT3A*-mutated group was unfavorably affected by the presence of *FLT3*/ITD (62.5% vs. 40.0%; P=0.068). Patients positive for *DNMT3A* mutation significantly more often suffered a relapse (60.0% vs. 40.8%; P=0.049). Among mutated patients, cases with Arg882 aberration relapsed less often than patients with any other mutation (33.3% vs. 57.1%; P=0.124). The overall survival (OS) was not affected by *DNMT3A* mutations (10.9 months in mutated vs. 13.6 in unmutated cases; P=0.386). Within the group of *FLT3*/ITD positive cases, *DNMT3A* mutations caused shorter OS (6.09 vs. 10.24 months; P=0.090). When *DNMT3A*-mutated cases were analyzed with respect to the *FLT3*/ITD status, those carrying both of these aberrations had significantly shorter OS than *FLT3*/ITD negative ones (6.09 vs. 18.87 months; P=0.002). Out of 20 patients harboring both of these mutations only one is still alive. Hematopoietic stem cell transplantation (HSCT) could salvage *DNMT3A*-mutated patients: 5/11 patients survive at 2 years post HSCT. *Conclusions.* We have confirmed high incidence of *DNMT3A* mutations in patients with AML with an intermediate cytogenetic risk. Patients with any mutation in *DNMT3A* gene tend to relapse more often than negative cases. Double-mutated (*FLT3*/ITD+*DNMT3A*) patients have a very poor prognosis. Patients with AML harboring *DNMT3A* mutation may benefit from HSCT.

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0036

AZACYTIDINE AND DECITABINE CONVEY ANTI-LEUKEMIC EFFECTS IN MDS/AML CELLS WITH A CONCOMITANT CORRECTION OF FOXO3A SIGNALING

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Background. Deregulated signalling via the transcription factor FOXO3a correlates with a poor prognosis in AML (Kornblau, Clin Cancer Res. 2010) most likely due to its aberrant nuclear localisation and subsequent loss of transcriptional activity. We here tested the capacity of hypomethylating agents to "correct"the pathogenetic events and correlated it with their established ability to induce apoptosis and cell cycle arrest. *Methods.* MDS (MOLM-13)- and AML- (HL-60)-derived cell lines were incubated with AZA (0,2-2µM) and DAC (0,2-2µM). The capacity of those drugs to induce apoptosis (DiOC6(3)/PI), cell cycle arrest (PI) and their impact on the expression level (immunoblot) and subcellular localization (immunoflourescence) of DDR-related proteins were concomitantly assessed. Functional relevance was determined by co-incubation with biochemical inhibitors of ATM (KU-55933), ATM/ATR (caffeine), Chk-1 (UCN-01), and Chk-2 (NSC-109555). Most important findings were recapitulated on MDS patient cells (n=7) after selection for CD34-positivity. *Results.* As previously described, both drugs induced dose- and time-dependent apoptosis and cell cycle arrest in G2/M. Noteworthy, DAC exhibit a higher capacity to induce to induce G2/M-arrest than AZA (G2/M-increase at 48h DEC: 20%). Most importantly, this increased anti-proliferative capacity was accompanied by an earlier and more efficient activation of FOXO3a (as demonstrated by a decreased phosphorylation on Ser253) and a concomitant nuclear translocation of non-phosphorylated FOXO3a. Noteworthy, this cytoplasmic-nuclear shift of FOXO3a was recapitulated in patient-derived cells responding to hypomethylating agents. Although the here assessed cohort of patients was small, the degree of Foxo3a translocation tended to augment with increased *in vitro* sensitivity to hypomethylating agents. Most importantly, in myeloid cell lines nuclear translocation of FOXO3a was accompanied by upregulation of its transcriptional

targets known to confer G2/M-arrest (p21, p27) as well as apoptosis (PUMA). To delineate the functional consequences of these observations, we recapitulated experiments in myeloid cell lines in the presence of biochemical cell cycle inhibitors. We thus show that both agents increase activation of the checkpoint-kinases-1 and -2 (phosphorylation of Chk-1-Ser317 and Chk-2-Ser68) as well as their downstream target γ-H2AX (phosphorylation on Ser139). Inhibition of ATM-mediated signalling by KU-55933 or ATM/ATR-mediated signalling by caffeine had little to no impact on AZA/DAC-induced apoptosis or inhibition of cell cycle progression, whereas inhibition of Chk-1 by UCN-01 (and to a lesser degree inhibition of Chk-2 by NSC-109555) abrogated G2/M-arrest induced by hypomethylating agents. *Conclusions.* We showed that hypomethylating agents can correct aberrant signaling pathways in MDS/AML, notably by "correcting"aberrant cytoplasmic localization of FOXO3a thereby (re-)establishing its function as a transcriptional regulator of cell-cycle-arresting and apoptosis-inducing molecules.

0037

MIR-155 UP-REGULATION IN FLT3 POSITIVE AML INVERSELY CORRELATES WITH EXPRESSION OF MYELOID-SPECIFIC TRANSCRIPTION FACTORS PU.1 AND CEBPBETA

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Background. Acute myeloid leukaemia (AML) arises from myeloid progenitor cells that are arrested at early stages of differentiation. It is a cytogenetically heterogeneous disorder with acquired recurrent chromosomal alterations detected in about 55% of adult patients, such as translocations, inversions, deletions, trisomies, and monosomies. In the remaining 45% of cases of normal karyotype AML, a number of novel molecular abnormalities have been described, such as the internal tandem duplication (ITD) or mutation (D835) of FLT3 gene, mutations of NPM1 gene, mutation of CEBPA gene and partial tandem duplication of the MLL gene. Several studies have shown that genome-wide gene expression profiling can clearly distinguish the major cytogenetic groups, so providing a better understanding of the underlying disease biology. Despite this progress, focusing on known genes will likely not suffice to uncover the molecular puzzle of AML. The integration of a whole genome approach including non-coding RNAs may lead to an improved understanding of AML biology. MicroRNAs (miRNAs) are a class of small noncoding RNAs that negatively regulate protein expression of specific mRNA by either translational inhibition or mRNAs degradation. There are several indications that miRNAs might be a new class of genes involved in human cancer and has been observed that distinct patterns of miRNA expression reflect different developmental lineage and different genetic categories of AML. I an our previous work we performed quantitative real-time RT-PCR to study the expression of 365 known human miRNA in a cohort of 29 primary selected AML characterized by common cytogenetic and molecular alterations and we identified distinctive miRNA expression patterns in some genetic groups. *Aims and Results.* According to the recent literature we found a strong up-regulation of mir-155 in normal kariotype AML carrying FLT3 mutations (Cammarata G. *et al.*, AJH 2010); so we decide to investigate expression levels of this miRNA in a larger cohort of 47 FLT3 AML patients (42 ITD and 5 D835) and 50 AML of other genetic categories. We found a 37,259 fold up-regulation of mir-155 in the first group compared to the second (P<0,0001). Several studies demonstrated that miR-155 directly repressed a broad range of target mRNAs implicated in myeloid hyperplasia and/or hematopoiesis. Using available prediction target algorithms we selected hypothetical mir-155 regulated genes such as PU.1 and CEBPβ, both genes codify for lineage specific transcription factors, indispensable for normal myeloid development. In the same cohort of 97 patients we found an inverse correlation between mir-155 expression levels and its predicted targets: PU.1 (Fold=0,471; P=0,005), CEBPβ (Fold=0,414; P=0,008). *Conclusions.* Based upon our current study, miR-155 appears to play a role in malignant haematopoiesis targeting the expression of central myeloid specific transcription factors that may contribute to block differentiation. Future functional analysis will clarify better the role of mir-155 in AML genesis and its molecular mechanism in the inhibition of myeloid differentiation

0038

TRANSCRIPTOME-WIDE IDENTIFICATION OF RNA-BINDING PARTNERS OF THE FUS PROTEIN CRITICALLY INVOLVED IN LEUKEMOGENESIS BY THE CHROMOSOMAL TRANSLOCATION T(16;21)

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Background. The translocation t(16;21)(p11;q22) is a recurrent primary abnormality in acute myeloid leukemia (AML), joining the two proteins FUS and ERG. The FUS-ERG fusion gene occurs in 1% of AMLs and is known to be associated with young age at diagnosis and poor prognosis. *Aims.* The biological mechanism by which these oncogenes disrupt the physiological developmental program during leukemogenesis is poorly understood. While most molecular studies have focused on the fusion protein, less effort has been dedicated to the analysis of the function of wild-type FUS. Gene expression in eukaryotes is extensively controlled at the post-transcriptional level by RNA-binding proteins (RBPs) modulating the maturation, stability, transport, editing and translation of RNA transcripts. FUS represents one of the rare examples of an RBP known to be altered in AML. We thus aimed to establish the role of FUS to better understand the impact of the loss of one of its alleles in FUS-ERG positive AMLs. *Methods.* We recently developed a crosslinking technology termed PAR-CLIP (photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation) for the transcriptome-wide identification of RBP binding sites by incorporation of the photoreactive nucleoside 4-thiouridine into mRNA of cultured cells. Using next-generation sequencing, this allows mapping the exact crosslink sites thus gaining insight into the RNA-recognition elements (RREs), the bound mRNA targets and thus the function of the studied RBP. *Results.* We have employed the PAR-CLIP method for FUS. The roughly 2,000,000 generated mRNA reads gave rise to around 30,000 clusters containing at least five reads and meeting our stringent quality criteria. We found that nuclear localized FUS predominantly binds intronic RNA segments. We identified and biochemically validated the RRE of FUS, which consists of a stem-loop opened by a non-Watson-Crick U-pyrimidine base pair and an A immediately 3' to the mispaired U in the loop region. Knockdown of FUS, despite its previously proposed function, did not significantly impact splice regulation nor alter mRNA stability in a binding-site-dependent manner. We are currently in the process of analyzing previously published genes known to be dysregulated in FUS-ERG AMLs to address the question whether FUS down regulation contributes to their dysregulation. Examples include RUNX1, CDK1, CSF3R, CD56, IL2RA and IL3RA. Additionally, we will generate expression profiles of FUS-ERG AMLs to correlate changes to our knockdown data. *Summary/Conclusions.* We did not find evidence of a suggested role of FUS in splicing regulation. Instead, its abundant, ubiquitous expression suggests a more general function in supporting basic nuclear RNA processing functions. This might implicate that the inactivation of one FUS allele amplifies the oncogenic effect of the FUS-ERG fusion, or that it possibly contributes additional oncogenic activity. Future research should not solely focus on the gain-of-function effects of the FUS-ERG fusion but rather follow a dual approach in also considering the loss-of-function effects of wild-type FUS. The latter approach can lead to the design of new inhibitors, such as RNA decoys containing a high affinity ligand for the RBP, or may identify downstream regulatory targets or pathways contributing to leukemogenesis. jessica.spitzer@med.uni-duesseldorf.de

0039

HEAT SHOCK PROTEINS EXPRESSION PROFILE FOR AML PATIENTS REVELS A DISTINCT SIGNATURE STRONGLY ASSOCIATED WITH FLT3 MUTATION STATUS- CONSEQUENCES AND POTENTIALS FOR PHARMACOLOGICAL INTERVENTION

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Background. Acute myelogenous leukemia (AML) is a heterogeneous malignancy characterized by bone marrow accumulation of immature leukemic cells. Even though the AML cell biology has been extensively

characterized, this has not translated into new therapeutic strategies with major improvements in overall disease-free survival. Heat shock proteins (HSPs) exhibit sophisticated cellular protection mechanisms, acting as molecular chaperones that prevent the formation of protein aggregates and assist proteins in their folding to native structures. HSPs are important for the regulation and remodeling of several proteins involved in leukemogensis (e.g. several transcription factors and kinases), and especially inhibition of HSP90 now emerges as a promising therapeutic strategy. *Aims.* We hypothesized that the HSPs level varies between AML patients, and that this variation can be used for subclassification of patients with regard to responsiveness to targeted therapy. The aim of the present study was to quantify the HSP levels for a large cohort of AML patients, and to compare HSP levels and the effect of the HSP90 inhibitor 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) in these patients. *Methods.* We included 75 consecutive patients. AML cells were isolated by density gradient separation and contained at least 95% leukemia blasts. Cell lysates were prepared from cryopreserved cells and protein levels of HSP27 (phosphor-Ser 15, phspho-Ser82), HSP40, HSP60, HSP70 and HSP90 determined by multiplex immunoassay for flow cytometric analyzes. The levels were compared with clinical and biological patient characteristics by using robust bioinformaticals tools including hierarchical clustering analyses. Functional characterization of 17-DMAG effects on AML cells included regulation of apoptosis, proliferation and release of angioregulatory cytokines. *Results.* AML cells derived from various patients differed in the expression level of different HSPs, and patients could be divided into two major clusters with low (cluster I) and high (cluster II) HSP levels. The two clusters did not differ with regard to AML etiology (de novo vs. secondary/relapse), differentiation (FAB classification, expression of CD34), cytogenetics or frequency of NPM1 mutations. In contrast, the frequency of FLT3 mutation was significantly higher in the high-HSP cluster II, thus the HSP levels are generally higher in primary AML cells with chemoresistant FLT3 mutations (p=0.0002). HSP90 inhibition showed a stronger proapoptotic effect in FLT3-ITD positive patients, whereas HSP90 inhibition had a strong antiproliferative and antiangiogenic effect in all the identified patient clusters. *Summary/Conclusions.* The HSP expression profile of primary human AML cells varies between patients, and especially patients with FLT3 mutations have special high intracellular protein levels of HSP. This is consistent with the hypothesis that HSP90 stabilizes this important oncogenic kinase. These differences may (i) be important for the responses to conventional chemotherapy and HSP90 inhibitors, or (ii) be used to identified patients with a high susceptibility to HSP90 inhibition.

0040

DIFFERENTIAL GENE EXPRESSION IN CYTOGENETICALLY NORMAL AML WITH RUNX1 MUTATIONS IS CHARACTERIZED BY HIGH EXPRESSION OF DNTT (DEOXYNUCLEOTIDYLTRANSFERASE, TERMINAL)

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The AML1/RUNX1 gene is a frequent mutational target in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). We have screened 95 cytogenetically normal AML (CN-AML) patients for RUNX1 mutations by capillary sequencing of genomic DNA. This led to the identification of 9 patients with RUNX1 mutations (9,5%). While RUNX1 mutations were significantly associated with older age, male sex, MLL-PTD mutations and poor clinical outcome, an inverse correlation with NPM1 mutations was observed. Patients with RUNX1 mutations showed a unique gene expression pattern with differential expression of 55 genes. The most significantly upregulated gene in RUNX1 mutation positive CN-AML was DNTT (deoxynucleotidyltransferase, terminal) which was previously shown to be associated with poor prognosis in AML (Venditti el., Leukemia, 1998). DNTT plays a role in early lymphoid differentiation where it generates antigen receptor diversity by synthesizing non-germ line elements (N-regions) at the junctions of rearranged Ig heavy chain and T cell receptor gene segments. Thus, high expression of DNTT in RUNX1 mutated AML might indicate abortive lymphoid differentiation during leukemogenesis in these patients. Interestingly, 21 out of 55 differentially expressed genes were reported to be also deregulated in RUNX1 mutated AML M0 (Silva *et al*., Blood, 2009) indicating that the expression of these genes is very likely to be influenced by RUNX1 mutations. These findings provide insights into the pathogenesis of RUNX1 mutation positive AML and may contribute to the identification of novel diagnostic markers and targets for therapy.

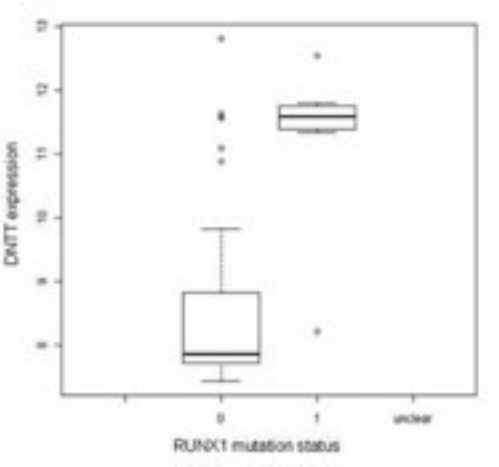

Figure 1. DNTT-Expression in RUNX1 wt vs RUNX1 mutated AML.

0041

THE DEACETYLASE INHIBITORS DACINOSTAT AND VORINOSTAT INHIBIT SELF-RENEWAL AND REPOPULATION CAPACITY OF AML1/ETO- AND PLZF/RARA-EXPRESSING MURINE STEM CELLS

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Background. Deacetylase inhibitors (DACi) are promising drugs leading to growth inhibition, cell cycle arrest, premature senescence and apoptosis in malignant cells. In acute myeloid leukemia, the most primitive population of leukemic stem and progenitor cells (LSC) are thought to account for relapses of the disease. As the efficacy of a DACi therapy depends on its capacity to eradicate the LSC compartment, we aimed to study function and gene expression in response to DACi treatment. *Aims.* Aim of our study was 1) to analyze the impact of an *in vitro* treatment with various DACi on the proliferation and self-renewing capacity of normal and leukemic stem cells and 2) to assess DACi-induced regulation of genes involved in survival of leukemic progenitor cells. *Methods.* Murine Sca1+/lin- hematopoietic stem cells (HSC) were retrovirally infected with AML1/ETO and PLZF/RARα known to induce an acute leukemia in C57BL/6N mice. Serial replating and day 12 spleen colony-forming unit assays (CFU-S) were performed to assess the impact of DACi on the functional capacity of LSC. For gene expression studies PLZF/RARα-, AML1/ETO- and mock-transduced 32D cells were analysed by Westernblot after treatment with valproic acid (VPA, 150µg/ml), dacinostat (2.5-20nM) or vorinostat (1 or 2µM). *Results.* Sca+/lin- HSC carrying AML1/ETO or PLZF/RARα had a serial replating capacity far exceeding that of mock infected controls (at least 6 vs. 2 rounds of plating, resp.). Of note, continuous presence of DACi in the methylcellulose resulted in a progressive loss of colony forming cells suggesting exhaustion of leukemic progenitors over time. The repopulation capacity of mock- as well as AML1/ETO- or PLZF/RARα-transduced HSC was analysed after a 7-day culture period in presence of cytokines +/- DACi. Compared to control cultures, the class I DACi VPA induced an up to 3-fold expansion whereas the more potent class I/II DACi dacinostat and vorinostat lead to a considerable cell loss by at least one log. When all the progeny grown were injected into lethally irradiated mice, the sparse dacinostat- and vorinostat-treated cells gave rise to a significantly lower number of spleen colonies compared to only cytokine-or VPA-treated controls (n=3 independent experiments). In the spleen of mice transplanted with AML1-ETO and PLZF-RARα-transduced HSC, oncogene expression was detected by qPCR. Dacinostat seemed to exert a deleterious effect on LIC, as we failed to detect an AML1/ETO- or PLZF/RARα-signal. VPA turned out to be effective in PLZF/RARα-, but not AML1/ETO-positive LIC and vorinostat gave conflicting results. In mock transfected Sca+/lin- HSC, dacinostat and vorinostat seemed to damage normal committed progenitor cells as measured by serial replating, but largely

spare the repopulating stem cell fraction responsible for establishing CFU-S. VPA significantly enhanced spleen colony formation as previously reported. Westernblot of PLZF-RARα- AML1-ETO- and Mock-infected 32D cells showed a concentration-dependent downregulation of Bmi-1 and c-myc after treatment with VPA, dacinostat and vorinostat which may have contributed to vanishing of leukemic progenitors. *Conclusion.* In summary, our data suggest that in contrast to the class I DACi VPA the potent class I/II DACi dacinostat and vorinostat inhibit both proliferation and self-renewal of LIC.

0042

THERAPEUTIC POTENTIAL OF TARGETING KEY HOXA-TALE GENES IN AML

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Background. HOX genes are master regulators of hematopoietic stem and progenitor cell (HSPC) development. HOX interaction with TALE (PBX or MEIS) proteins is pivotal to their function. The prevailing hypothesis is that HOX/TALE expression underlies HSPC self-renewal while dysregulated HOX/TALE expression underpins maintenance of the leukemia initiating cell. Gene expression profiling studies support this hypothesis and highlight the importance of the HOXA cluster and TALE genes in hematopoiesis and leukemogenesis. HOXA6 and HOXA9 form part of the leukemia-associated 'HOX code' identified from human samples and murine models of this disease. *Aims.* To identify and therapeutically target the AML-associated HOXA-TALE axis in intermediate subtype human AML. *Methods.* HOXA cluster and leukemia-associated TALE gene expression was evaluated in 145 favourable and intermediate AML patient samples by Affymetrix microarray and expression of the significantly different genes were confirmed by RQ-PCR assays in an independent patient cohort. Immunoprecipitation assays were used to identify HOXA-TALE protein interactions using tags in overexpressing fibroblasts and endogenous interactions were determined AML cells. HOXA/TALE was transiently silenced using Nucleofection or viruses to package and deliver shRNA to OCI AML3 (NPM1-mut) and U937 (NPM1-WT) AML cells. Viability was assessed using CellTiter-Glo® and direct cell counting whilst cell death was determined using the Caspase-Glo®assay. The proportion of cells within specific phases of cell cycle was established using FACS and propidium iodide staining. Cell responses were examined following target gene knockdown alone or in combination with chemotherapy (Cytarabine or Mylotarg). Furthermore altered gene expression was examined using the Roche AmpliChip containing 1500 leukemia-associated probesets. *Results.* HOXA1-A2, A4-A10, PBX3 and MEIS1 displayed differential ($p \leq 0.01$) expression between favourable and intermediate AML groups and confirmed an association of HOXA/TALE with nucleophosmin gene mutations (NPM1-mut) recently identified as a favourable prognostic marker in AML. HOXA-PBX3-MEIS1 protein interactions were identified in AML cell lines suggesting functional relevance of these complexes for which silencing may alter cell survival. HOXA9 is an established oncogene and HOXA6 was identified as the most consistently and highly expressed 'HOX code' gene in 318 AML samples by microarray thus these HOXA and their TALE partners were selected for further study. Specific shRNAs were transiently transfected into OCI AML3 and U937 cells and knockdown was validated by RQ-PCR and western blotting where compatible antibodies were available. Preliminary data indicate that HOXA6, HOXA9, MEIS1 and combined HOXA6/HOXA9 silencing greatly reduced cell growth compared to controls and was additive with chemotherapies at IC50 values used in treatment of AML. Furthermore altered expression of cell cycle-associated and adhesion-associated genes was identified in the HOXA knockdown cells by microarray. *Summary.* HOXA/TALE knockdown impairs growth and sensitises cells to chemotherapy independently of NPM1 mutation status. *Conclusion.* These findings suggest silencing of clinically significant HOXA cluster genes and their critical co-factors or modulation of downstream pathways could offer a novel strategy in the treatment of AML.

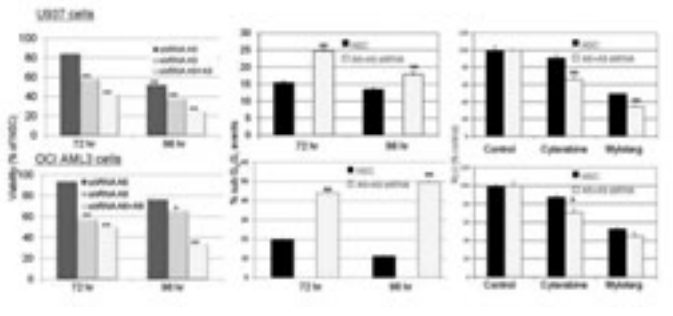

Figure 1. Reduced HoxA6/A9 +chemotherapy reduces cell growth.

0043

THE ROLE OF CYSTEINE PROTEASES IN PAEDIATRIC LEUKAEMIA

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Background. Calpains and cathepsins are cysteine proteases known to be expressed in ALL and AML. We have previously shown that treatment with Calpain Inhibitor III (*MDL28170*) reduces cell viability and clonogenicity in a dose-dependent manner, demonstrating a more potent effect in cells carrying the AML1/MTG8 fusion gene (Kasumi-1) than in another M2 cell line (HL-60) lacking this translocation. *MDL28170* also inhibits Cathepsin B and Cathepsin L. These lysosomal enzymes are both involved in the regulation of apoptosis, cell adhesion and cell cycle control. They are highly expressed in many malignant cell types and their secretion has been identified as being an important mediator of solid tumour invasion and metastasis. The intracellular roles of these cathepsins and their effects in leukaemia are less well understood. *Aims.* We aimed to identify which calpains and cathepsins are important in leukaemic maintenance and to functionally analyse their roles. We aim to test and validate these proteases as potential drug targets. *Methods.* Specific inhibitors of cathepsin B (*CA074Me*), Cathepsin L (*Cathepsin L inhibitor 3*) and Calpain 1&2 (*PD150606*) were chosen in addition to *MDL28170* which inhibits all of the above proteases. Knockdown of cysteine protease expression was achieved using siRNA and confirmed using RTPCR and Western Blot. Cell-cycle analysis and apoptosis assays were also performed in the presence of the inhibitors. LD50 was determined using the WST-1 cell viability assay in t(8:21)(q22;q22) positive and negative cell lines, including some bearing the t(4;11) poor prognostic marker. The effects on clonogenicity of the inhibitors and of cysteine protease knockdown using siRNA were assessed. *Results.* Calpain and cathepsin inhibition has a dose-dependent cytotoxic effect in the AML and ALL cell lines tested. The effects of simultaneous calpain, cathepsin B and cathepsin L inhibition using *MDL28170* are more pronounced than those seen when inhibitors meant to target specific cysteine proteases are used. Cathepsin inhibitors have a greater cytotoxic effect than calpain inhibitors. *MDL28170*, *CA074Me* and *Cathepsin L Inhibitor 3* have a greater effect on Kasumi-1 clonogenicity than calpain inhibition in isolation using *PD150606*. Knockdown of the calpain small subunit and Calpain-2 using siRNA also reduced clonogenicity of Kasumi-1 cells by up to 80%. *Summary/Conclusions.* In summary, we show that calpains are important mediators of leukaemic maintenance and clonogenicity. In addition, Cathepsins B and L are play a role in proliferation, clonogenicity and survival of AML1/MTG8 expressing cell lines and other leukaemic populations. Further functional analysis is ongoing to examine the potential links between these pathways.

0044

ROLE OF DEATH CELL-RECEPTORS PATHWAY IN APOPTOSIS INDUCED BY THE HISTONE DEACETYLASE INHIBITOR SODIUM BUTYRATE IN NPM1-MUTATED AML CELLS

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AML carrying NPM1 mutations [Falini B *et al.*, NEJM 2005;352:254-266] accounts for about one-third of adult AML, shows distinctive biological and clinical features [Falini B *et al.*, Blood 2007;109:874-885] and has been included as a provisional entity in the 2008 World Health Organization (WHO) classification of myeloid neoplasms. In spite of the relatively good prognosis of NPM1-mutated AML, there are still cases that show poorer outcome, especially those associated with FLT3-ITD mutation and elderly patient population. Therefore new therapeutic strategies need to be explored. Here, we investigated the effect of sodium butyrate, a short-chain fatty acid which has long been known to be a histone deacetylase inhibitor (HDACi) able to induce maturation in normal and tumor cells, in cellular models of NPM1-mutated

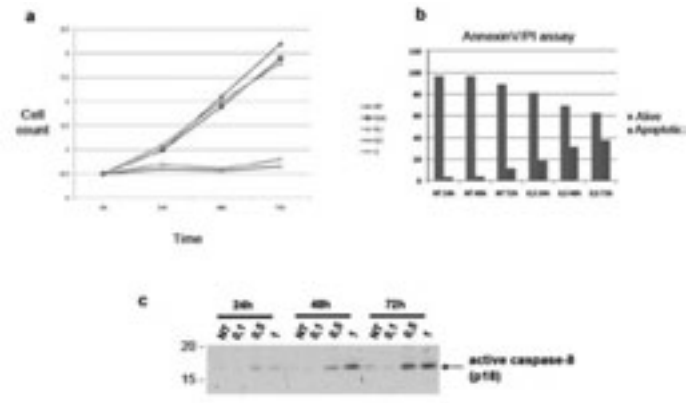

Figure 1.

AML: i) the OCI/AML3 cell line, previously identified as a human AML cell line carrying cytoplasmic mutated NPM1 in the absence of FLT3-ITD; ii) primary AML cells originated from a patient with NPM1-mutated AML bearing FLT3-ITD mutation (MONT1) and propagated as cell line in NOD/SCID mice; and iii) primary AML cells from 4 NPM1-mutated AML patients at diagnosis. In either cell lines or patients' primary AML cells carrying NPM1 mutation, but not in the U937 or OCI/AML2 cell lines (not harboring NPM1 gene mutation) used as control, growth arrest and pro-apoptotic effects were evident after 24 hrs and marked after 48 hrs of treatment with doses of drug of 0.5-1 mM (Figure 1a and b). In particular, no signs of differentiation were evident at morphological examination of treated cells. Interestingly, induction of apoptosis was associated with activation of caspase-8 (Figure 1c), suggesting involvement of the death cell receptors pathway. Indeed, flow cytometric analysis showed increased expression of TRAIL-receptor DR5 upon drug treatment. Moreover, concomitant treatment with a specific caspase-8 inhibitor prevented cell growth arrest and markedly reduced apoptosis. Levels of either NPM1 mutant or wild-type protein did not appear significantly affected by treatment with sodium butyrate. Further studies are needed to further characterise the molecular mechanisms of sodium butyrate-induced apoptosis in NPM1-mutated AML cells.

0045

2(β-PIPERIDINO-ETHYL)9-HYDROXYELLIPTICINIUM BICHLORIDE (ELP10) EXHIBITS ANTI-LEUKEMIC ACTIVITY IN VITRO AND IN VIVO

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Background. ELP10 is a 9-hydroxyellipticine derivative namely 2(β-piperidino ethyl)9-hydroxyellipticinium-bichloride. Since it was developed as an anti-cancer agent, we here assessed its anti-neoplastic efficacy with particular emphasis on its anti-leukemic activity in MDS and AML. *Methods.* To delineate the anti-neoplastic activity of ELP10, a screening of 68 NCI-listed malignant cell lines was carried out. The IC50 was confirmed by retesting the drug impact on 32 selected cell lines (solid tumors, and leukemia cell lines) by employing a sulforhodamine B (SRB) assay. The exact dose-and time-dependency of ELP10's anti-leukemic efficacy in AML-/MDS-derived cell lines was determined by assessment of apoptosis (by staining with DioC23/PI and FACS quantification at 24h, 48h and 72h) and cell cycle progression (PI staining and FACS quantification at 24h and 48h) on MDS/AML-derived cell lines (KG-1, KG-1a, HL-60, MOLM-13, Kasumi-1). Finally, ELP10's anti-leukemic efficacy *in vivo* was characterized in Balb/c mice injected intraperitoneally with L1210 or P388 murine leukemia cells and treated with increasing dosages of ELP10 (ranging from 1,6mg/kg to 50mg/kg). *Results.* In the NCI cell line bank ELP10 exhibited anti-neoplastic activity in a large series of cell lines representative of solid tumors as well as leukemias. The anti-neoplastic activity was highest in cell line models of acute leukemia, notably of AML-derived cell lines, which exhibited the lowest IC50. Confirmatory experiments in a broad spectrum of MDS- and AML-derived cell lines established ELP10's ability to induce time-and dose-dependent induction of apoptosis and cell cycle arrest in G2/M. Apoptosis- and/or cell cycle-arresting effects of ELP10 were observed - albeit to a different degree - in all tested myeloid cell lines, thus 72h of incubation of HL-60- or Kasumi-1 cells with 1μM ELP10 induced apoptosis in about 50% of cells, and increased G2/M-arrest by about 40% (24h) of cells. Finally, in Balb/c mice, ELP10 increased life-span by about 50% in P388-grafted mice, and by about 30% (low dose ELP10) to 90% (high dose ELP10) in L1210-grafted mice. *Conclusions.* These studies provide evidence for an *in vitro* and *in vivo* anti-leukemic activity of ELP10, especially in AML- and MDS-derived cells, even at low dose levels.

0046

FUNCTIONAL CHARACTERIZATION OF THE PROMOTER REGION OF EVI1 IN ACUTE MYELOID LEUKEMIA

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The EVI1 gene (3q26) codes for a transcription factor with important roles in development and leukemogenesis. Aberrant expression of EVI1 has been reported in patients with 3q26 rearrangements; however, about 9-20% of acute myeloid leukemia cases with no 3q abnormalities overexpress EVI1, and this is also associated with an unfavorable outcome. These rearrangements were the only known mechanism that led to EVI1 overexpression; although recently it has been described that MLL fusion proteins selectively activate EVI1 in HSC-derived MLL leukemic cells. Our aim was to functionally characterize the promoter region of the human EVI1 gene, and to identify transcription factors involved in its regulation. *Material and Methods*. Bioinformatic analysis, validation by chromatin immunoprecipitation (ChIP), siRNA analysis, and luciferase assays. *Results.* To determine the minimal promoter region, we generated seven 5'-truncation constructs of the EVI1 promoter upstream of the luciferase reporter gene. These constructs were transfected into HEK293T, HEL, HEPG2 and A549 cell lines. These experiments revealed that a 318bp region retains more than the 50% of the full length construct activity. To identify the upstream regulatory factors in this EVI1 minimal promoter region, we examined the 318bp sequence using MATCH, TFSEARCH and MatInspector softwares. Site-directed mutagenesis allowed us to define the contribution of the hypothetical binding sites found. Tansfection of the mutated constructs in the HEK293T and A549 cell lines showed a marked decrease in the promoter activity when the ELK1 and RUNX1 binding sites were mutated. ChIP in HEL and HEK293T cell lines demonstrated that ELK1 and RUNX1 bind to the proximal promoter region of EVI1. Moreover, knockdown of RUNX1 and ELK1 by siRNA caused decrease of EVI1, demonstrating their involvement in the transcriptional regulation of EVI1. Taking together, this approach allowed us to identify a functional region of 318bp in the proximal promoter region of EVI1, with several binding sites for transcription factors with important roles in hematopoiesis. These results also showed that RUNX1 and ELK1 regulate the transcription of EVI1. Further studies to confirm the role of the transcription factors RUNX1 and ELK1 in acute myeloid leukemia cases with overexpression of EVI1 are in progress. E-mail address: mmaicas@alumni.unav.es

Acute myeloid leukemia - Clinical 1

0047

STRATIFICATION ACCORDING TO TREATMENT RESPONSE IMPROVED OUTCOME IN CHILDHOOD ACUTE MYELOID LEUKEMIA

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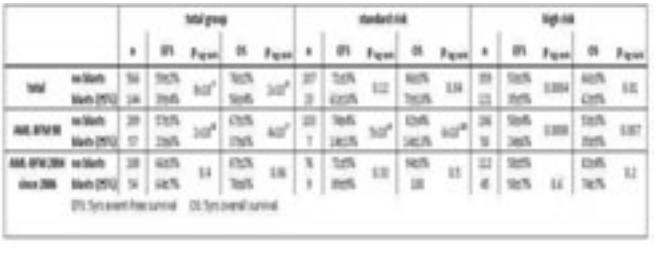
Background. Treatment response is generally considered as prognostic factor; however, studies of minimal residual disease did not confirm independent significance.[1] Since 2006 the AML-BFM study group stratified poor responders after 1st and 2nd induction to intensified therapy, either an additional element or eligiblility for allogeneic matched donor stem cell transplantation (SCT). *Patients and Methods.* Sevenhundred-ten children from the German AML- BFM trials 98 (n=346) and 2004 (n=364) were included. Excluded were children with acute promyeloblastic leukemia (n=39), myeloid leukemia of Down syndrome (n=122), early death (n=35) or incomplete data (n=89). 230 children (32%) were treated in the SR-group* and 480 in the HR-group (68%). Complete remission (CR) was determined after induction, late complete remission (LCR) at the end of intensive therapy. Bone marrow smears were centrally analyzed during treatment. Immunophenotyping was performed to confirm the morphology results. In a 4-color approach, the following antibodies were used: CD33, CD34, CD2, CD7, CD56, CD15, CD117, CD123, CD13, and CD19. *Results.* A good correlation was found between morphology and immunophenotyping (coefficient Pearson 0.86). In 28% of the patients regenerating blasts could be distinguished from malignant blasts. Persistence of blasts was detected in 144 patients (20%); 53 patients showed more than 5% blasts (SR n=13, 2%, HR n=40, 5%) and 90 children more than 10% of blasts (SR n=13, 2%, HR n=77, 11%). Whereas in Study 98 only 13%of the SR patients were shifted to HR therapy, since 2006 it increased to 21%. In the HR patients with persistent blasts, SCT in 1st CR or PR increased from 22 to 33%. Vice versa, in patients without detectable blasts after 1st induction the frequency of SCT decreased from 9% to 6%. Overall, blasts persistence after 1st induction was associated with an impaired event-free and overall-survival (EFS 39±4% vs. 59±2% $p_{log\ rank}$ 8x10-7; OS 56±4% vs. 76±2% $p_{log\ rank}$ $8x10^{-7}$). This was mainly an effect by the HR-group (EFS 35±5% vs. 53±3% $p_{log\ rank}$ 0.0004; SR: EFS 61±10% vs. 71±3% $p_{log\ rank}$ 0.12). Since 2006, the prognostic significance of "blasts after 1st induction"was lost (table 1).

Table 1.



Analyzing the effect of further therapy including SCT in patients with blasts after 1st induction, in study 98 36 out of 55 patients (65%) achieved CR and 41 (75%) LCR. This significantly improved in study 2004 (CR 71%, LCR 88% and since 2006 (CR 78%, LCR 95%, p=0.007). In conclusion, using treatment response for stratification, EFS and OS could be improved in childhood AML.

Reference

1. Langebrake *et al*. J Clin Oncol. 2006Aug1;24(22):3686-92.

* Standard risk (SR) group: FAB M1/M2 with Auer rods/ t(8;21), FAB M4eo/inv(16) and blasts <5%; High-risk (HR) group definition: all others.

0048

A PHASE IB STUDY OF ORAL PANOBINOSTAT IN COMBINATION WITH CYTARABINE AND MITOXANTRONE AS SALVAGE THERAPY FOR REFRACTORY OR RELAPSED ACUTE MYELOID LEUKEMIA (AML)

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Background. Panobinostat is an oral pan-deacetylase inhibitor that increases acetylation of proteins involved in cancer cell growth and survival. Preclinical studies demonstrated that panobinostat potentiates the activity of cytarabine (ara-C) and fludarabine and has synergistic activity in combination with doxorubicin in acute myeloid leukemia (AML) cells. Single-agent panobinostat has demonstrated complete responses (CR) in patients with AML in a previous phase I trial. AML is associated with poor prognosis, particularly in patients with relapsed or refractory disease. The addition of panobinostat to a chemotherapeutic regimen that is active in patients with AML who have relapsed or are refractory to prior induction therapy has the potential to improve therapeutic outcomes in this setting. *Aims.* The primary objective of this phase Ib, multicenter, open-label study is to determine the maximum tolerated dose (MTD) of panobinostat in combination with fixed dose ara-C and mitoxantrone in patients with relapsed or refractory AML by determining the incidence of dose-limiting toxicities (DLTs) within the first cycle. Secondary and exploratory objectives include safety and anti-leukemic activity. *Methods.* The study is comprised of 3 parts: 1) dose escalation to determine the MTD of panobinostat in combination with ara-C and mitoxantrone, 2) dose expansion to assess safety and preliminary activity at the MTD, and 3) optional dose extension to assess safety of single-agent panobinostat in responding patients who are not eligible for other therapies. Panobinostat was orally administered (20 mg starting dose) thrice weekly on days 1, 3, 5, 8, 10, and 12, in combination with intravenous ara-C (1 g/m^2) on days 1-6, and intravenous mitoxantrone (5 mg/m^2) on days 1-5 of a 28-day cycle. Patients receive a maximum of 3 cycles of combination therapy. Patients who are eligible for optional dose extension received single-agent panobinostat (60 mg thrice weekly). *Results.* As of February 14, 2011, 29 patients have been treated, 5 in cohort 1 (20 mg panobinostat), 7 in cohort 2 (30 mg panobinostat), 10 in cohort 3 (40 mg panobinostat) and 7 in cohort 4 (50 mg panobinostat). Safety and efficacy analyses are available for these patients. The median age was 53 years (range 19-72). All patients received prior ara-C. One DLT was observed (sepsis and tachyarrhythmia) at 40 mg panobinostat. Adverse events (AEs) were observed in 24 patients (83%). Frequent hematologic AEs included thrombocytopenia and febrile neutropenia (45% each), anemia (31%), leukopenia (27%), and neutropenia (24%). Frequent non-hematologic AEs included: nausea (69%), diarrhea (59%), vomiting (55%), decreased appetite and hypokalemia (48% each), fatigue and pyrexia (45% each). The most frequent grade 3/4 AEs were febrile neutropenia (45%) and thrombocytopenia (41%). Encouraging clinical efficacy was observed in 15 patients (52% ≥ partial response [PR]; 8 CR, 3 CR with incomplete blood count recovery, and 4 PR), particularly at higher doses of panobinostat. *Summary/Conclusions.* The combination of panobinostat with ara-C and mitoxantrone showed no unexpected toxicities with promising activity in patients with relapsed or refractory AML. The MTD has not been reached, and the study is ongoing at the 60 mg cohort of the dose-escalation phase.

0049

ADDITION OF PURINE ANALOGUE EITHER CLADRIBINE OR FLUDARABINE TO INDUCTION REGIMEN IS ASSOCIATED WITH IMPROVED SURVIVAL OF AML PATIENTS WITH HIGH-RISK KARYOTYPE

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Prognosis of AML patients with unfavorable cytogenetic features is poor with reported long term survival of approximately 20% despite the use of alloHSCT. So far, any modifications of induction chemotherapy did not result in improved survival. Between 2004-2008 the Polish Adult Leukemia Group conducted a randomized trial comparing standard induction (daunorubicin 60 mg/m2 for 3 days + AraC 200 mg/m2 for 7 days, DA) with the same regimen combined with either cladribine 5 mg/m2 on days 1-5 (DAC) or fludarabine 25 mg/m2 on days 1-5 (DAF). Inform consent was obtained. The goal of the current analysis was to evaluate if the addition of purine analogue affects outcome in a cohort of patients with high-risk karyotype, as defined according to SWAG criteria. Among 652 patients with newly diagnosed AML included in the PALG DAC vs. DAF vs. DA study, 111 presented with unfavorable karyotype. The median age was 50 (19-60) years. 35 patients were randomly assigned to DA arm, 35 to DAC, and 41 do DAF. In the respective study groups the rate of CR was 37%, 60%, and 54% (DA vs. DAC+DAF, p=0.05). Among CR patients 41% received consolidation followed by alloHSCT from either HLA-matched related or unrelated donor (DA, 23%, DAC 48%, DAF 45%). With the median follow-up of 2.8 yaers the 3-years probability of the overall survival was 20% for DA arm, 36% for DAC and 37% for DAF (DA vs. DAC+DAF, p=0.01) (see: figure). Leukemia-free survival equaled 45%, 31% and 56%, respectively (p=NS). No significant differeneces could be demonstrated with regard to remission duration and non-relapse mortality. All three regimens were characterized by comparable hematological and non-hematological toxicity. We conclude that the addition of purine analogue either cladribine or fludarabine to standard induction regimen based on daunorubicin combined with AraC is associated with improved survival of patients with newly diagnosed AML and high-risk karyotype. The advantage is mainly a consequence of increased rate of complete remission.

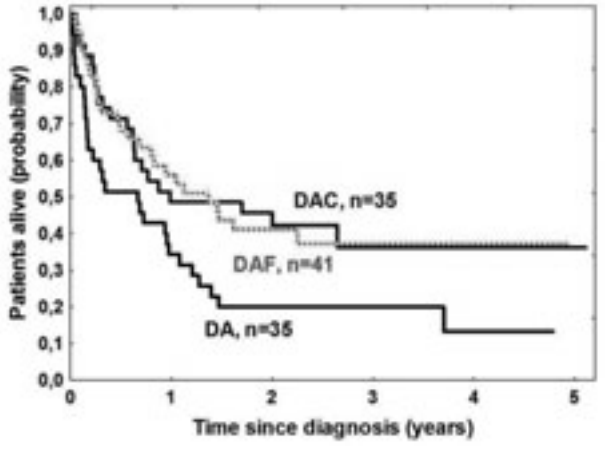

Figure 1.

0050

MANAGEMENT AND OUTCOME OF OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) IN FIRST RELAPSE: A STUDY FROM THE ACUTE LEUKEMIA FRENCH ASSOCIATION (ALFA)

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Background. Older patients with AML in first relapse are frequently targeted for new drug evaluation. There are, however, few published data on their optimal management and current outcome. *Aim.* We performed an analysis of 393 patients aged 50y+ (median, 64y) with AML in first relapse after prospective inclusion in ALFA-9801/9803 trials (232/161). *Patients.* At relapse, median CR1 duration was 9 months (1-69); median WBC was 3.4 x 109/L; ECOG-PS was 2+ in 28% of the patients. Only 12 patients relapsed after HSCT. Diagnosis cytogenetics was: CBF, 16 (4%); intermediate, 240 (61%) including 173 normal karyotype (NK); adverse, 76 (19%); and not done 61 (16%). Twenty-nine of the 174 patients tested at diagnosis had FLT3-ITD (17%), while incidences of NPM1, double CEBPA, IDH1, IDH2, or WT1 mutation were 24%, 2%, 9%, 10%, and 2%, respectively. Among 86 NK-AML patients tested, 24 had a favorable genotype (NPM1 or double CEBPA without FLT3-ITD). *Results.* By physician choice, salvage was intensive chemotherapy (ICTx) in 209 (53%), low-dose cytarabine (LDAC) in 48 (12%), best supportive care (BSC) in 124 (32%), and unknown in 12 (3%) patients. Overall, CR2 rate was 30% with a significant impact of CR2 on post-relapse survival (median PRS, 16 vs 4 months; P<0.001). Duration of CR1 (<6m, 29%; 6-12m, 37%; >12m 34%) strongly influenced CR2 rate (12 vs 32 vs 47%; P<0.001) and PRS (4 vs 7 vs 11

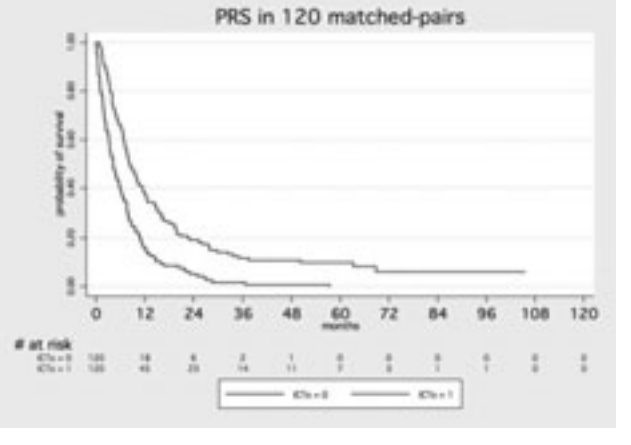

Figure 1. Post relapse Survival in 120 matched pairs.

months; P<0.001). As also expected, patients with CBF-AML had higher CR2 rate (69 vs 27%, P<0.001) and longer PRS (16 vs 6 months, P=0.02). They were not included in subsequent analyses. In patients tested, FLT3-ITD predicted shorter PRS (3 vs 7 months, P=0.03). Patients with NK-AML and favorable genotype had similar CR2 rate and PRS than other patients with normal, or intermediate, or even unfavorable karyotype. In multivariate analysis, CR1<12m (HR, 1.8; P<0.001), ECOG-PS 2+ (HR, 2.0; P<0.001), age (P=0.001), and WBC (P=0.03) predicted shorter PRS, while adverse cytogenetics and FLT3-ITD did not. Patients who received ICTx displayed higher CR2 rate (52 vs 4%, P<0.001) and longer PRS (9.5 vs 4 months, P<0.001) than LDAC/BSC patients, without significant difference in PRS between LDAC and BSC (5 vs 3 months). When ICTx vs LDAC/BSC was added in the multivariate model, ICTx (HR, 0.5; P<0.001), CR1 <12m (HR, 1.7; P<0.001), ECOG-PS 2+ (HR, 1.8; P<0.001), and WBC (P=0.006) but not age remained significant, suggesting younger age was an important decision criteria for ICTx. To further evaluate the role of intensive salvage, we performed a one-to-one matching based on the propensity score for ICTx vs LDAC/BSC calculated with the following variables: age, ECOG-PS, WBC, CR1 duration, prior HSCT, and intermediate versus adverse cytogenetics. In 120 matched-pairs, ICTx was strongly associated with longer PRS (8.1 vs 4.2 months; HR, 0.50; P<0.001). *Conclusion.* These results confirm the value of favorable cytogenetics and CR1 duration as important prognostic factors in older patients with AML in first relapse. Awaiting new effective therapies, they also indicate that ICTx salvage should be proposed each time the patient status allows it.

0051

CD34+ PEAK IN PERIPHERAL BLOOD DURING MOBILIZATION IS AN INDEPENDENT PROGNOSTIC FACTOR IN ACUTE MYELOID LEUKEMIA PATIENTS IN 1ST CR TREATED WITH ALLOGENEIC OR AUTOLOGOUS TRANSPLANTATION

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Ninety-six AML patients in 1st complete remission (CR) were evaluated for peak CD34+ cell levels in peripheral blood (PB) during PBSC mobilization and harvest. Distribution of CD34+ cell peaks was determined and cases were grouped on the basis of 50th and 75th percentile: Group A, those having a CD34 peak ≤70 × 109/L (n.48); Group B, those having a CD34+ cell peak between 70 and 183 × 109/L (n.24); Group C, those having a CD34+ peak >183 x 109/L (n.24). Irrespectively of post remissional treatment received, Group A had a disease free survival (DFS) of 73%, Group B a DFS of 51% and Group C of 30% (P=0.0003). In intermediate cytogenetic risk stratum, those patients treated by autologous transplantation had a DFS of 68%, 33% and 14% in groups A, B and C, respectively, (P=0.01) while after allogeneic transplantation DFS was 87% in Group A+B versus 50% in Group C,(P=0.009). Peak of CD34 in PB was selected by multivariate stepwise analysis as independent predictor for DFS while harvested number of CD34 cells and infused CD34 cell were not entered the model. Others factors important in univariate analysis were age, cytogenetic risk, FAB classification and FLT3-ITD. Peak of CD34+ cells in PB was predictor for DFS also when these factors were considered in multivariate Cox proportional hazard analysis. In conclusion peak of CD34+ cells reached after 1st consolidation cycle is a simple and reliable prognostic factors. Its clinical value should further studied and compared to others known predictors of prognosis.

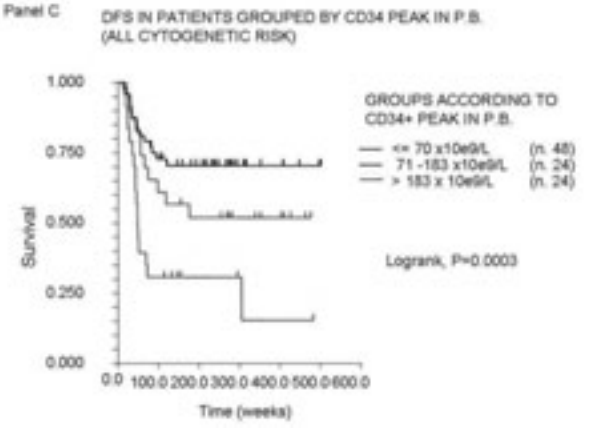

Figure 1.

0052

ACUTE MYELOID LEUKEMIA IN ADOLESCENTS AND YOUNG ADULTS IN THE NORDIC COUNTRIES - OUTCOME ACCORDING TO PEDIATRIC AND ADULT TREATMENT PROTOCOLS

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Background. Recent studies on adolescents and young adults undergoing treatment for acute lymphoblastic leukemia (ALL) have shown promising results for implementation of more intensive pediatric treatment protocols in the adult setting. The comparison of treatment outcomes in young acute myeloid leukemia (AML) patients has not yet been studied as extensively. *Aim.* To investigate disease characteristics, treatment and outcome for adolescents and young adults treated for AML according to pediatric and adult protocols within the Nordic countries. *Patients/Methods.* A mainly population-based retrospective survey of all patients aged 10-18 years diagnosed with AML, according to the WHO-classification, at pediatric clinics in Denmark, Finland, Iceland, Norway and Denmark 1993-2009 and all patients 15-30 years diagnosed in adult clinics in Denmark 2000-2009, Sweden 1997-2009 and a majority of the Norwegian patients diagnosed 1996-2008 were investigated. All FAB-classes were included. Secondary leukemias and patients with Down syndrome were excluded. *Results.* 166 patients aged 10-18 years (median 13 years) with de novo AML treated according to the NOPHO 1993 and 2004 protocols were compared with 253 patients 15-30 years (median 24 years) treated in adult centres according to different national or regional guidelines. The incidence of de novo AML 2000-2009 was 5.5 per million inhabitants in the age group 10-14 years, 5.9 in 15-18 years old, and 6.2 in 19-30 years old in Sweden, the corresponding figures for Denmark were 3.9, 7.7 and 7.9, respectively. The disease characteristics differed regarding the frequency of acute

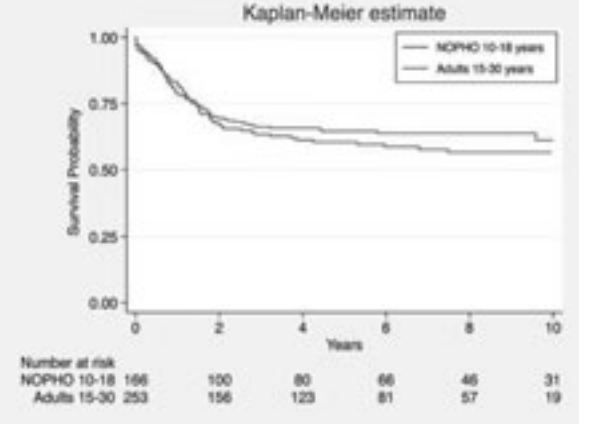

Figure 1.

promyelocytic leukemia (APL) and cytogenetic features between the cohorts. APL was more common in the adult cohort, 19.8% of adult leukemias compared to 5.4 % in the pediatric cohort. Core binding factor (CBF) leukemia was more common in the pediatric cohort 21% versus 14.2 % in the adults. Total favorable cytogenetics (defined as t(15;17), inv(16), and t(8;21)) were thus more frequent in the adult cohort with 33.2% versus 26.5%. The MLL-rearrangements, t(9;11) and 11q23, were more frequent in the pediatric cohort, 9.6%, than in the adult cohort with 6.0%. Overall survival (OS) at 5 years was similar for the pediatric and adult cohorts, 60.4% (52.2-67.7%) vs. 64.6% (58.1-70.3%). OS at 5 years excluding APL-patients was almost identical, 60.3 % (51.9-67.8%) vs.59.9% (52.4-66.5%). OS at 5 years was better, but not significantly so, for female than for male AML-patients in both cohorts; in the pediatric cohort 65.3% (59.0-75.5%) vs. 56.8% (45.8-66.4%) and in the adult cohort, 70.6% (61.6-77.9%) vs. 58.2% (48.6-66.7%). *Conclusion.* Differences in disease biology with less APL, more CBF-leukemia and more MLL-leukemia in the pediatric patients were found. No difference in outcome for AML patients aged 10-30 years, treated either in pediatric protocols or adult national/regional guidelines, was found. Age could not be shown to be an independent prognostic marker in this mainly population-based material from the Nordic countries.

0053

THE ADDITION OF LOW DOSE GEMTUZUMAB OZOGAMICIN TO INDUCTION, CONSOLIDATION AND MAINTENANCE THERAPY OF ELDERLY PATIENTS WITH NON M3 AML: UPDATE OF GENOA EXPERIENCE AND ANALYSIS OF PROGNOSTIC FACTORS

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Background. Elderly AML patients and patients with AML evolved from MDS or therapy related (sAML) display a very poor prognosis. We showed that adding low dose gemtuzumab ozogamicin (GO) to fludarabine, Ara-C and anthracycline (MY-FLAI3) may improve DFS and OS without increasing toxicity compared to a historical cohort of patients treated with fludarabine, Ara-C and idarubicin (FLAI), (Clavio 2007). *Aims.* We updated our experience and reviewed the prognostic impact of cytogenetics and molecular biology (mutations of FLT3 and NPM genes and expression of WT1 and BAALC genes) at diagnosis. *Patients and Methods.* Eighty-five consecutive CD33+ AML patients (≥ 60 years) were treated between May 2004 and June 2010. The median age was 68 years (60-82), the karyotype was unfavourable in 21 patients (25%), intermediate in 63 (74%) and favourable in 1 (1%). Twenty-eight patients had secondary AML (33%). The induction therapy consisted of Fludarabine 25 mg /sqm, Ara-C 1 g /sqm, idarubicin 5 mg / sqm, all for 3 days, followed by GO 3 mg / sqm at day 4. Complete responders received the same regimen as consolidation therapy and started a twelve-month maintenance therapy consisting in alternating GO (3 mg/sqm) with chemotherapy (Cytarabine, 1 g /sqm every 12 hours for three times), every 3 months. *Results.* Neutrophil (N > 0.5 x 109 / l) and platelet (Plt > 25 x 109 / l) recovery required a median of 15 days (range 10-26) and 16 days (range 7-30) from the end of therapy, respectively. There were four early deaths during induction (5%). Twenty-six major infectious complications were recorded (sepsis in 15 patients, pneumonia in 3, aspergillosis in 5, other infections in 2). Non-haematological toxicity was very mild. In particular, none of the patients experienced grade 3 or 4 hepatic toxicity. Forty-seven patients (55%) achieved a complete remission (CR) (seven after a second induction course). No clinical or molecular parameters were significantly associated with CR rate, whereas complete remission rate was 64% and 28% in good-intermediate /poor karyotype patients, respectively (p 0.03). Median duration of CR and OS were 9 months (range 1-70) and 12 months (range 1-72), respectively; 36-month projected EFS and OS were 12% and 18%, respectively. Cox regression analysis disclosed that good-intermediate karyotype patients had a better EFS (p 0.006) and OS (p 0.002) compared to those with poor risk cytogenetics. Patients with denovo AML had a better EFS compared to patients with sAML (p 0.029). Among molecular markers only BAALC expression < 1000 affected OS, although with borderline statistical significance (p 0.05). *Conclusions.* The addition of low dose GO to induction, consolidation and maintenance therapy is well tolerated and associated with a good antileukemic activity. In elderly AML patients treated with MY-FLAI classical prognostic factors such as karyotype and clinical history (de novo vs secondary disease) appear to be more important than molecular markers.

0054

BLASTIC PLASMACYTOID DENDRITIC CELL LEUKEMIA: PRELIMINARY RESULTS OF A RETROSPECTIVE ITALIAN MULTICENTRIC STUDY

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Background. Epidemiological and clinical information on blastic plasmacytoid dendritic cell leukemia (BPDCL) are rarely reported. *Aims.* To evaluate in patients (pts) with BPDCL the clinical features, the prognostic factors, and the efficacy of treatments. *Methods.* A multicenter retrospective cohort study was carried out between January 2005 and December 2010 in 26 Italian hematology divisions. *Results.* According to WHO-2008 classification, a total of 42 evaluable cases of BPDCL were collected (M/F 31/11; median age 57 y.o., range 20-81). At diagnosis the median values of hemoglobin, white blood cell count, and platelet counts were 5.2 g/dL (range 4.5-16.6), 2.1 x 10^9/L (range 0.3-54.1), 27 x 10^9/L (range 8-428), respectively. The median bone marrow infiltration was 90%. In 92% of cases constitutional symptoms (fever, fatigue, weight loss) were present, while only 3 were asymptomatic; 33 pts (78%) had peculiar skin lesions, while lymph node and/or spleen involvements were documented in 22 (52%). Extramedullary disease was described in 5 cases and included: CNS (3), nasal cavities (1), and pleural fluid (1). In 22 pts (52%) cytogenetic study was performed, revealing an unfavourable karyotype in 9 (40%), in 6 characterized by complex chromosome aberrations. Molecular biology studies were performed in 10 pts, showing in 3 the FLT-3 ITD mutation. Forty pts received an acute leukemia-like induction therapy (2 died early, receiving only palliative treatments), that consisted of antracycline and cytarabine or equivalent (AML-like regimen) in 29 (72%), and of dexamethasone, vincristine, cyclophosphamide, metotrexate (ALL-like regimen) in 11 (28%); 1 pt received in addition radiotherapy for cutaneous localizations. Nine pts (22%) underwent allo-HSCT as part of first-line (2) or salvage treatment (7). A complete (CR) or partial remission (PR) after induction therapy was achieved in 19 (45%) and 6 pts (14%) respectively (overall response rate 59%). It were registered 8 CR and 2 PR after ALL-like regimen, and 11 CR e 4 PR after AML-like regimen, with a significant advantage for ALL-like chemotherapy (p=0.04). The median overall survival (OS) was 8 months (range 0.2-60); 22 months (range 2-31) and 8 months (range 0.2-60) in pts received ALL-like regimen and AML-like regimen respectively (p=0.05). In HSCT-pts the median OS were 31 months (range 3-60), with a significant advantage with respect to non-transplanted pts (median 6 months, range 0.2-26, p=0.004). In pts obtaining a complete remission, the median disease free survival was 9 months (range 3-60); among them 18 pts relapsed, after a median time to diagnosis of 4 months (range 1-9). As for the transplanted pts, 4 was still alive in CR at last follow up. The overall mortality rate was 43% at 6 months and 73% at 12 months. *Summary/Conclusions.* BPDCL is a

rare hematopoietic neoplasia, preferentially involving skin, bone marrow, and lymph nodes, characterized by a poor prognosis. Initial response to acute leukemia-like chemotherapy is good, but relapse occurred very rapidly after a median time of only 4 months; allo-HSCT performed in first remission may lead to long-term survivor and disease control in selected cases, but more data are needed to confirm these preliminary results.

0055

THE EXPRESSION PATTERN OF MICRORNAS (MIRNA) MIGHT ADD RELEVANT PROGNOSTIC INFORMATION TO MOLECULAR CATEGORIZATION OF INTERMEDIATE RISK CYTOGENETIC ACUTE MYELOID LEUKEMIA

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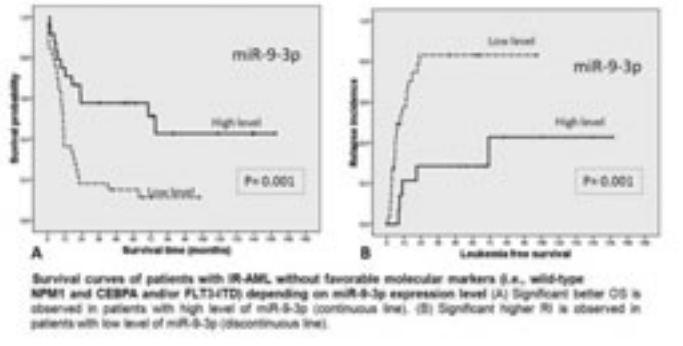
Background. The prognosis of AML patients within the intermediate cytogenetics category is mainly determined by the mutational status of some relevant genes, such as NPM1 mutations (NPMmut), or biallelic CEBPA mutations (CEBPAmut), associated with a favorable outcome, and with the presence of FLT3 internal tandem duplication (FLT3-ITD), which correlates with an adverse prognosis. Nonetheless, additional biological features such as miRNA expression pattern might contribute to refine prognosis and guide therapy in this setting. *Aim.* To investigate whether miRNA expression is associated with molecular characteristics and clinical outcome in intermediate-risk AML patients (IR-AML). *Methods.* We have analyzed samples from 85 IR-AML patients (median age, 52 [range, 18-71]; 52% males) who received intensive therapy from 1994 to 2009. Forty-three patients (51%) harbored NPMmut, 37 (44%) harbored FLT3-ITD (including 23 with NPMmut), and 11 (13%) harbored CEBPAmut, including 7 with biallelic mutations. The expression of 670 mature miRNAs was analyzed by multiplex Real Time PCR using TaqMan Human MicroRNA Arrays (Applied Biosystems). All PCR reactions were performed using an ABI 7900 HT sequence detection system. miRNA expression data was analyzed by the $2^{-\Delta\Delta}$Ct method, using RNU48 as endogenous control. Statistical analysis was performed with BRB Array Tools, SPSS version 15.0.1 and R software version 2.9.0. *Results.* Supervised analysis by means of t-test based on multiplex permutations (class comparisons analysis, p<0.001) revealed a distinctive miRNA signature in patients with NPMmut, with overexpression of miR-10a, miR-10a*, miR-10b and miR-196b, and downregulation of miR-126, miR126*, miR-424, miR-424* and miR-335, as well as patients with biallelic CEBPAmut, characterized by downregulation of miR-196b and upregulation of miR-181a. Response rate in this series of patients was 84%, with 5-year survival of 43±11% and relapse incidence (RI) of 55±14%. Multivariate analysis for survival identified age, absence of NPMmut, and FLT3-ITD as unfavorable variables together with low expression of miR-9-3p (p<0.001; HR=3.3, 95% CI: 1.7-6.4), and increased level of let-7a* (p=0.026; HR=5.1, 95% CI: 1.21-21.5) and miR-196b (p=0.056; HR=7.27, CI: 0.95-55.6). Concerning risk of relapse, the absence of NPMmut, FLT3-ITD and increasing leukocyte count were associated with a higher RI. Remarkably, decreased miR-9-3p expression (p=0.011; HR=3.3, 95% CI: 1.3-8.2) and miR-135a (p=0.02; HR=4.2, 95% CI: 1.2-14.2), together with higher levels of miR-23a* (p<0.001; HR=6.2, 95% CI: 2.61-14.7) were independently associated with a higher relapse risk. Of note, a decreased miR-9-3p level retained its adverse prognosis value in the subgroup of patients without favorable molecular markers (i.e., wild-type NPM1 and CEBPA and/or FLT3-ITD;p=0.001, see figure). On the contrary, let-7a* levels segregated subgroups of patients in the category of favorable genotype (i.e., mutated NPM1 without FLT3-ITD p=0.027). *Conclusions.* In this series of patients of intermediate-risk cytogenetic AML, measurement of expression levels of several miRNAs such as miR-9-3p, miR-135a, let-7a* or miR-23a* showed independent prognostic value, and might contribute to predict the outcome within specific molecular subgroups. Nonetheless, confirmation of the prognostic impact of these miRNAs and investigation of possible underlying mechanisms account for this effect require future studies.



Survival curves of patients with IR-AML without favorable molecular markers (i.e., wild-type NPM1 and CEBPA and/or FLT3-ITD) depending on miR-9-3p expression level (A) Significant better OS is observed in patients with high level of miR-9-3p (continuous line). (B) Significant higher RI is observed in patients with low level of miR-9-3p (discontinuous line).

Figure 1.

0056

EFFICACY OF HISTAMINE DIHYDROCHLORIDE AND INTERLEUKIN-2 IN MORPHOLOGIC SUBTYPES OF ACUTE MYELOID LEUKEMIA

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Background. The combination of histamine dihydrochloride (HDC) and low-dose interleukin-2 (IL-2) was recently approved in Europe as relapse-preventive immunotherapy in acute myeloid leukemia (AML). HDC has been shown to block the formation of immunosuppressive oxygen radicals in normal myeloid cells that express a radical-generating NADPH oxidase and histamine H2 receptors (H_2R). HDC thus preserves anti-leukemic functions of natural killer cells and cytotoxic T cells. *Aims.* 1) To investigate the expression of NADPH oxidase components and H_2R on leukemic cells in morphologic subtypes of newly diagnosed AML. 2) To determine the outcome of HDC/IL-2 immunotherapy in morphologic subtypes of AML. *Methods.* Flow cytometry was employed to screen freshly recovered leukemic cells from the blood or bone marrow of patients with morphologic subtypes of newly diagnosed AML and to monitor the expression of H_2Rs and the NADPH oxidase component gp91phox. Leukemia-free survival (LFS) was determined in patients receiving HDC/IL-2 or no treatment (control) in a phase III trial as described in detail elsewhere (Brune *et al.*, Blood 108:88-96). *Results.* H_2R and gp91phox were commonly co-expressed in a fraction (10-60%) of malignant cells recovered from patients with AML of M4 and M5 FAB classes. In contrast, H_2R or gp91phox were undetectable on leukemic cells from patients with FAB/M2 AML. In line with these findings, a post-hoc efficacy analysis of a phase III trial using HDC/IL-2 in AML revealed that patients with FAB/M2 AML in first CR did not benefit from HDC/IL-2 immunotherapy, even when the analysis was carried out in patients with a strong overall benefit of HDC/IL-2 (age <60, p>0.7, HR=1.14, n=41). In contrast, corresponding HDC/IL-2-treated patients with non-M2 AML displayed significantly higher LFS than control patients (52 vs. 23 % LFS at 3 years, p<0.001, HR=0.43, CI: 0.27-0.69, n=113). The LFS benefit was pronounced in patients with M4 or M5 AML (63 vs. 27 % at 3 years, p<0.01, HR=0.37, CI: 0.18-0.75, n=58). *Summary and Conclusions.* These finding imply that HDC/IL-2 immunotherapy predominantly targets monocytic forms of AML, which may be explained by co-expression of histamine receptors and gp91phox on leukemic cells of the monocyte lineage.

0057

RASGRP1/APTX RATIO STRONGLY CORRELATES WITH CLINICAL RESPONSE AND SURVIVAL IN AML PATIENTS TREATED WITH TIPIFARNIB-BORTEZOMIB COMBINATION

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Background. We conducted a phase I-II study aiming to assess efficacy and toxicity of tipifarnib-bortezomib treatment in elderly AML patients. RASGRP1/APTX genetic ratio, which is associated with treatment response in patients treated with tipifarnib alone, was tested.

Methods. Eighty patients were enrolled with secondary-AML: 14 had high risk cytogenetics; 42 were previously untreated. Seventy-five patients were treated. *Results.* Nine patients achieved complete remission (CR), 1 patient obtained a partial response (PR) and in 2 cases an hematological improvement (HI) was documented for an overall response rate (ORR) of 19%. Median time to response was 112 days, corresponding to 4 cycles (range 2-14). Marrow response (CR+PR) was significantly associated with overall survival (OS) (p<0.0001). RASGRP1/APTX was evaluated before treatment initiation on bone marrow (BM), peripheral blood (PB) or both. The median RASGRP/APTX value on BM was 15.3 (15-19.8) in responder patients and 2.2 (0.5-25.9) in non responders, respectively (p=0.00006). Its median value on PB was 31.6 (19.3-35.5) in responders and 6.4 (0.5-27.1) in non responders, respectively (p=0.00001). Interestingly, no marrow responses were recorded in patients with marrow RASGRP1/APTX ratio <8, while the response rate was 43% [how many were CR?] in patients with RASGRP1/APTX >8 (p<0.0001). Finally, RASGRP1/APTX levels significantly correlated with OS (p=0.001) with a median OS of 490 days and 162 days in patients with RASGRP1/APTX >8 and <8 respectively. *Conclusion*. We conclude that the clinical efficacy of the combination of tipifarnib and bortezomib was evident. We confirmed that the RASGPR1/APTX BM or PB level is an effective predictor of response and survival and our now studying the response of such patients to tipifarnib, alone.

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0058

DNMT3A EXON 23 MUTATIONS IN ACUTE MYELOID LEUKEMIA: A SINGLE CENTER EXPERIENCE

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Background. It has been recently shown that DNMT3A mutations are recurrent in patients with intermediate-risk cytogenetic AML and are associated with a poor outcome (Ley T *et al.*, NEJM, 2010). We have assessed the clinical characteristics and prognostic impact of DNMT3A exon 23 mutations (DNMT3Am) in a series of 210 patients treated at the Hematology department of Toulouse, France. *Aims.* To assess the prognosis impact of DNMT3Am in AML patients treated with intensive chemotherapy. *Methods.* We used a real-time High Resolution Melting (HRM) polymerase chain reaction (PCR) to identify a somatic mutation in the exon 23 of DNMT3A. Positive cases detected by the HRM analysis were sequenced to confirm the mutation. FLT3 and NPM mutations were assessed by multiplex PCR and capillary electrophoresis. We used samples from 210 AML patients (65y or younger) stored at the HIMIP cell bank in Toulouse University Hospital. Patients were treated by a "3+7"induction chemotherapy (between 01/2000 and 12/2009). Responding patients with HLA-identical sibling were allocated to allogeneic stem cell transplantation (allo-SCT) in first CR, others to autologous-SCT or high dose Ara-C. Study endpoints: complete response rate, cumulative incidence of relapse, disease-free and overall survival. *Results.* DNMT3Am were detected in 39 patients (18.6%): 17 males, median age 47y (20-62), median WBC 36.8 G/L (1.2-253), platelets (58 g/L, 8-814), FAB M1/2 (31%), M4/5 (62%), normal karyotype 25/38 (66%), intermediate Ka 37/38 (97%). As compared with DNMT3A exon 23 wild type (wt), DNMT3Am were significantly associated with both FLT3-ITD (17 FLT3-ITD out of 38 DNMT3Am, p=0.007) and NPM1 mutations (21 NPM1c/26, p<0.0001). Analysis of therapeutic outcome was performed only for patients with intermediate cytogenetic risk (n=180). The complete response rate was 31/37 (84%) in the DNMT3Am group vs 115/143 (80%) in the DNMT3Awt group (p=0.65). In CR patients, allo-SCT was performed in 37 (DNMT3Awt) and 15 (DNMT3Am) patients, respectively. With a median follow up of 24 months, we did not detect any impact of DNMT3A mutation on DFS (DNMT3Awt, median: 19.1 vs 94.7 months, DNMT3Am, p=0.15), CIR (DNMT3Awt, median: 22.8 vs 94.7 months, DNMT3Am, p=0.42) and OS (DNMT3Awt, 26.3 vs 99.9 months, DNMT3Am, p=0.3). In this series, the only significant prognostic factor for DFS and OS was NPM1 mutation. Although we did not find any impact of DNMT3Am in FLT3-ITD patients, DNMT3Am could worsen the outcome of NPM1m/FLTwt patients. Furthermore, in patients who received allo-SCT, there was a trend for improved DFS and OS in DNMT3Am patients (medians not reached) as compared with DNMT3Awt patients (median DFS, 28.4 months; median OS, 104.1 months) but the difference did not reach statistical significance. *Summary / Conclusions.* The mutation of DNMT3A exon 23 had no clear prognostic impact in AML with intermediate-risk cytogenetics. However, the favorable outcome of DNMT3Am patients after allo-SCT suggests that this therapeutic strategy could erase the prognostic impact of DNMT3A mutations. Many more patients are needed to determine if DNMT3Am could help in the refinement of therapeutic decision for patients with a favorable molecular risk (NPM1m/FLT3wt).

0059

DEVELOPING DIGITAL MRNA COUNTING AS A NEW TECHNIQUE FOR ACUTE LEUKEMIA DIAGNOSIS

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Introduction. The diagnosis of malignant hematological diseases has become increasingly complex during the last decades. It is based on the interpretation of results from different laboratory analyses, which range from microscopy to gene expression profiling. Recently, a new method for the analysis of mRNA phenotypes has been developed, the nCounter technology (Nanostring® Technologies), which allows for the quantification of hundreds of mRNA molecules in biological samples. We evaluated this technique in a Swiss multi-center study on samples from acute leukemia (AL) patients. *Material and Methods.* Peripheral blood (PB) and bone marrow (BM) samples were obtained from healthy controls (5 PB, 11 BM) and from patients with AL and various other hematological diseases (38 AML, 8 ALL, 2 CML, 3 CLL, 3 MDS), and referred to the Geneva Haematology Service. 43 AL samples were obtained from other Swiss Centers. For each patient a hematological work-up was performed, including a detailed flow cytometric analysis. Leftover material was used for the quantification of a set of 85 different mRNAs using the nCounter technology. Initially, 100 ng of purified RNA from each sample were hybridized with probes specific for the corresponding 85 surface antigens, and every specific hybridization product was then quantified. Subsequently, we exploited one of the many advantages of the nCounter technology, namely the fact that crude RNA without any enzymatic treatment is hybridized, and thus used total cell lysates in the hybridization. Correlation coefficients were calculated for the most relevant 18 antigens using mRNA values (number of molecules/sample) and values obtained from flow cytometry (% of positive cells/sample, in comparison to isotype controls). *Results.* mRNA and protein profiles were established for normal PB and BM samples. Relative signal intensities and expression patterns of the various surface antigens were similar to those described in the literature and found in previously performed Affymetrix microarray analyses. Acute leukemia samples were analyzed with this validated set of antigens and the Spearman Correlation Coefficients for nCounter and flow cytometry results calculated, based on >1600 comparisons. Highly significant values between 0.4 and 0.9 were found for the 18 antigens tested. A second correlation analysis performed on a sample basis and specifically on the blast population resulted in concordant results between flow cytometry and nCounter in 54 - 100% of the antigens tested, depending on the number of blasts present and the type of leukemia (AML versus ALL). Discordant results could be attributed mainly to samples with mRNA expression but lacking expression of the corresponding protein on the cell surface, and to myeloid antigens with an already high mRNA and protein expression in normal non-leukemic samples. *Conclusion.* The nCounter allows the fast and easy establishment of a mRNA profile from hematological samples. Correlation between the values for mRNA quantity obtained by this technique and protein expression, as measured by flow cytometry, is excellent for most of the antigens tested. Potential advantages of this new technology in the diagnosis of

AL, especially of rare AL cases and in MPAL (mixed phenotype acute leukemia), will be discussed.

0060

COMPARATIVE ANALYSES OF GENETIC ALTERATIONS IN PAIRED SAMPLES FROM 118 ADULT AML PATIENTS AT DIAGNOSIS AND RELAPSE

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Background. Acute myeloid leukemia (AML) represents a clinically and biologically heterogeneous malignancy with uncontrolled proliferation of hematopoietic precursors. Although the majority (70-80%) of AML patients achieve a complete remission (CR) after induction chemotherapy, about a half of these patients relapse with a gravid prognosis. However, the genetic alterations at relapse have not been extensively studied. *Aims.* In this study, we aimed to compared the genetic aberrations between AML at diagnosis and at relapse to determine their roles in AML development and disease progression. *Methods and Materials.* Comparison of genetic alterations, including Class I mutations, such as mutations of *N-RAS, K-RAS, PTPN11, JAK-2, KIT* tyrosine kinase domain of FLT3 (*FLT3/TKD*) and internal tandem duplication of FLT3 (*FLT3/ITD*) and Class II mutations, such as mutations of *CEBPA, AML1/RUNX1,* and partial tandem duplication (PTD) of MLL as well as *NPM1* and *WT1* mutations, at relapse with those at diagnosis was performed in paired samples from 118 patients with de novo AML. *Results.* One hundred and one (85.6%) patients had at least one molecular gene mutation and 56 (47.5%) had at least two mutations at diagnosis. At relapse, 85 (72%) out of the 118 patients had at least one molecular gene mutation, and 40 patients (33.9%) had two or more mutations. Among the patients with gene mutations, concurrent Class I and Class II or Class I and *NPM1* mutations, which behave more like Class II mutations, could be demonstrated in 46.5% (47/101) at diagnosis and in 38.8% (33/85) at relapse. Mutational shifts occurred in 55 patients (46.6%). Class I mutations, which activate signal transduction, were lost more frequently (around 50%) at relapse than Class II mutations, which perturb transcription factors. Genetic evolution with acquisition of novel mutations at relapse were identified in 14 individuals (11.9 %), all involving Class I or *WT1* mutations, but not Class II or *NPM1* mutation. The patients with genetic alterations at relapse had poorer overall survival. Stepwise Cox proportional hazards modeling showed that presences of *FLT3/ITD*, *WT1*, *KIT* or *MLL/PTD* at relapse were independent poor prognostic factors. *Conclusions.* The findings from this study support the two-hit theory not only in the development but also in the progression of AML. The fact that Class I mutations are frequently lost and can be acquired at relapse, in contrast to Class II mutations or *NPM1* mutation, suggests that Class II and *NPM1* mutations may play a role as the first hit in the leukemogenesis, while Class I mutations act as the second hit. In addition, genetic alterations at relapse can predict the clinical outcome.

0061

GERM-LINE MUTATIONS IN THE DNA DAMAGE RESPONSE GENES BRCA1, BARD1 AND TP53 IN PATIENTS WITH THERAPY-RELATED MYELOID NEOPLASMS

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Background. Therapy-related myeloid neoplasms (t-MNs) are severe long-term sequelae of cytotoxic treatments for a primary, often malignant disorder accounting for 10% of all MDS/AML cases. Increasing evidence from animal and human studies pinpoints a strong influence of genetic predisposition in the pathogenesis of these secondary malignancies. *Aims.* Cases with t-MNs frequently exhibit a remarkable family history of cancer. We therefore hypothesized that cancer predisposition syndromes are prevalent in this cohort of patients and aimed at identifying germ-line mutations in respective candidate genes. *Methods.* A "nuclear pedigree"consisting of all first- and second degree relatives was obtained from 51 adult and pediatric index patients with t-MNs and evaluated for cancer predisposition syndromes according to established criteria. In conspicuous cases, genomic DNA from cultured skin fibroblasts of t-MN patients was analyzed for deleterious germ-line mutations by PCR and direct sequencing as well as for large genomic rearrangements by MLPA. Mutations and genetic variants were classified according to public databases. Deleterious heterozygous germ-line mutations were further assessed for loss of the wildtype allele in CD34+ sorted leukemic cells by PCR, direct sequencing and SNP/CNV microarrays (Affymetrix GeneChip Human Mapping SNP 6.0 arrays), respectively. *Results.* Twenty-five of 51 (49%) patients with t-MNs had a hematological malignancy and 26 (51%) a solid tumor as primary disease. Non-Hodgkin's lymphoma (29%), breast cancer (BC) (25%) and sarcoma (8%) were the most frequent primary neoplasms found in more than 60% of all patients. Six pedigrees indicated a hereditary breast and ovarian cancer syndrome and ten a Li-Fraumeni (LF) or LF-like syndrome initiating a search for *BRCA1*, *BRCA2*, *BARD1* and *TP53* germ-line mutations, respectively. Deleterious, heterozygous germ-line mutations were found in 5/51 (9.8%) individuals: two in *BRCA1* (c.5251C>T, p.R1751*; c.3112G>T, p.E1038*), one in *BARD1* (c.1670G>C, p.C557S) and two in *TP53* (c.1146delA, p.K382fs*40; c.849-852insGGCG, p.R283fs*22). The *TP53* mutations have not been described previously. Both, *BRCA1* c.3112G>T and *TP53* c.849-852insGGCG germ-line mutations showed homozygosity in sorted CD34+ leukemic cells and SNP/CNV microarray analysis revealed loss of the wild-type allele in either case. *Conclusion.* Given the estimate that approximately 5% of cancers arise in the context of hereditary cancer predisposition syndromes, deleterious germ-line mutations are found with increasing prevalence in this cohort of t-MN patients. Preliminary data indicate that these mutations contribute to therapy-related leukemogenesis. Furthermore, our data may have clinical implications with respect to genetic counseling of these patients and their relatives.

0062

EXPRESSION PATTERN OF WT1 ISOFORMS IN PATIENTS WITH CHILDHOOD AML

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Background. Wilm's tumour gene 1 (WT1) is overexpressed in a large proportion of acute leukaemias and other haematological malignancies. It has been demonstrated that WT1 protein is produced in more than 36 different isoforms. These variants have distinct, partially overlapping functions and their ratio is supposed to influence the final effect of WT1. However, only limited information on WT1 isoforms has been published so far and the relevance of their expression pattern remains unclear. *Aims.* We determined the expression pattern of four WT1 isoforms characterized by the presence or absence of exon 5 and KTS insert (A[-/-], B[+/-], C[-/+], D[+/+]) in childhood AML using a specific qPCR system. *Methods.* We designed a unique qPCR system for detection and quantification of 4 major WT1 isoforms and verified the sensitivity, specifity and reproducibility of the results in extensive testing. With this assay we analysed WT1 isoforms expression pattern in 7 leukemic cell lines (Kasumi-1, MV4;11, NB-4, REH, NALM6, UOCB6, RS4;11), diagnostic bone marrow samples of 64 children with AML and 26 healthy controls. *Results.* We found an excellent correlation between the total WT1 expression and the sum of WT1 isoforms levels (rho=0,973, p<0.0001). Analysis showed diverse expression patterns of WT1 isoforms in the particular leukemic cell lines (p<0.0001). Interestingly, we found a similar pattern of WT1 isoforms in cell lines with MLL/AF4 rearrangement, independent of the lineage (myeloid - MV4;11 and lymphoid - RS4;11). The analysis of healthy controls (bone marrow or peripheral stem cells) failed to define the physiological ratio of WT1 isoforms. Very low levels of total WT1 in these samples precluded detection of WT1 isoforms since we reached the limit of the sensitivity of qPCR method (as defined by limiting dilution experiments). For the same reason of very low total WT1 levels, 11 patients (mostly AML M5) were excluded from the further analyses. Rather surprisingly, in all analysed patient diagnostic samples (N=53) a uniform WT1 isoforms expression pattern was present (A<C<B<D) with

Exon5[+] variants overexpression. We observed a trend to a higher level of isoform C in M1 and M3 patients and PML/RARa+ patients (Kruskal-Wallis p=0.0307 and p=0.0063, respectively). *Conclusion.* This is the first report of the analysis of WT1 isoforms expression pattern in childhood AML using a unique qPCR method for the detection of WT1 variants. Although the ratio of WT1 isoforms may vary in different tissues and cell lines, our data suggest that the WT1 isoforms expression pattern is rather uniform in paediatric AML, with predominant expression of Exon5[+] isoforms and possible variations in isoform C levels depending on the morphological and genetic characteristics. Careful pre-analytical testing of the detection system parameters suggests the technical limitations in detection of WT1 isoforms in samples with very low total WT1 levels. Therefore, the previously reported differences in WT1 isoforms ratio between normal bone marrow and AML samples should be interpreted with caution.

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0063

LEVEL OF MINIMAL RESIDUAL DISEASE (MRD) AND WHITE BLOOD CELL COUNT (WBCC) DISCRIMINATE CATEGORIES OF PATIENTS WITH DIFFERENT OUCOME AMONG ADULTS WITH FAVOURABLE-RISK ACUTE MYELOID LEUKEMIA

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Background. Core binding factor acute myeloid leukaemia (CBF-AML) and AML with mutated Nucleophosmin (NPM) without FLT3-ITD mutation are currently regarded as favourable-risk AML. Recent findings suggest a biological and prognostic heterogeneity of this AML subgroup, particularly of CBF-AML. *Aims.* The aim of our study was to assess whether MRD detection was able to identify patients with increased risk of relapse. *Material and Methods.* MRD was determined by multiparametric flow cytometry (MPFC) on bone marrow (BM) samples collected at the end of consolidation therapy. The threshold for MRD negativity was set below the level of 3.5x10e-4 residual leukemic cells. We evaluated 59 patients with de novo AML, enrolled sequentially in AML10/AML12 (n=48) and AML17 (n=11) EORTC/GIMEMA randomized trials between 1995 and 2007. In AML10/AML12 protocols, patients aged ≤ 60years received induction treatment that combined standard or high dose of cytarabine (ARA-C), according to randomization, etoposide, and anthracycline. As consolidation, all patients received intermediate dose of ARA-C and anthracycline. Thereafter, those with an HLA-compatible sibling were allografted. Patients without a donor underwent autologous stem cell transplantation (AuSCT). Patients aged > 60 years (AML17) received mitoxantrone, ARA-C, and etoposide and 2 cycles of a consolidation program consisting of idarubicin, cytarabine, and etoposide. All patients were randomized before induction, to receive or not, gemtuzumab ozogamicin, repeated on day 1 of each consolidation cycle. Median age was 48 yrs (range 18-75), 11 patients were older than 60 years, 36 were males and 23 females and 48 (81%) had white blood cell count (WBCc) <50 x 10e-9/L. Twenty-nine CBF-AML [21 with t(8;21) and 8 with inv(16)] and 30 NPM-AML were evaluated. Overall 24 patients (41%) relapsed, 3 NPM-AML patients experienced an early relapse after consolidation therapy. After first consolidation, 21 patients underwent AuSCT, 13 AlloSCT and 23 did not received any transplant procedure: 11 because of age, the remainders due to refusal or medical reasons (2 of 12 were consolidated with high dose ARA-C). *Results.* MRD positive status after consolidation (MRDpos) and WBCc ≥50 x 10e-9/L were significantly associated to relapse (p=0.017 and 0.0001, respectively). At 4 years, DFS for patients MRDneg vs MRDpos and with WBCc <50 vs ≥ 50 x 10e-9/L was 70% vs 44% and 59% vs 21% (p=0.018 and 0.011, respectively). Accordingly, cumulative incidence of relapse (CIR) at 4 years for patients MRDneg vs MRDpos and with WBCc <50 vs ≥ 50 x 10e-9/L was 21% vs 55% and 33% vs 83% (p= 0.005 and <0.001, respectively). Therefore, we identified 3 different groups of patients based on the combination of MRD status after consolidation and WBCc. At 4 years, DFS for MRDneg/WBCc<50 x 10e-9/L, MRDpos/WBCc<50 x 10e-9/L and MRDposWBCc≥50 x 10e-9/L was 77%, 49% and 15%, respectively (p=0.001) and CIR at 4 years was 12%, 47% and 90% (p<0.0001). *Conclusions.* Combined evaluation of WBCc and post-consolidation MRD status enables identification of patients at higher risk of relapse in spite of a favorable risk genetics/cytogenetics profile for whom intensification by AlloSCT should be considered.

0064

TREATMENT OF MOLECULAR RELAPSE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Evidence for beneficial effect of minimal residual disease (MRD) monitoring and early intervention (=pre-emptive therapy) in non-APL acute myeloid leukemia (AML) pts. are very limited. *Aims.* To assess usefulness, efficacy and toxicity of various treatment regiments in the therapy of molecular relapse in non-APL AML patients. *Methods.* We have performed a retrospective analysis of molecular relapses in AML pts. with molecular target (RUNX1-RUNX1T1, CBFB-MYH11, MLL fusion and NPM1) and its treatments. RQ-PCR was used for the MRD monitoring. Molecular relapse was defined as confirmed reappearance of the fusion transcript or mutated gene detection or its 10-fold increase in pts. with persistent positivity and corresponding bone marrow cytology, immunophenotype and cytogenetic analysis remained negative. *Results.* In the study period 1/1/2003 - 31/12/2010 we have treated 30 molecular relapses in 19 pts. Median follow up was 24 months (range 8-95 months). The median time from the end of previous therapy to molecular relapse was 5.6 months (range 0.3-31 months) - for RUNX1-RUNX1T1 positive patients 5 months, for CBFB-MYH11 pts. 4 months, for patients with MLL fusion 9.5 months and 6 months for patients with NPM1 mutation. Following pre-emptive treatment regimens were used: conventional chemotherapy ("5+2"like regimens) (20% of relapses), clofarabine (30%), gentuzumab ozogamicine - GO (17%) and immunomodulation after allogeneic hematopoietic stem cell transplantation - allo HSCT (30%). The overall response rate was 66% (CMoR - 53%, PMoR - 13%, SD - 13%, progression - 20%). The highest response rate was achieved with clofarabine - 88% (CMoR - 66%, PMoR - 12%, SD - 0%, progression - 22%) and conventional chemotherapy - 84% (CMoR - 50%, PMoR - 34%, SD - 16%, progression - 0%). The response rate for GO was 60% and for immunomodulation after HSCT 44%. We observed neutropenia and thrombocytopenia gr. III-IV (according to CTCAE 4.0) in 100% cases treated with clofarabine, conventional chemotherapy or GO and only in 11% and 0% respectively in patients after immunomodulation after HSCT. Severe complications occured in 17% cases treated with conventional chemotherapy (one death for toxicity) and in 11% cases treated with clofarabine, but not in patients treated with GO or immunomodulation. 30% of patients underwent allo HSCT. New relapse during follow up period was observed in 58% with median 7 months (range 3-18 months). However only 50% (8/16) of cases that achieved CMoR after treatment of molecular relapse relapsed in follow up period; in opposite 100% (3/3) patient with only PMoR after treatment of initial relapse relapsed in follow up period. One-year disease free survival was 56%, one-year overall survival was 74%. *Summary/Conclusions.* Our study has shown feasibility of MRD monitoring and pre-emptive strategy using molecular targets in non-APL AML pts. This approach had reasonable toxicity. Different pre-emptive treatment strategies led to response in 2/3 of molecular relapses and clofarabine led to the highest % of CMoR (66%). However, even using allo HSCT as the part of molecular relapse treatment, in 58% of responders new relapse occurred with median of 7 months.

0065

AGE-DEPENDENT ANALYSIS OF THE PROGNOSTIC IMPACT OF MINIMAL RESIDUAL DISEASE (MRD) IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Although the therapy of acute myeloid leukemia (AML) has witnessed remarkable progress over the last 2-3 decades in, two thirds of young adults still die of their disease. In older adults, who represent the majority of patients with AML, the results are even more unsatisfactory. In fact, while 40-50% can achieve a complete remission (CR), less than 10% are long-term survivors. Based on this, age is universally recognized as a pivotal prognosticator affecting outcome and treatment choice. In consecutive series of adult patients with de novo AML, we have repeatedly demonstrated the prognostic role of (MRD) detection, by flow cytometry. In particular, we have found that a level

of MRD ≥ 3.5x10e-4 leukemic cells at the end of consolidation is associated with a relapse rate of 70-80%. *Aims.* In the present study we evaluated whether the prognostic impact of MRD assessment after consolidation remained unaltered even in age-stratified (< 60 and > 60 years) populations of adult patients with de novo AML. *Methods.* For the purpose of this study, we analyzed 128 young adults (median age 46, range 18-60) and 55 elderly adults (median age 67, range 61-78). All patients under study, achieved CR after induction therapy of EORTC/GIMEMA protocols AML10, LAM99P and AML12 (for patients < 60 years) or AML13, AML15A and AML17 (for patients > 60 years). The two cohorts were well balanced in terms of frequency of FLT3-ITD and NPM1 mutated cases. A lower frequency of good-risk karyotypes was observed in elderly vs young patients (4% vs 20%, p=0.014). *Results.* The frequency of MRD negative measurements was lower among elderly patients as compared to the younger ones [7 (13%) vs 42 (33%), p=0.005]. Among 48 MRD positive elderly, 40 (83%) have relapsed and 8 (17%) have not; among 7 MRD negative, 4 (57%) have relapsed and 3 (43%) have not. Overall survival (OS) and disease free survival (DFS) were significantly longer for patients who were MRD negative at the post-consolidation time-point (p<0.001 for both comparisons). On the opposite, among 86 MRD positive younger patients, 51 (59%) experienced a relapse and 35 (41%) did not whereas among 42 MRD negative, 11 (26%) have relapsed and 31 (74%) are in continuous remission (p<0.001). OS and DFS were significantly longer for patients who were MRD negative at the post-consolidation time-point (p<0.001 and p=0.002, respectively). Cumulative incidence of relapse (CIR) was significantly lower for the MRD negative group both in elderly (57% vs. 88%, p<0.001) and younger patients (30% vs. 70%, p=0.002). *Conclusions.* in elderly adults, flow-cytometric MRD negativity defines a subgroup of patients with a CIR that is significantly lower than that of MRD positive patients (57% vs 88%, p=0.002). Nonetheless, elderly patients infrequently become MRD negative 13% vs 33%, p=0.005 and, even when MRD negativity is obtained, the rate of relapse doubles that of the younger counterpart (57% vs 26%, p=0.005), confirming that age represents by itself a poor-risk features in AML.

0066

THE IMPACT OF PRIOR HYPOMETHYLATING AGENT TREATMENT AMONG SECONDARY AML PATIENTS TREATED WITH CPX-351 OR 7+3 CHEMOTHERAPY

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Background. CPX-351 encapsulates cytarabine and daunorubicin at a 5:1 molar ratio predicted *in vitro* to maximize synergy. CPX-351 liposomes accumulate within bone marrow with preferential uptake by leukemia cells followed by intracellular release of drug. Multiple CRs in advanced AML patients in Phase I led to a randomized Phase 2b study comparing CPX-351 (85 pts) vs. standard cytarabine + daunorubicin (7+3) (41 pts) for untreated older patients with AML. CPX-351 showed improvement in CR + CRi rate, 60 day mortality, EFS and OS. Significant improvement in OS was observed among 51 (40%) patients entered with secondary AML (prior antecedent hematologic disorders (AHD): MDS, MPN, CMMoL, and treatment related AML. Previously treated patients with AHD, particularly prior hypomethylating agent therapy for MDS, identifies a subgroup with poor outcomes. Further analyses were performed to see whether the apparent improvement in outcomes following CPX-351 treatment of secondary AML patients might extend to patients with prior hypomethylating agent treatment. *Aims.* To evaluate the impact of prior hypomethylating agent therapy on the outcome of AML induction treatment with CPX-351 compared to 7+3. *Methods.* Previously untreated de novo or secondary AML patients, aged 60-75, PS= 0-2, S_{Cr} <2.0 mg/dL, total bilirubin <2.0 mg/dL, ALT/AST <3 x ULN, and LVEF >50%, were eligible. Patients were randomized 2:1 to receive up to 2 induction and 2 consolidation courses of CPX-351 (100 u/m^2; d1, 3, 5; 90 min infusion, 1 unit= 1 mg cytarabine + 0.44 mg daunorubicin) or standard 7+3 treatment (cytarabine 100 mg/m^2/d CI x 7d and daunorubicin 60 mg/m^2 d1, 2, 3). Consolidation with hematopoietic stem cell transplantation (HSCT) was permitted. Endpoints included: CR + CRi rate (1° endpoint) and duration, EFS, aplasia rate, survival at 1-year, and death at day 30 and 60. *Results.* This analysis focuses on the group of secondary AML patients randomized to CPX-351 (32 pts) and 7+3 (19 pts), analyzing the impact of prior hypomethylating agent treatment. Twenty (39%) of 51 patients with secondary AML included in this study received prior hypomethylating agents. Three patients on CPX-351 and 2 on 7+3 had prior treatment with other agents and are not included in the table. Prior treatment with hypomethylating agents was associated with reduced response rate, EFS and proportion alive at 1 year. With small numbers of secondary AML patients CPX-351 treatment was associated with improved outcomes. *Summary/Conclusions.* Newly diagnosed secondary AML patients treated with CPX-351 demonstrated higher response (CR + CRi) rate, lower induction mortality, improved EFS, and improved survival (p=0.01) compared to 7+3 chemotherapy. Prior hypomethylating therapy diminished treatment efficacy in both arms of the study, but CPX-351 treatment was associated with improved response and survival in this poor prognosis group.

Table 1.

		CPX-351 (n=32)		7+3 (n=19)	
Sex (m)		66%		47%	
Age (median, %>70 yo)		68y, 41%		68y, 42%	
ECOG PS 2		22%		21%	
Adverse Cytogenetic Risk		28%		32%	
Prior AHD	MDS	59%		58%	
	CMMoL	13%		11%	
	MPD	13%		16%	
	MDS/MPD	3%		5%	
Treatment-related AML		13%		11%	
OUTCOMES		Prior Hypomethylating Agent Treatment			
		YES (n=13)	NO (n=16)	YES (n=7)	NO (n=10)
Early Death (<Day 60; %)		1 (8)	1 (6)	2 (29)	3 (30)
Responses (CR+CRi; %)		7 (54)	11 (69)	2 (29)	4 (40)
Median EFS (months)		3.8	4.9	1.2	1.4
Median Survival (months)		9	NR	6	5.6
Alive by Day 365 (%)		5 (38)	8 (50)	1 (14)	2 (20)

0067

THE PROGNOSTIC IMPACT OF GERMLINE 46/1 HAPLOTYPE OF JANUS KINASE 2 IN CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA

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Background. Risk stratification according to acquired genetic alterations received increasing attention in acute myeloid leukemia (AML) in recent years, although current prognostic factors are not sufficient to accurately predict outcome especially in AML with normal karyotype (NK-AML). Inherited polymorphisms are also candidates for the explanation of the heterogeneous prognosis. Germline Janus kinase 2 (JAK2) haplotype designated as 46/1 haplotype was reported to be associated with an inherited predisposition to JAK2 V617F positive and negative myeloproliferative neoplasm, and also to NK-AML. *Aims.* The aim of this study is to assess the prognostic impact of 46/1 haplotype on disease characteristics (age at disease onset, history of AML, morphology, associations with cytogenetic and molecular genetic alterations) and treatment outcome (complete remission and relapse rate, disease free survival [DFS] and overall survival [OS]) in AML. *Methods.* JAK2 rs12343867 SNP tagging 46/1 haplotype was genotyped by LightCycler technology applying melting curve analysis with the hybridization probe detection format in 176 patients with AML diagnosed consecutively in a single center. The participants signed informed consents, and the study was approved by the Institutional Ethics Committee. *Results.* The allele C of JAK2 rs12343867 co segregates with the 46/1 haplotype, while allele T of rs12343867 is linked to the wild type haplotype.46/1 haplotype carrier frequency was similar in de novo and in myelodysplasia- or therapy-related AML subgroups. Distribution of morphological subtypes were different between haplotype-carriers and non carriers (p=0.047). There were considerably more FAB M2 morphological

cases within 46/1 non-carriers vs. carriers [17% (15/87 TT) vs. 6% (5/89 TC and CC), p=0.018], while myelomonocytoid and monocytoid variations were more frequent in 46/1 haplotype carriers [32% (28/87 TT) vs. 55% (49/89 TC and CC), p=0.003]. Similar distribution was observed in case of NK-AML. There was a tendency of increased 46/1 haplotype carrier frequency in NK-AML compared to AML with abnormal karyotypes [58% vs. 44%, p=0.069, OR (95%CI): 1.79 (0.98-3.26)]. FLT3 ITD and NPM1 mutation distribution was equal in 46/1 carriers and non-carriers. Considerably higher remission rate (94% vs. 79%, p=0.064) and fewer deaths in complete remission or in aplasia caused by infections (24% vs. 47%, p=0.038) were observed in 46/1 non-carriers compared to 46/1 haplotype carriers. SNP rs12343867 had no prognostic impact in the entire AML group, where age, karyotype, NPM1-FLT3 ITD combined mutation status were independent prognostic factors. C allele carriership of the SNP 12343867 was an independent adverse prognostic factor for DFS [HR (95%CI): 1.88 (1.07-3.29), p=0.028] and OS [HR (95%CI): 1.88 (1.07-3.29), p=0.027] in NK-AML. *Conclusions.* JAK2 46/1 haplotype may be a novel, independent unfavorable risk factor in NK-AML.

0068

MLN4924 A NOVEL INVESTIGATIONAL NEDD8-ACTIVATING ENZYME (NAE) INHIBITOR, IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) OR HIGH-GRADE MYELODYSPLASTIC SYNDROMES (MDS): RESULTS FROM A PHASE 1 STUDY

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Background. NAE regulates the NEDD8 conjugation pathway and is required for the activity of cullin-RING E3 ligases (CRLs), which control degradation of substrates involved in cell-cycle regulation, signal transduction, DNA replication, and stress response, including proteins important for AML cell survival. Inhibition of NAE with MLN4924, a novel, investigational, first-in-class small molecule NAE inhibitor, results in accumulation of CRL substrates and leads to apoptosis, as demonstrated in AML preclinical models. *Aims.* The primary objectives of this multicenter study were to evaluate the safety and tolerability of MLN4924, and to establish the MTD and the recommended phase 2 dose of MLN4924 in patients with AML and high-grade MDS. Secondary objectives included a preliminary assessment of efficacy, and analysis of pharmacokinetics and pharmacodynamics. *Methods.* Patients aged ≥18 years, with ECOG performance status 0-2, who had AML or high-grade MDS and were not candidates for potentially curative therapy, received MLN4924 as a 60-minute IV infusion on days 1, 3, and 5 of a 21-day cycle for ≤12 months or until documented disease progression. Dose escalation from 25 mg/m^2 proceeded using a standard '3+3' escalation method. Informed consent was obtained. *Results.* Twenty-nine patients (19 males, 28 AML, 1 MDS) were treated, including 3, 4, 5, 13, and 4 at dose levels of 25, 33, 44, 59, and 78 mg/m^2, respectively. Median age was 57.7 years (range 20-84 years); by cytogenetics, 1, 6, and 12 patients had good-risk, intermediate-risk, and poor-risk disease. Seven patients have received ≥6 cycles; 4 remain on treatment. Two DLTs, multi-organ failure and reversible ALT elevation, were reported at the 78 mg/m^2 dose level. Accrual has been completed and the MTD has been established as 59 mg/m^2. The most common treatment-emergent grade ≥3 AEs were febrile neutropenia (n=9, 31%), elevated ALT/AST (n=5, 17%), thrombocytopenia (n=4, 14%), and pneumonia (n=3, 10%). Four patients achieved a CR. A 29-year-old woman with relapsed AML following allogeneic SCT achieved CR after Cycle 1 (25 mg/m^2) before developing PD during Cycle 8. An 82-year-old man with high-risk azacitidine-refractory MDS that evolved into AML had a PR in Cycle 8 and a CRi in Cycle 10 (33 mg/m^2), becoming transfusion-independent before progressing after Cycle 12. A 71-year-old man with de-novo AML refractory to standard cytarabine plus daunorubicin induction achieved CRi during Cycle 1 (44 mg/m^2); the patient has reduced transfusion dependence in Cycle 15. A 51-year-old man with refractory AML following allogeneic SCT achieved a marrow CRi after Cycle 1 (59 mg/m^2). Plasma $AUC_{0\text{-}24hr}$ of MLN4924 increased linearly with increasing dose from 25-44 mg/m^2 after Cycle 1 Day 1 dosing in 9 evaluable patients. MLN4924 exerted predicted pharmacodynamic effects in peripheral blood and bone marrow, including increased transcription of the targets of CRL substrates in blood and the formation of MLN4924-NEDD8 adduct in bone marrow. *Conclusions.* NAE inhibition by MLN4924 demonstrates evidence of anti-tumor activity in patients with AML. MLN4924 appears generally well tolerated. Pharmacodynamic analyses show anticipated effects of tumor target inhibition. Further dosing schedules are being investigated to enable higher doses and greater MLN4924 exposure.

0069

IDENTIFICATION OF A SUBSET OF AML CASES WITH CYTOPLASMIC NPM1 LOCALIZATION WITHOUT DETECTABLE KNOWN NPM1 MUTATIONS

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Background. Mutation of the nucleophosmin gene (NPM1) is detected in about 30% of all patients with acute myeloid leukemia (AML). AML with mutated NPM1 shows distinctive biological and clinical features and is a provisional entity in the 2008 WHO classification. One of the important clinical features of NPM1-mutated AML is its favorable prognosis in the absence of FLT3-ITD. Mutation of NPM1 leads to aberrant accumulation of nucleophosmin in the cytoplasm of leukemic cells. This feature is often used to examine the presence of an NPM1 mutation in AML blasts by immunohistochemistry (IHC). *Aim.* To evaluate the sensitivity and specificity of NPM1 IHC in predicting NPM1 mutation status. *Methods.* Immunohistochemical stainings of decalcified bone marrow biopsies derived from AML patients were performed with a Benchmark XT. Slides were incubated with 1:50 diluted supernatant of the biotinylated anti-NPM1 antibody. Streptavidin with multimer horse radish peroxidase (HRP) and color development was carried out using DAB as a chromogen. NPM1 mutation analysis was performed by cDNA fragment analysis with a fluorescent labeled forward primer. For sequencing of the NPM1 gene RT-PCR was performed and PCR products were sequenced. *Results.* A total of 119 patients diagnosed with AML in the University Medical Center Groningen from 2005 to 2010, excluding those with favorable cytogenetic abnormalities, were analyzed in this study. The mean age was 57 years (range 17 to 81 yr) and 52% was female. Cytogenetics revealed that 65 patients (55%) had a normal karyotype and 26 (22%) had an unfavorable karyotype. An internal tandem duplication of the FLT3 gene (FLT3-ITD) was found in 23% of cases. Screening for NPM1 mutations by fragment analysis revealed mutated NPM1 in 34 out of 119 patients (29%). Screening for NPM1 mutations by IHC revealed cytoplasmic NPM in 33 out of 119 patients (28%) of these cases (Table 1). However, 5 cases had mutant NPM1 by fragment analysis but nuclear localization of NPM1 by IHC, and, reversibly, 4 cases had no NPM1 mutation detected by fragment analysis but cytoplasmic localization of NPM1 by IHC. Additional sequencing of exons 9 and 11 of the NPM1 gene of the latter cases did not reveal mutations at these sites. Consequently, in this study considering fragment mutation analysis as a gold standard, the positive predictive value of cytoplasmic NPM1 was 89% and the negative predictive value was 94%. The sensitivity of the IHC analysis was 85% with a specificity of 95%. In addition, we performed quantitative RT-PCR studies of several HOX genes, MEIS1, PBX3, BAALC and MN1. The cases with nuclear staining of NPM1 by IHC but a NPM1 mutation by fragment analysis showed a gene-expression profile as expected for NPM1 mutated AML. Interestingly, also the gene-expression patterns of the AML cases with cytoplasmic NPM1 by IHC but no known NPM1 mutation closely resembled those of mutated NPM1 controls. *Conclusion.* In this study, cytoplasmic localization of NPM1 by immunohistochemistry often but not always predicted mutation of the NPM1 gene. However, discrepant cases with cytoplasmic expression without detectable mutations may represent a distinct subgroup.

Table 1.

Correlation of immunohistochemistry and fragmentanalysis for NPM1
Number of patients are indicated

Fragment analysis	Immunohistochemistry: cytoplasmic	Immunohistochemistry: nuclear
mutant	29	5
wild type	4	81

Basic science in bleeding disorders

0070

MODULATION OF IMMUNE RESPONSES TO SELF AND NON-SELF ANTIGEN BY ACTIVATED PLATELETS IN VITRO

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Background. Platelets are abundantly present in the circulation, and activated platelets may have a role in regulating immune responses. Recent studies have indicated that activated platelets may stimulate maturation of monocyte-derived dendritic cells and regulate B-cell activity via shedding of soluble and platelet-derived microparticle bound CD40L. The impact of platelets on T-cell function is largely unknown, however. *Aims.* In this study we examined how activated platelets influence the cytokine production and CD4+ T-cell proliferation elicited by self- and non-self antigens in cultures of mononuclear cells (MNCs) from healthy donors. *Methods.* MNCs were isolated from 10 healthy donors (3 females, mean age 38 years, SD ± 16 years) and labelled with carboxyfluorescein succinimidyl ester (CFSE). Platelets were isolated by centrifugation and activated by thrombin-receptor activating protein (TRAP). The MNCs were cultured in media containing autologous serum and stimulated with tetanus toxoid (TT), thyreoglobulin (Tg), autologous platelets (plts) or combinations of TT + plts or Tg + plts. Platelets were added to the cultures in concentrations ranging from 0.5 - 3.4 x1010/L. Culture supernatants were harvested at day 1 and analysed for Th1/Th2 cytokine production. CD4+ T cell proliferation was measured at day 7 by flow cytometry. MNCs and platelets from 10 healthy donors (5 females, mean age 23 years, SD ± 10 years) were subsequently isolated and stimulated correspondingly, but were harvested after 16 hours for analysis of IL-10 secretion. *Results.* Addition of activated platelets caused an increased production of IL-10 in MNC cultures stimulated with Tg (p=0.0005), and reduced the production of TNF-a (p=0.0237). In cultures stimulated with TT, addition of activated platelets resulted in an increased production of IL-6 (p= 0.012). IL-10 secretion assays revealed a significant higher percentage of IL-10 producing CD4+ T cells in cultures stimulated with Tg in presence of platelets compared to cultures containing platelets alone (p=0.045)(Fig. 1A, horizontal bars represent means). Moreover, activated platelets inhibited both the Tg- and the TT-induced proliferation of CD4+ cells (p=0.002 and p=0.037, respectively) (Fig. 1B, horizontal bars represent means). *Conclusion.* Addition of activated autologous platelets to MNC cultures increases the IL-10 production by i.a. CD4+ T cells and, accordingly, inhibits the proliferation of CD4+ T cells elicited by the self-antigen Tg *in vitro*. Inhibition of the CD4+ T-cell proliferation elicited by the foreign antigen TT was also observed. The results of this study suggest that platelets may have a role in regulating the adaptive immune system.

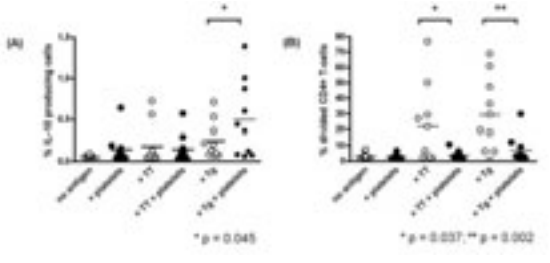

Figure 1. (A) IL-10 secretion (B) CD4+ T-cell proliferation.

0071

TWO NOVEL VON WILLEBRAND FACTOR GENE MUTATIONS THAT RESULT IN A UNUSUAL TYPE 2N VWD WITH DEFECTIVE MULTIMERISATION AND REDUCED VWF LEVEL

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VWD is a congenital bleeding disorder which results from quantitative or qualitative defects of von Willebrand factor (VWF). It is the most common inherited bleeding disorder in humans with a prevalence ranging from 3-4 /100,000 to 1.3% of the population. The current classification of VWD consists of 6 distinct types. Types 1 and 3 result in a quantitative VWF deficiency, whilst the 4 type 2 variants cause qualitative VWF defects. Type 2N defect result from defective VWF binding to F8 and consequently low levels of circulating F8. Inheritance is autosomal recessive. In this report our non consangious Asian family had significantly reduced levels of plasma VWF and lacked high molecular weight multimers. Analysis of the VWF gene sequence in DNA from affected members of the family detected 2 novel mutations. See tables 1 and 2. Type 2N mutations are normally found in regions essential for F8 binding, either the D' (amino acids 769-865) and D3 (amino acids 866 to 1244) domains. Mainly exons 18-20 with a few outside this. The novel exon 19 mutation, c.2546 G>T, p.C849F, this is a non conservative change in the D' region of VWF, responsible for binding to F8. Additionally, similar loss/gain of cysteine residues have previously been associated with reduce/absent high molecular weight multimers in individuals with type 2N VWD. This mutation is consistent with a contribution to the diagnosis of type 2N VWD. A second variant is expected to contribute to the reduced VWF level seen in this family. The novel c.8155+6 T>A mutation in intron 50 of the VWF gene. The sequence change has not been reported but a c.8155+6T>C mutation affecting the same nucleotide, has been reported to lead to skipping of exon 50 of the VWF gene. In silico splice site predictions also indicate a deleterious effect on splicing of the c.8155+6T>A sequence change. The three affected children have 2 novel mutations that are consistent with the diagnosis of Type 2N VWD with absent high molecular weight multimers. Whereas mum is a carrier of the type 2N mutation and dad is a carrier of the intron 50 mutation.

Table 1.

Phenotype

	1:1	1:2	2:1	2:2	2:3	Normal Range
VWF:Ag (IU/mL)	72	114	23	29	26	50-200
Factor VIII:Ag (IU/mL)	Normal	Normal	Substantially decreased	Substantially decreased	Substantially decreased	46-146
Factor VIII: C (IU/mL)	154	201	14	22	11	50-200
RCoF (IU/mL)	70	97	7	18	25	50-172
Multimers	Normal	Normal	Absent HMWM	Absent HMWM	Absent HMWM	

Table 2.

Genotype

Family Member	Exon 19	Intron 50	Multimers
1.1 (father KK)		c.8155+6 T>A	
1.2 (mother EA)	c.2546 G>T, p.C849F		
2.1 KaK	c.2546 G>T, p.C849F	c.8155+6 T>A	Absent HMWM
2.2 Huk	c.2546 G>T, p.C849F	c.8155+6 T>A	Absent HMWM
2.3 HaK	c.2546 G>T, p.C849F	c.8155+6 T>A	Absent HMWM

0072

POLYMORPHISMS IN IMMUNE RESPONSE GENES IN SEVERE HAEMOPHILIA A PATIENTS WITH INHIBITORS IN INDIA

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Background. The development of alloantibodies or 'FVIII inhibitors' to infused FVIII, is perhaps the most serious complication of FVIII replacement therapy. It leads to a considerable increase in mortality and cost of management of bleeds among these patients. In developing countries such as India, where resources are limited, an increased incidence of post-operative inhibitor development has been reported, which usually proves disastrous during this critical time of wound healing. A number of genetic and non-genetic risk factors have been implicated, but there is no data on the predisposing risk factors for inhibitor development in Indian haemophilia A patients. *Aims.* We studied polymorphisms in the *IL1β, IL4, IL10, CTLA-4* and *TNFA* genes, in 100 Indian severe hemophilia A patients, i.e. 40 inhibitor positive patients (and also 20 inhibitor concordant or discordant haemophilic family members), and 40 inhibitor negative control patients, to attempt to find a marker for the differential immune response to FVIII. The polymorphisms selected for study have been shown to influence antibody production in various autoimmune disorders, and some are reported to be associated with FVIII inhibitors in other populations. *Methods.* The *IL1β* rs1143634 C/T and *IL4* rs2243250 C/T single nucleotide polymorphisms (SNPs); as well as the *CTLA-4* rs5742909 C/T and rs231775 A/G SNPs, were analysed by the PCR-RFLP technique. Six *TNFA* SNPs; rs1800629 G/A, rs361525 A/G, rs1800630 C/A, rs4248158 C/T, rs1799724 C/T, and rs3093662 G/A were also studied in these patients by DNA sequencing. Cost-effective and quicker allele-specific PCRs have been designed to analyze the *TNFA* rs1799724 C/T and rs3093662 G/A SNPs. Genotyping for the *IL10* promoter dinucleotide microsatellite, *IL10G*, was carried out using a 6-FAM fluorescent labeled forward primer and GeneMapper analysis software (v.4.0). *Results.* The 'C/T' genotype of the *TNFA* rs1799724 C/T polymorphism was found to be significantly higher in inhibitor positive patients (OR: 4.645, P:0.0228, 95%CI:1.200-17.982; Fisher's exact test). The other cytokine related gene polymorphisms showed no strong association, in contrast to reports on the association of certain polymorphisms with inhibitors in other populations. *Summary/Conclusions.* This is the first report from India on the association of polymorphisms in immune response genes with inhibitors in severe haemophilia A patients. These findings could influence the study of other immune response gene polymorphisms as well as other genetic risk factors of inhibitor development. This could provide useful insights into the immune response to FVIII in inhibitor positive haemophilia A patients, and possibly influence the timely prediction and prevention or treatment of FVIII antibodies.

0073

A RARE CASE OF COMBINED INHERITED DYSFIBRINOGENEMIA AND FACTOR VII DEFICIENCY FROM MUTATIONS IN THE FGB AND F7 GENES

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Background. Dysfibrinogenemia and factor (F)VII deficiency are autosomally inherited rare coagulation disorders. The genetic backgrounds of the disorders are mutations in the fibrinogen Aα (*FGA*), Bβ (*FGB*) or γ (*FGG*) gene and *F7* gene, respectively. Clinical manifestations of both disorders are variable from asymptomatic form to life-threatening hemorrhage. *Aims.* We herein report the first genetically confirmed case of dysfibrinogenemia combined with FVII deficiency. *Methods.* The patient was a 51-year-old man referred for prolonged PT that was accidentally detected on preoperative screening. Past medical history revealed no history of bleeding tendency even on several surgeries to treat hemorrhoid, injured intervertebral disks of the lumbar spine, and sprain of right rotator cuff. Routine coagulation studies revealed prolonged prothrombin time (PT) at INR 1.31-1.35, marginally prolonged activated partial thromboplastin time (aPTT) at 41.2 sec (reference range, 29.1-41.9 sec), prolonged thrombin time (TT) at 42.2 sec (14.3-16.5 sec), and decreased fibrinogen level at 57 mg/dL (182-380 mg/dL, Clauss method). Special coagulation tests revealed a decreased FVII activity at 44% (68-149%). On suspicion of dysfibrinogenemia and hereditary factor VII deficiency, direct sequencing analyses were performed for *FGA*, *FGB*, *FGG* and *F7* genes. *Results.* The patient was found to be heterozygous for a point mutation in exon 8 of *FGB*, replacing the last stop codon to tryptophan (c.1475A>G, p.X492TrpextX13). The mutation was previously reported in dysfibrinogenemia (Fibrinogen Magdeburg II). In addition, the patient was heterozygous for a known missense mutation in exon 6 of *F7* (c.466G>A, p.Gly156Ser). Collectively, the patient was confirmed to have dysfibrinogenemia and heterozygous FVII deficiency by molecular genetic studies. *Summary/Conclusions.* To our knowledge, the present patient is the first genetically confirmed case of dysfibrinogenemia and factor VII deficiency. It was suggested that these autosomally inherited coagulation disorders might not be very rare and have potentially been underdiagnosed in Koreans.

0074

HYPOFIBRINOGENEMIA ASSOCIATED WITH A MUTATION IN THE BETAC-DOMAIN OF FIBRINOGEN

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Background. Fibrinogen, a 340 kDa glycoprotein, is composed of three sets of different polypeptide chains termed Aα, Bβ, and γ. It plays a crucial role in hemostasis and other physiological processes. Hypofibrinogenemia is characterized by a low fibrinogen level and bleeding tendencies may occur. *Aims.* The aim of the study was to characterize pathological preoperative coagulation findings in a patient with decreased both functional and total fibrinogen level. *Methods.* Routine coagulation testing was performed on STA-R coagulation analyzer (Diagnostica Stago). Both, fibrin polymerization and fibrinolysis were measured by turbidimetrical method at 350 nm after addition of human thrombin to the patient`s plasma. Kinetics of fibrinopeptide release was measured by RP-HPLC method. Gene sequencing was performed by a Sanger method. Scanning electron microscopy (SEM) was performed on VEGA Plus TS 5135 electron microscope (Tescan sro). *Results.* The patient presented with a low fibrinogen level as determined by both Clauss and immunoturbidimetric method and did not indicate any haemorrhagic or thrombotic complications. Both fibrin polymerization and fibrinolysis were found to be normal. Proteomics studies did not show any evidence of the mutant chain in the patient's plasma. DNA analysis revealed a heterozygous point mutation Bβ Asn381Lys. The mutation is situated approximately in the middle of the βC-domain. Molecular modeling revealed no stable rotamer of the mutated fibrinogen. The replacement of neutral charged asparagine residue by the positively charged lysine residue presumably changes the conformation of its neighborhood, which is unallowable for a correct Bβ chain assembly and subsequent fibrinogen folding. Aberrantly folded molecules may be then either degraded or stored by hepatocytes; and the mutated molecules are not present in the circulation. There is no evidence of a liver disease in the patient so we presume that misfolded molecules are rather degraded than stored by hepatocytes. *Conclusion.* The mutation found in the patient may be the direct cause of hypofibrinogenemia. E-mail: kotlinr@uhkt.cz

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0075

SYSTEMATIC REVIEW OF PHARMACOKINETIC OF FACTOR IX

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Background. The substitutive therapy of hemophilia B relies approximately for half on recombinant and the other half on plasma derived products, on a worldwide basis. More than 10 years after the first marketing authorization of recombinant factor IX (rFIX), the quantities used have more than doubled without significant change in therapeutic practices. It seems that the number of patients treated cannot explain such a difference *Aims.* . It raises the question of real pharmacokinetic equivalence or not between plasma-derived products and the recombinant one. Indeed, in 2003 Kisker *et al.* already suggested that, based on pharmacokinetics modelisation, therapy using rFIX may be more expensive.

This prompted us to review all available data. *Methods.* Systematic review of PubMed indexed literature has been performed, leading to the identification of 51 studies. 7 studies on animals and 2 on continuous infusion were not integrated, yielding to 42 analysed studies. Among these studies only 15 (36%) were in accordance with international sampling recommendations and 19 (45%) were conformed to recommendations of doses for injection. Concerning recovery (IU/dl/IU/kg), only 20/42 were considered as methodologically acceptable with data available for 26 studies . *Results.* Recovery data indicate for plasma-derived FIX (pdFIX) 1.17 ± 28 vs rFIX 0.81 ± 0.14 showing a significant advantage for pdFIX (p<0.0001). Only 4 paired studies were available, showing also a very clear difference between 1.41 ± 0.33 for pdFIX and 0.82 ± 0.05 for rFIX p=0.03. The comparison of clearance (ml.h-1.kg-1) could be achieved among 22 studies for pdFIX and only 3 studies for rFIX, showing also a significant difference: 5.6 ± 1.9 for pdFIX and 8.5 ± 4.5 for rFIX with p<0.05. *Conclusion.* These data may explain why lower quantities may be enough for treating hemophilia B patients when using pdFIX as previously suggested.

0076

MARKERS OF COAGULATION ACTIVATION AND ENHANCED FIBRINOLYSIS IN GAUCHER TYPE 1 PATIENTS: EFFECTS OF ENZYME REPLACEMENT THERAPY

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Background. Low-grade coagulation activation and enhanced fibrinolysis have been reported in Gaucher patients (GP). Data concerning the effects of enzyme replacement therapy (ERT) on that aspect of Gaucher disease are rare and controversial. *Aim.* To assess the parameters of coagulation and fibrinolysis activation in our GP and the impact of ERT on them. *Methods.* From 2005 to 2008, 21 Serbian treatment-naive type 1 GP (M/F 11/10; median age 37 years, range 2-74; splenectomized 8/21) were studied. Chitotriosidase activity (CA) was measured by spectrofluometry method. Spleen volume (SV) was assessed with CT. Fibrinogen was measured according to standard methods. Plasminogen, protein C (PC), antithrombin (AT) and plasminogen activator inhibitor (PAI) were measured using spectrophotometric assay. Thrombin-antithrombin complexes (TAT), prothrombin fragments (F1+2) and D-dimer were measured by ELISA. All GP were treated with ERT (Imiglucerase). Haemostatic parameters were assessed after 6, 12 and 24 months (ERT6, 12, 24). *Results.* Pre-treatment: Mean values of markers of coagulation activation (TAT and F1+2) and mean value of marker of enhanced fibrinolysis (D-dimer) were elevated although disseminated intravascular coagulation (DIC) was not registered (DIC score: mean 2, range 0-4),Table 1. A significant positive correlation between CA and D-dimer (p=0.047) as well as a significant inverse correlation between CA and PC level (p=-0.048) were registered. Non-splenectomized GP had significantly reduced AT and PC level compared to the splenectomized ones (p=0.0285 and p=0.0599). After ERT: CA and SV significantly decreased during ERT (p=0.0001). Fibrinogen (p=0.001), TAT (p=0.0001), F1+2 (p=0.0001) and D-dimer (p=0.05) significantly decreased while PC (p=0.006) and PAI (p=0.02) significantly increased at ERT12 and remained unchanged at ERT24. At ERT24 the mean D-dimer was still elevated while the mean TAT and F1+2 remained in normal.ranges. *Conclusion.* Our results suggest that a significant number of our GP had ongonig low-grade coagulation activation and enhanced fibrinolysis without an overt DIC, which might contribute to the bleeding tendency of GP. D-dimer and PC levels corelated with total CA and SV suggesting a relationship beetwen Gaucher cells burden and deriving pronflamatory cytokines on one side and coagulation activation on the other side. ERT significantly decreased the level of coagulation activation and enhanced fibrinolysis.

Table 1. Markers of coagulation activation and fibrinolysis.

Parameter	Normal range	N° pts with abnormal values	Mean ERT_0	Mean ERT_6	Mean ERT_{12}	Mean ERT_{24}	N° of pts with abnormal values
Fibrinogen (g/L)	2-4.5	4	3.3	2.9	2.7	3.1	1
CA (nmol/ml/h)	6-162	20	6776	4821	3083	1977	18
SV (cm3)		21	1904	1442	1200	1013	21
AT (%)	80-120	0	116	116	110	110	0
PC (%)	70-140	2	93	92	102	103	0
Plg (%)	70-140	0	109	92	91	93	0
PAI (U/ml)	0.3-3.5	1	2.2	2.9	3.21	3.5	2
D-dimer (µg/L)	<125	16	297	208	180	178	15
TAT (µg/L)	1.0-4.1	10	4.2	2.9	2.1	2.6	0
F_{1+2} (nmol/L)	0.4-1.1	6	1.2	0.7	0.7	0.5	0

0077

RARE COAGULATION DISORDERS: A STUDY OF 70 CASES IN THE EGYPTIAN POPULATION

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Background. Few Rare Bleeding Disorders registries (RBD) exist, none in Africa.Rare coagulation defects, such as factor (F)I, FII, FV, FV +FVIII, FVII, FX, FXI and FXIII deficiencies are transmitted as autosomal recessive traits with more prevalence in Muslim countries where consanguineous marriages are frequent.However, epidemiological information on the real distribution of these deficiencies is still limited and consequently when compared with the common bleeding disorders, most of these rare disorders are not well characterized clinically and do not have well-established treatment strategies.The clinical data of some big rare coagulation disorders registries such as the Italian, Iranian and North American have been reported.Only several small scale studies have been reported in Africa with none to date in the Egyptian population. *Aim.* To compare the clinical spectrum of some RBD in Egypt with other published data and to see if they behave differently in a population with many historical ethnic variations. *Methods.* A local hospital registry was started and patients were studied over 3 years.Assessment included detailed bleeding history, diagnostic events,clinical manifestations and treatment recieved. FX and FV deficiencies were classified according to the North American Rare Bleeding Disorders Registry and FVII deficiency according to the severity scoring system. Treatment was provided according to UKHCDO guidelines in view of resource constraint and patient's previous bleeding history. *Results.* We report the full clinical data of 70 patients, 61.4 % males and 38.6% females with the vast majority the offspring of consanguineous marriages .Afibrinogenemia is the most prevalent constituting 28.6% of the rare coagulation disorders, FV deficiency(10 homozygous and one heterozygous) constituting 15.7% and 20% of patients had FVII deficiency (6 severe, 7mild and 1moderate) .FX deficiency (11homozygous and 2heterozygous) was reported in 18.6% and FXIII deficiency in 11.4%. One patient had combined FV and FVIII deficiency and 3had FII deficiency. Intracranial hemorrhage occurred in 10% of our study group, joint bleeds in 12.9%, muscle bleeds in 7.1%, oral bleeding in 48.6%, epistaxis in 50%, bleeding per rectum in 17.1%, hematuria in 7.1%, post-circumcision bleeding in 14.3% and umbilical bleeding in 30% mainly in afibrinogenemia patients. Most patients received on demand therapy usually on initial presentation and 5 are on prophylactic therapy. *Conclusions.* The prevalence of these rare coagulation deficiencies and bleeding symptoms is different in the Egyptian population than elsewhere and so further studies including bigger numbers of patients could serve as an important source for clinicians in different parts of the world in view of rarity of these conditions.

0078

CARDIAC RESYNCHRONIZATION THERAPY DEVICE IMPLANTATION OR REPLACEMENT IN PATIENTS ON ORAL ANTICOAGULATION TREATMENT. EFFICACY AND SAFETY OF A REDUCED-DOSE PROGRAM

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Background. Many patients who require cardiac resynchronization therapy (CRT) are on oral anticoagulant therapy (OAT). Current guidelines recommend discontinuation of OAT and the initiation of anticoagulant 'bridging' therapy with heparin during these procedures. But some patients may be exposed to bleeding and/or thromboembolic complications. *Aims.* Our purpose was to evaluated the efficacy and the safety of CRT-device (CRT-D) implantation or replacement without interruption of OAT, applying a dose reduction program. *Methods.*A group of patients attending the Cardiology Department with an indication of CRT that were previously on chronic oral anticoagulation, was prospectively

analyzed. We collected their main baseline characteristics: age, sex, previous clinical history, blood cell counts, biochemistry (including renal function parameters), OAT indication, and concomitant use of antiplatelet drug. The type (implantation or replacement) and duration of the procedure, and the type of CRT-device, were also recorded. We established the hemorrhagic-thrombotic risk of each patient. We applied a dose adjustment of the OAT to achieve, just the day of surgery, an internacional normalized ratio (INR) between 1.5 and 2.0 for patients at low risk of thrombosis, and about 2.0 in case of high risk. Among others, the incidence of bleeding and thrombotic complications was analyzed. For statistical analysis, we used the Fischer's exact test for qualitative variables and the Mann-Whitney test for quantitative variables. *Results.* A total of 31 consecutive patients (mean age 73 years, range 36-90, males 24) were enrolled: 14 cases of CRT-D replacements (13 pacemakers and 1 defibrillator) and 17 CRT-D implantations (13 pacemakers, 3 re-synchronizers and one defibrillator). The mean time spent in the procedure was 53 minutes (range 15-150). In 18 patients (58%) the indication for OAT was a permanent or paroxysmal atrial fibrillation, and in six (19%) a mechanical valve prosthesis. A high bleeding risk was established in 19% of patients and a medium-high thrombotic risk in 52%. Five patients (16%) were carriers of coronary stent and six patients (19%) were also taking antiplatelet agents. The mean INR value on the day of surgery was 1.76 ± 0.34. Peri-procedure, 5 patients (16%) had complications: four cases of mild-moderate hematomas and one mild thrombotic complication. Forty-five days after the procedure, all complications had resolved. The baseline conditions of the patients associated with a higher rate of complications were a terminal renal failure status (p=0.037) and a prosthetic mechanical valve (p = 0.06). *Conclusions.* Our findings suggest that implantation/replacement of a CRT-D with a dose reduction of AOT constitutes an effective and safe alternative to interruption or routine "bridging"therapy. Complications were rare and never serious in our series, and the patients at increased risk were those with advanced renal failure or mechanical valve prostheses. Further studies involving a larger number of cases are needed to confirm these preliminary results.

0079

ASSOCIATION BETWEEN LEPTIN LEVELS AND BONE DISEASE IN HEMOPHILIAC PATIENTS

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Background. Hemophilia A and B has been associated with increased prevalence of osteopenia or osteoporosis. Leptin may play a key role in the pathogenesis of osteoporosis. *Aims.* The purpose of the present study was to investigate serum leptin levels in hemophiliac patients with or without bone disease. *Methods.* 81 male patients (73 with hemophilia A, 8 with hemophilia B) aged 45.4±15 years were screened. Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DXA) in lumbar spine (LS), femoral neck (FN) and total hip (TH). *Results.* Low bone mass was diagnosed in 20 patients (24.7%). The mean leptin levels in the whole cohort were 9.26 ± 9.89 ng/ml (range 0.05-51.97), being 12.61 ± 11.05 ng/ml (range 3.15-44.72) in patients with low bone mass (n=20) and 8.16 ± 9.32 ng/ml (range 0.05-51.97) in those with normal BMD (n=61). Serum leptin concentrations were strongly associated with body weight (rs = 0.457, P = 0.0001) and body-mass index (BMI) (rs = 0.491, P = 0.0001). In unadjusted analysis leptin was inversely associated with BMD in LS (rs = -0.255, P = 0.023), but not in FN and TH (rs = -0.205, P = 0.068 and rs = -0.191, P = 0.090, respectively). However, after adjusting for BMI and body weight, leptin was inversely associated with BMD in FN (F1,76 = 7.727, P = 0.007, β = -0.371, ΔR2 = 0.089) and TH (F1,76 = 4.533, P = 0.036, β = -0.290, ΔR2 = 0.054), but not in LS (F1,75 = 2.076, P = 0.154, β = -0.202, ΔR2 = 0.026). No association was found between age, presence of HBV, HCV or HIV infection or alkaline phosphatase and leptin levels. No association was also found between the severity of arthropathy (assessed by Pettersson score for knees and ankles: rs = 0.120, P = 0.334, rs = 0.110, P = 0.375, respectively and with Arnold-Hilgartner classification system: rs = 0.092, P = 0.461, rs = 0.226, P =0.066, respectively) and leptin levels. *Conclusions.* Our study showed a negative correlation between circulating leptin and BMD in patients with hemophilia A or B independently of body weight and BMI.

Chronic lymphocytic leukemia - Biology 1

0080

THE CYCLIN-DEPENDENT KINASE INHIBITOR CR8 INDUCES APOPTOSIS IN PROLIFERATING CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background. Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the Western world, and follows a heterogeneous clinical course. The finding that CLL patients with undetectable minimal residual disease (MRD) post-treatment possess a survival advantage has prompted the development of novel agents for incorporation into clinical trials, with the aim of eradicating MRD. *Aim.* To determine whether a novel cyclin-dependent kinase inhibitor (CKI) CR8 can overcome pro-survival and pro-proliferative signals induced in the tumor microenvironment, thus promoting apoptosis in CLL cells. *Methods.* Peripheral blood samples were obtained, after informed consent, from patients with a clinically confirmed diagnosis of CLL. The effect of CR8 treatment on CLL cells was determined by culturing them either in isolation, or by co-culturing on the mouse fibroblast cell line NT-L, or NT-L cells transfected with CD154 *in vitro*, to replicate *in vivo* survival- or proliferation-promoting environments, respectively. Flow cytometry, Western blotting and quantitative-PCR were performed to eludicate the mechanism utilised by CR8 to induce CLL cell apoptosis. *Results.* Our research demonstrates that the CKI CR8 (a novel derivative of roscovitine) is 100-fold more potent at inducing apoptosis in primary CLL cells than roscovitine in isolated culture (EC_{50}: 0.14 µM and 14.99 µM, respectively). Treatment of distinct CLL prognostic subsets with 100 nM CR8 revealed that cells isolated from previously-treated patients with more advanced disease were significantly less sensitive to CR8 than cells derived from untreated patients. While there was a trend towards reduced CR8 sensitivity in samples exhibiting poor prognostic markers (ZAP-70+, 11q/17p cytogenetic abnormalities), this did not reach significance. Importantly, 85% apoptosis was noted in all CLL subgroups upon treatment with 300 nM CR8. While *in vitro* co-culture systems that mimic the *in vivo* microenvironment of CLL proliferation centres rendered CLL cells resistant to fludarabine, CR8 was capable of inducing apoptosis, targeting actively proliferating CLL cells, albeit at a reduced potency compared with cells treated in isolated culture. Moreover, CR8 reduced the ability of cells to proliferate, causing a block at the G_1 phase of the cell cycle. CR8-induced apoptosis in CLL cells was mediated, at least in part, by an inhibition in expression of the anti-apoptotic proteins XIAP, Bcl-xL and Mcl-1. Indeed, reduced expression of XIAP and Mcl-1 was likely due to the ability of CR8 to inhibit CDK7 and CDK9 activities, both of which control transcription. Interestingly, we identified a non-dividing population of CLL cells resistant to CR8, when cultured in a pro-proliferative environment. *Summary*. These studies demonstrate that the novel CKI CR8 overcomes pro-survival and -proliferative signals to induce apoptosis, but reveal a discrete population of chemo-resistant cells that may be responsible for MRD in CLL patients.

0081

FC GAMMA RECEPTOR IIB ON HUMAN B CELLS PROMOTES RITUXIMAB INTERNALISATION AND REDUCES CLINICAL EFFICACY

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Background. The anti-CD20 monoclonal antibody (mAb) rituximab is invaluable in the treatment of B-cell neoplasms, but resistance remains a significant problem. Anti-CD20 mAb can be classified as type I (rituximab, ofatumumab) or type II (tositumomab, GA101) according to their activities in various *in vitro* effector assays. We recently reported that type I mAb, unlike type II, are internalised from the surface of normal and malignant B cells. Such internalisation is important because

it consumes mAb and reduces therapeutic efficacy. Here, we report that the inhibitory Fc gamma receptor (FcγRIIb) on target B-cells is a key regulator of this process *Aims.* The aim of the study is to uncover the mechanism behind the internalisation of rituximab. *Methods.* Internalisation of anti-CD20 mAb in primary tumour material was analysed using an *in vitro* flow cytometry-based fluorescence quenching assay. Western blotting and confocal microscopy were used to elucidate the events post-internalisation. *Results.* Rapid internalisation was particularly evident in most cases of chronic lymphocytic leukaemia and mantle cell lymphoma (MCL), but not from the majority of follicular or diffuse large B-cell lymphoma samples, possibly explaining their differing clinical responses to rituximab. Within each disease, the speed and extent of internalisation was heterogeneous. Internalisation of rituximab correlated strongly with FcγRIIb expression on tumour cells regardless of lymphoma subtype. Transfection of FcγRIIb converted FcγRIIb- Ramos cells into rapid internalisers in a dose-dependent manner. Internalisation also resulted in reduced macrophage phagocytosis of mAb-coated targets and could be inhibited by blocking FcγRIIb. Internalisation was largely cell-intrinsic, independent of contact with other FcγRIIb-expressing cells, in a process wherein FcγRIIb was phosphorylated and internalised along with CD20 and mAb prior to lysosomal degradation. This data is supported by clinical results showing that high FcγRIIb expression predicted less durable responses following rituximab-containing regimens in a small cohort of MCL patients. *Summary/conclusions.* High FcγRIIb expression provides a potential biomarker of response to rituximab-containing therapy and may identify patients for which treatment with type II anti-CD20 mAb may be preferable.

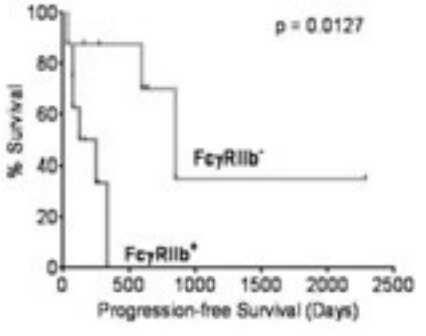

Figure 1. PFS of MCL patients by FcgRIIb expression.

0082

MONOALLELIC EXPRESSION OF THE CANDIDATE TUMOR SUPPRESSOR GENE C13ORF1 IDENTIFIED BY PROMOTER-RESTRICTED H3K4ME2

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Monoallelic expression is a mechanism of gene regulation especially relevant in the context of tumor suppressor genes (TSG), where inactivation of the single expressed copy can lead to a complete loss of tumor suppressive function. Monoallelic gene expression has been proposed to correlate with promoter-restricted enrichment of dimethylated lysine 4 of histone H3 (H3K4me2). We used this epigenetic mark to predict monoallelic expression of candidate tumor suppressor genes in a critical region in chromosomal band 13q14.3 telomeric to RB1 that is recurrently deleted in tumors. While the biallelically expressed gene KPNA3 and the monoallelically expressed candidate tumor suppressor gene RFP2 localized in 13q14.3 showed no promoter-restricted enrichment of H3K4me2, the monoallelically expressed candidate tumor suppressor ncRNA genes DLEU1 and DLEU2 carry significantly more H3K4me2 marks in their promoters compared to their first exons. Interestingly, based on the promoter-restricted enrichment of H3K4me2 in peripheral blood mononuclear cells, we identified the potential tumor suppressor gene C13ORF1 to be also monoallelically expressed in a parent-of-origin independent manner. We suggest that this gene might be involved in the pathomechanism of chronic lymphocytic leukemia (CLL), where more than 50% of patients display loss of one allele in the leukemic cells and where monoallelic expression will at least in a subset of patients cause a complete loss of C13ORF1 function.

0083

TARGETED RE-SEQUENCING TO ESTABLISH FREQUENCIES OF MUTATIONS IDENTIFIED BY WHOLE GENOME SEQUENCING IN B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA

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B-cell chronic lymphocytic leukaemia (CLL) is characterised by clinical and biological heterogeneity. Its pathogenesis remains largely unknown and it is unclear which genes are involved in disease maintenance and progression. Next generation sequencing technology has the ability to analyse the whole genome for sequence variations and is a sensitive tool to elucidate the complexity of cancer genomes. We therefore performed whole genome sequencing (WGS) on 2 patients with relapsed/refractory CLL and identified multiple protein code changing mutations. The aim of the present study was to validate our findings in a larger cohort of CLL patients. *Methods.* Discovery WGS was carried out to an average of >30-fold depth on blood samples of 2 patients plus matched germline buccal swab controls. Sequencing employed SBSv5 chemistry on the HiSeq2000 instrument. Paired 100-base reads were aligned to the human reference GRCh37.1/hg19 and candidate single nucleotide variants (SNVs), insertions, deletions and copy number variants (CNVs) were detected in both genomes. Potential candidate mutations were selected on the basis of: (1) functional annotations, (2) predicted impact on protein function using SIFT and Polyphen analysis, and (3) high-resolution SNP array data on a large cohort of CLL patients which identified copy number alterations (CNAs) affecting genomic regions in the potential candidates affected by targeted re-sequencing. Next, 40 CLL patients with treatment naïve or relapsed/refractory CLL were selected and subjected to capillary sequencing of the coding exons of candidate mutations. *Results.* WGS of 2 CLL patients revealed on average 20 non-synonymous, nonsense or frame-shift mutations per patient. Of the genes selected, many are involved in cancer pathways (eg MEK1, ASXL1, FAT3, NRG3) or are important in B-cell development or regulation of the innate immune response (ADAD1, SAMHD1, BCL2L13). Besides, we identified mutations in wnt pathway members. *Conclusions.* WGS has the potential to efficiently detect mutations in promising candidate genes involved in CLL pathogenesis. Work is ongoing to elucidate the frequency and dynamics of these mutations in a larger cohort of patients to provide insights into understanding CLL biology and help direct future therapeutic options.

0084

This abstract has been withdrawn.

0085

IMPACT OF THE MEVALONATE PATHWAY AND HIF/PGP AXIS IN MODULATING MULTIDRUG-RESISTANCE IN UNMUTATED CLL CELLS

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Background. The mutational status of the tumor immunoglobulin heavy chain variable region (IGHV) is a very reliable prognosticator in chronic lymphocytic leukemia (CLL): patients with unmutated (UM) IGHV have a worse prognosis than patients with mutated (M) IGHV. The tumor microenvironment actively supports the survival and *in vivo* accumulation of CLL cells and confer a multidrug resistance (MDR) phenotype to CLL B cells. MDR is due to the over-expression of membrane transporters, like P-glycoprotein (Pgp), which actively extrudes

several anticancer drugs. The activity of Pgp is under the positive control of the transcription factor Hypoxia-Inducible-Factor-1-alfa (HIF-1α), which is in turn activated by Ras/Rho-dependent signaling. A low mevalonate pathway activity, is known to be associated to a reduced production of isoprenylated small G-proteins, which are crucial factors for Pgp activity. Little is known on the role of metabolic and molecular pathways regulating chemoresistance in CLL cells. *Aim.* The aim of this study was to investigate the role of metabolic and signaling pathways involved in MDR modulation in M and UM CLL cells, in order to identify specific targets of therapeutic interventions. *Methods.* M and UM CLL cells negatively selected by magnetic beads isolation were cultured in standard medium in the presence or in the absence of murine stromal cells (M2-10B4) and CLL-derived bone marrow stromal cells (BMSC). The Mevalonate pathway activity was measured by cells radiolabelling with [14C]-mevalonic acid, lipid extraction and thin layer chromatography. RhoA/Ras isoprenylation, ERK1/2, Akt activity and HIF-1 phosphorylation were detected by Western blot. Pgp expression was measured by Real Time-PCR and Western Blot and its activity was evaluated by measuring the efflux of rhodamine 123. The Cytotoxicity induced by Doxo was analysed by annexin V and propidium iodide (PI) staining. *Results.* UM CLL cells showed a significantly more active Mev pathway than M CLL cells. The higher levels of Mev pathway activity were associated to a higher expression of Ras/Rho and to a higher activity of downstream kinases Akt and ERK1/2. This higher activity of ERK/Akt pathway was paralleled by a higher expression of phosphorylated HIF-1α, which is a potent inducer of mdr1/Pgp gene. As expected, UM CLL cells displayed higher levels of mdr1 mRNA expression, lower accumulation of intracellular Doxo and higher *in vitro* viability upon Doxo exposure. BMSCs induced the upregulation of Rho/Rho Kinase and Pgp proteins in UM CLL cells, protecting them from Doxo-induced cytotoxicity. Targeting of the Mev pathway by Zoledronic acid determined a significant reduction of the Rho/Rho kinase and Pgp activity, and abrogated BMSCs-mediated chemoresistance. *Conclusions.* Our data demonstrate that Hif/Pgp axis is more active in UM CLL cells compared to M CLL cells, leading to higher levels of chemoresistance. Specific targeting of the Mevalonate pathway and/or downstream signalling pathways may represent a promising strategy of therapeutic intervention.

0086

PI3-K-ALPHA ISOFORM AS A POTENTIAL TARGET FOR THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. There is evidence on the involvement of PI3-K/Akt pathway in the pathophysiology of CLL. We have recently shown that this pathway is activated *in vivo* and *in vitro* upon the interaction between CLL cells and primary bone marrow stromal cells (BMSC). Several inhibitors of this pathway has been tested and few such as PI3-K-delta inhibitor is already in clinical evaluation. However, the pattern of expression of different PI3-K isoforms and the significance of selective targeting of other isoforms remains to be explored. *Aims.* The aim of this study is to evaluate the expression of the four isoforms of PI3-K-p110 catalytic subunit (alpha, beta, gamma and delta) and to test the *in vitro* response of CLL cells to pan- and isoform-selective inhibitors of the PI3-K. *Methods.* Peripheral blood mononuclear cells of 15 CLL patients and 7 healthy individuals were analysed for the expression of the four isoforms of PI3-K-p110 at the mRNA and protein levels. The effect of pan-PI3-K inhibitors (Wortmannin, LY-294002 and PI-103) and PI3-K-isoform inhibitors against alpha, beta and gamma isoforms on cell viability was valuated. Overall cell viability was estimated by annexin V/PI staining, FACS analysis and MTT assays. Selective effect on cellular subpopulations was evaluated by flowcytometry using antibodies against CD3, CD14 and CD19 in combination with 7AAD. The effect of the inhibitors on the phosphorylation of the downstream targets Akt and PTEN was evaluated by western blotting. *Results.* RT-PCR analysis demonstrated the expression of the four PI3-K-p110 isoforms (alpha, beta, gamma and delta) in CLL samples. The mRNA expression was higher in CLL samples compared to healthy individuals. Western blot experiments confirmed that CLL cells express all four PI3-K-p110 isoforms at higher levels in comparison to healthy persons. Intracellular staining using isoform-specific antibodies demonstrated that more than 50% of the leukemic cells (CD19+/CD5+) were positive for p110 isoforms compared to less than 30% of CD19+ cells of healthy individuals. Exposure of CLL cells to pan- and isoform specific PI3-K inhibitors showed a significant induction of apoptosis with variable potency. PI3-K-p110-alpha selective inhibitor (morpholino-thienopyridine compound) was most effective at nanomolar concentrations with IC50 ranging between 10-20 nM with maximum effect at 40 nM concentration. This was followed by p110-beta inhibitor (morpholino-pyrimidinone/TGX-221) and p110-gamma inhibitor (thiazolidinedione compound). Co-culture experiments showed that p110-alpha inhibitor is most effective in inhibiting the supportive effect of BMSC. The induction of apoptosis was accompanied by dephosphorylation of Akt at Thr308 leading to Akt inactivation. This was also associated with dephosphorylation of PTEN at amino acid residues Ser380 consistent with the activation of PTEN phosphatase function. *Conclusion.* CLL cells express all four PI3-K-p110 isoforms at the mRNA and protein at higher levels than samples of healthy individuals. Targeting the PI3K pathway with alpha isoform-specific inhibitors significantly induces apoptosis in CLL cells and overcome the supportive effect of BMSC. These data confirm the value of PI3-K as a potential target for therapy in CLL and point to the significance of targeting the PI3K-p110 alpha isoform in particular.

0087

STEREOTYPED B-CELL RECEPTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA: A COMPENDIUM FROM A MULTICENTER ITALIAN COHORT

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Background. In chronic lymphocytic leukemia (CLL), a fraction of cases carries highly homologous "stereotyped"B-cell receptors (BCR) characterized by non-random combinations of immunoglobulin heavy-chain variable (IGHV) genes and heavy-chain complementarity determining region-3 (HCDR3). *Aim.* We performed sequencing analysis to characterize IGHV regions in a large panel of CLL patients investigated by a multicenter Italian study group and investigated possible correlations between stereotyped HCDR3 subsets and IGHV usage or biological/molecular and clinical features. *Methods.*The analysis of stereotyped subsets was performed based on previously reported criteria (Stamatopoulos *et al*. Blood 2007; Murray *et al*. Blood 2008; and Bomben *et al*. BJH 2008). A pair-wise alignment of sequences was adopted in order to discover novel potential subsets (HCDR3 identity > 60%). Evaluation of the time to first treatment (TTFT) was performed only in Binet A patients. *Results.* A total of 1382 CLL patients were investigated for productive IGHV-D-J rearranged sequences; twenty-two carried a double in-frame rearrangement. Based on the 98% homology criteria, 504/1404 sequences (35.9%) were classified as unmutated (UM). Stereotyped HCDR3 sequences were found in 28.6% (402/1404) of the patients, 66.6% (268) of which were UM; the most recurrent stereotyped subsets identified in our panel were #1 (39 cases), #7 (30), #4 (27), #3 (16), #9 (14) and #2 (14). Of note, the global alignment procedures within our dataset sequences allowed to identify 31 new putative subsets in 65 patients. Concerning the correlations between the most recurrent stereotyped subsets and genomic aberrations we found that (i) subset #1 (IGHV1-5-7/IGHD6-19/IGHJ4) was significantly associated with del(17)(p13); (ii) subsets #8 (IGHV4-39/IGHD6-13/IGHJ5) and #10 (IGHV 4-39 or IGHV2-5/IGHD2-2/IGHJ6) with trisomy 12;(iii) subset #4 (IGHV4-34) with a favorable cytogenetic profile. Despite previous reports (Marincevic *et al*. Haematologica 2010) we did not observe the presence of del(11q)(23) in all the cases (9 pts) included in subset #2 (IGHV3-21). Of clinical relevance, we found that subset #1 patients showed a significantly reduced TTFT compared to all UM patients, UM not stereotyped patients or UM patients with the same heterogeneous IGHV genes. Concerning previous reports, we confirmed the more unfavorable clinical course of subset #2 but not the difference in disease progression between subset #5 (IGHV1-69/IGHD3-10/IGHJ6), not stereo-

typed IGHV1-69 and subset #3 (IGHV1-69/IGHD2-2/IGHJ6) patients. *Conclusion.* Our findings suggest that distinct stereotyped BCRs in CLL are characterized by specific cytogenetic profiles and clinical course. In particular, we found a significant prognostic relevance of subset #1 in the context of UM CLL patients that would be validated in larger prospective series of patients.

0088

NURSE-LIKE CELLS SHOW DEREGULATED EXPRESSION OF GENES INVOLVED IN IMMUNOCOMPETENCE

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Chronic lymphocytic leukemia (CLL) cells convert CD14+ cells from patients into "nurse-like"cells (NLCs). CLL cells can also convert CD14+ peripheral blood mononuclear cells (PBMCs) from healthy donors into cells with morphological similarities to NLCs (CD14CLL-cells). However it is unclear whether this conversion process is only induced by CLL cells. We show that CD14+ PBMCs from healthy donors can also be converted into differentiated cells (CD14B-cells) by non-malignant B-cells. Intriguingly, non-malignant CD19-sorted B-cells were also able to differentiate CD14+ PBMCs from healthy donors into cells (CD14B-cells) that support survival similar to NLC and CD14CLL-cells. Moreover, gene expression levels of survival-associated genes like APRIL, BAFF and PECAM1 were comparable in all three differentiated cell types (NLC, CD14CLL- and CD14B-cells). In order to identify changes specifically induced by CLL cells, we compared gene expression profiles of NLCs, CD14CLL-cells and CD14B-cells. CD14+ cells cultured with CLL cells were more similar to NLCs than those cultured with non-malignant B-cells. The most significant changes induced by CLL cells were deregulation of the antigen presentation pathway and of genes related to immunity. NLCs had reduced levels of lysozyme activity, CD74 and HLA-DR *in-vitro* while expression of inhibitory FcGR2B was increased. These findings suggest an impaired immunocompetence of NLCs which, if found in-vivo, could contribute to the immunodeficiency in CLL patients.

0089

SNP BEAD-ARRAYS: TOWARDS A ROUTINE CLINICAL USE IN CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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Background/Aims. Determine if SNP random bead-arrays (Illumina, using oligonucleotides probes immobilized onto beads, that correspond to Single-Nucleotide Polymorphisms along the human genome) are more sensitive than Interphase Fluorescence In Situ Hybridization (I-FISH) or Conventional metaphase Cytogenetics (CC) to detect specific chromosomal abnormalities associated with prognosis in B-CLL, e.g. deletions at 17p, 11q, 13q and trisomy 12. *Methods.* A blood sample from 24 patients with B-CLL at diagnosis was analysed simultaneously by I-FISH, CC and SNP bead-array. FISH: LSI p53, ATM and CEP12 Single Color Probes (Abbott) were used, to detect del(17p13.1), del(11q22.3) and tri12, respectively. For detection of del(13q14.3), we tested in parallel the LSI D13S319 (~130 kb) Single Color (Abbott) and 13q14.3 (~400 kb)/ 13qter Dual Color probes (Cytocell). In normal lymphocytes, an average of 6% nuclei showed a loss of signal (truncated nuclei or random colocalized signals), and the cut-off to ascertain a chromosomal deletion was defined over 10% (m+3SD) CC: blood lymphocytes are cultivated for 72 hours with immunostimulants (DSP30 and IL-2) SNP: DNA (200 ng) was hybridized on the Illumina HumanCNV370 BeadChips (~373,397 markers; 4.9 kb median marker spacing; mean 7.8 kb). *Results.* FISH identified del(13q) (13-95% of nuclei, m=47%), del(11q) (35-54%) and tri12 (25-49%) in 18 (75%) [including 2 biallelic deletions], 3 (12.5%) and 4 cases (17%) respectively (5 cases presented associated abnormalities). SNP-arrays detected all of those abnormalities [del(13q), size: 0.49-33 Mb; del(11q), 35-42 Mb], with signals intensity correlating with percentage of FISH-positive nuclei. Moreover, SNP-arrays showed 4 del(13q) detected neither by the FISH probes used (>150 kb) nor by CC: cryptic deletions (39-82 kb) located at 13q14.3 (n=2) or q14.1 (n=1); 30 Mb deletion (13q13-q22) undetected by I-FISH (2% of nuclei with loss of signal, low clonal infiltration) (n=1). In that case, a biallelic deletion (20% nuclei) was detected by FISH performed 6 months later. In one case with del(13q) and tri12 (~25% of nuclei by FISH), an additional del(11q) was detected by CC (5/24 mitoses) and SNP-array (38 Mb, including ATM). In addition, SNP-arrays enabled to define more precisely the size and location of the abnormalities. For example, in 4 cases with del(13q) detected by FISH, SNP-arrays showed coexistence of several clones with variable sizes. No del(17p) was detected. Only one of the 24 cases (37% of CLL cells) was negative by FISH, CC and SNP-array, for the loci studied. *Summary/Conclusions.* The SNP random bead-array platform is more sensitive than molecular or conventional cytogenetics techniques. In our study, 92% (22/24) B-CLL cases presented with del(13q) at diagnosis. We report the best resolution (deletion of 39 kb) described. Higher density SNP-arrays (> 5 million markers) may be used to improve sensitivity, and closely determine involved genes. Our study confirms usefulness of SNP bead-arrays for prognostic evaluation in B-CLL, and sensitivity (abnormality detected in 2% of tumoral cells). This could allow to define a more homogeneous set of patients for prospective therapy trials.

0090

HGF-CMET INTERACTION PROLONGS SURVIVAL OF CHRONIC LYMPHOCYTIC LEUKEMIA CELLS THROUGH TYR705STAT3 PHOSPHORYLATION

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We have recently reported that chronic lymphocytic leukemic (CLL) cells express the hepatocyte growth factor (HGF) receptor c-MET, a tyrosine kinase, at high levels. HGF is a pleiotropic cytokine that may induce survival, proliferation, adhesion and migration of malignant cells. We demonstrated that mesenchymal cells (MSC) producing HGF in elevated quantities, such as bone marrow stromal cells (BMSC) or trabecular bone marrow cells (TBMC) or an osteoblast-like cell line, protect CLL cells from spontaneous apoptosis, when co-cultured *in vitro* and we suggested that HGF may contribute to the enhancement of CLL survival in the bone marrrow microenvironment. In agreement with this observation recombinant human HGF, exogenously added in cultures *in vitro*, also induced increased viability of CLL cells.In order to clarify and confirm the critical role played by HGF in MSC/CLL crosstalk we utilized a neutralizing anti-HGF antibody, or a c-MET inhibitor, in functional studies and further silenced HGF secretion by siRNA in mesenchymal cells. Moreover through western blot and cyto-fluorographic analysis we studied the activation of signaling molecules potentially induced by CLL/MSC co-cultures or HGF treatment of leukemic B cells . We demonstrated a significant inhibition of the prolonged survival of CLL cells by the use of an anti-HGF neutralizing antibody or of the c-MET inhibitor SU11274 in experiments where CLL cells were co-cultured with different mesenchymal cell lineages. Silencing of HGF production by siRNA significantly reduced the anti-apoptotic effect induced by the osteoblast-like cell line MG63 in co-culture experiments. We further observed that co-culture of CLL cells with conditioned media from BMSC or MG63, or their treatment with recombinant HGF induced phosphorylation of STAT3 in TYR705 after 10-40 min. A putative role played by pSTAT3 in CLL survival by MSC co-culture or HGF treatment was further confirmed by blocking CLL cells viability with a STAT3 inhibitor.Our data underlines a pivotal role of HGF in prolonging CLL cells survival and potentially in contributing to apoptosis resistance of the leukemic B cell clone at the level of particular bone marrow niches. Molecules involved in the HGF/c-MET signaling pathway(s) may be further forseen as possibile therapeutical targets in the treatment of chronic lymphocytic leukemia.

0091

HETEROGENEOUS FUNCTIONAL OUTCOMES AFTER TOLL-LIKE RECEPTOR STIMULATION IN DIFFERENT SUBGROUPS OF CHRONIC LYMPHOCYTIC LEUKEMIA ARE UNDERLINED BY DISTINCT TRANSCRIPTIONAL PROGRAMS

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We have recently reported that subgroups of patients with chronic lymphocytic leukemia (CLL) defined by distinctive molecular characteristics of the clonotypic B cell receptors (BcRs) exhibit distinct expression profiles of the Toll-like receptor (TLR) signaling pathway-associated genes as well as differential outcomes after TLR stimulation. The aim of this study was to investigate whether specific TLR-induced transcriptional modulation is observed and if it may be implicated in differential functional effects. To this end we analyzed 4 CLL cases each belonging to stereotyped subset #1 (unmutated IGHV1/5/7-IGKV1(D)-39 BcRs) or #4 (mutated IGHV4-34/IGKV2-30 BcRs). We selected these particular cases prompted by our recent finding of subset-biased response patterns to certain TLR ligands; in particular, imiquimod (ligand for TLR7) was active in subset #1 and induced CD25 expression while Pam3CSK4 (ligand for the TLR1/2 heterodimer) was active in subset #4 and induced CD86 expression. In addition, we included in the analysis 3 cases utilizing the IGHV4-34 gene, in mutated albeit non-stereotyped rearrangements (non #4). In order to study transcriptional changes, we negatively selected CD19+ B lymphocytes and stimulated the cells with specific TLR ligands. The gene expression profile of the TLR signaling pathway (overall, 83 genes) was determined by the RT² Profiler® PCR Array kit (PAHS-018A array, SABiosciences) in CLL cells unstimulated or stimulated with TLR ligands for 24 hours. Stimulation of TLR7 with imiquimod in subset #1 cases led to a specific transcriptional program characterized by significant changes of only a few genes with up-regulation of HSPD1 and TNF and down-regulation of HMGB1, MAP2K3 and IFNG mRNA levels. In stark contrast, stimulation of TLR1/2 with Pam3CSK4 in subset #4 cases affected the expression of a much greater number of genes. The effect was uniform in that all genes with significant changes showed down-regulated mRNA levels. Notably, the genes with altered expression include receptors, adaptors, effectors and NFκB/ MAPK signaling molecules which are all critical for TLR signaling; this suggests that a negative feedback loop may occur in this subset. Finally, in non #4 cases, which are generally non responsive to TLR1/2 stimulation, we observed that HSPD1, IL6, MAP2K4, MAP4K4, NFKB2, SARM1, TICAM2, TLR6 and TLR9 were up-regulated, while only SIGIRR, a negative regulator of the TLR signaling pathway, was down-regulated. In conclusion, the TLR pathway is competent for signaling in CLL with subset-biased functional outcomes; specifically, distinct transcriptional programs are activated by different TLR that may regulate TLR responsiveness and/or tolerance to additional TLR ligands.

0092

IDENTIFICATION OF 'STEREOTYPIC' PATTERNS EXCLUSIVE OF UNMUTATED IGHV1-69-DERIVED CLL B-CELLS

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Background. IGHV1-69/51p1 identifies ~30% unmutated CLL (U-CLL) and has a marked restriction in the HCDR3, with ~50% assigned to subsets with stereotypic patterns. The most representative pattern is subset-6 (>1% of all CLL), including virtually identical HCDR3s encoded by IGHV1-69, IGHD3-16 in reading-frame 2 (RF2), and IGHJ3. In healthy individuals, ~20% circulating naïve B-cells using IGHV1-69/IGHJ6 combinations have stereotypic patterns similar to U-CLL, suggesting tumor origin from the natural naïve B-cells. If U-CLL using different IGHV1-69/IGHJ combinations (including those assigned to subset 6) also derive from a similar repertoire is unknown. *Aims.* The aims of the present study were i) to detect stereotypic patterns using IGHV1-69/J3 rearrangements in the circulating B-cells of healthy donors and ii) to identify any counterparts of the unusually highly conserved subset-6 sequence in the normal blood. *Methods.* The functional IGHV1-69/D/J3 nucleotide sequences (n=165) derived from 7 healthy individuals were aligned to IMGT sequence directory (http://imgt.cines.fr/) and analyzed for IG usage. The derived HCDR3 amino acid (AA) sequences were aligned to those from a pre-established reference database of IGHV1-69+ve CLLs (n=654, including 78 IGHV1-69/J3 rearrangements) and to each other, and analyzed for identity with public and new CLL subsets, in concordance with established criteria. *Results.* One-hundred-forty-five normal rearrangements (87,9%) were unmutated and 20 (12,1%) were mutated, similarly to CLL (66/78 [84,6%] U-CLL and 12/78 [15,4%] M-CLL, p=.5). Frequencies of IGHD genes of IGHV1-69/J3 normal B-cells were significantly different from those of IGHV1-69/J3 CLL (p<.001). In particular IGHD3-16 frequency was markedly lower in normal (7/165, 4,2%) than in CLL (41/78, 52,6%, p<.001). IGHD3-16 was in RF2 less frequently in normal (5/7, 71,4%) than in CLL (39/41, 95,1%, p<.029). Stereotypic IGHV1-69/J3 patterns were found in 62/165 normal (37,6%) and 51/78 CLL (65,4%, p<.001). Remarkably, none of the normal stereotypes associated with the CLL stereotypes and viceversa. None of the normal IGHV1-69/D3-16/J3 rearrangements were assigned to subsets while CLL IGHV1-69/D3-16/J3 rearrangements were assigned to subset-6 in almost all circumstances (39/41, 95,1%, p<.001). A seminested PCR with a subset-6 specific degenerate primer was used to extract IGHV1-69/D3-16/J3 rearrangements assignable to subset-6 from the naïve B-cell repertoire. We identified 46 unique sequences and 10 clonal expansions from 114 clones, giving a total of 56 sequences with different HCDR3. Thirty-eight CLL subset-6 HCDR3 derived-AA sequences were available for comparison. All CLL used a common CAR(GGxYD) motif at codons 107-111 comprising the V-N1-D joining region). By sequence logo analysis, it emerged that the GGxYD motif was completely disrupted and never repeated in any individual normal B-cell. Disruption was also in terms of length of the N1 junction (5-15 AA in normal vs constantly 5 AA in CLL). *Summary/Conclusions*. These data suggest existence of CLL-specific motifs in the N1 junction region of subset-6-associated HCDR3, that are not found among circulating naive B-cells. This unusual feature of a highly frequent (>1%) HCDR3 motif in CLL is the most representative example of HCDR3 selection by "antigen" in CLL. One important question is to search the antigen or autoantigen that provides this CLL-specific driving force.

0093

THE MIR-17~92 CLUSTER FAMILY DETERMINES THE RESPONSIVENESS OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS TO TOLL-LIKE RECEPTOR 9 TRIGGERING

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Background. Chronic lymphocytic leukemia (CLL) cells expressing unmutated (UM) IGHV, a phenotype associated with aggressive clinical courses, can be more efficiently induced to proliferate by stimulating Toll-like Receptor 9 (TLR9) with unmethylated CpG oligonucleotides (CpG) than mutated (M) CLL cells. MicroRNA (miRNAs) are 18- to 22-nucleotide-long non-coding RNA molecules that regulate gene expression and play a key role in several biological process including oncogenesis. *Aim:* Elucidating miRNAs involvement in regulating activation/proliferation processes of CLL cells. *Methods:* Freshly-isolated negatively-selected CLL cells from 17 CLL patients (9 UM and 8 M) were stimulated with CpG (18 hours) or left unstimulated. miRNA and Gene Expression Profiling (GEP) were performed with Agilent Technologies. In-silico prediction of miRNAs modulation was made analyzing GEP data by the T-REX and GSEA software; validations were performed by qRT-PCRs. *Results:* miRNA profiling and GEP were separately evaluated in UM and M-CLL. In UM-CLL, 21 miRNAs resulted up-regulated and 3 down-regulated upon CpG stimulation. GEP data analysis selected 30 significant miRNAs, upregulated in CpG-stimulated cells, as allegedly responsible for GEP perturbation, of which 3 of them (miR-17, miR-20a, and miR-20b) in common with the miRNA signature of CpG-stimulated cells. These 3 miRNAs and additional 4 miRNAs

(miR-17*, miR-18a, miR-19b-1* and miR-92a-1*) again comprised in the miRNA signature, all belonged to the miR-17~92 cluster, a well-known miRNA cluster over-expressed in a variety of B-cell lymphomas. The GEP of CpG-stimulated UM-CLL cells included genes involved in cell cycle regulation and NF-kappaB cascade, in agreement with the proliferative effect of CpG in UM-CLL cells. In particular, among these genes: i) E2F5, TP53INP1, TRIM8, and ZBTB4, all target of miR-17, were downregulated in CpG-stimulated cells and knocked-out upon miR-17 transfection in six primary UM-CLL cells; ii) the miR-17 gene regulator MYC and the MYC target genes CAD, PGK1 and TFAM were upregulated in CpG-stimulated UM-CLL cells. Time-course experiments using primary UM-CLL cells showed that MYC and miR-17 were both upregulated upon CpG stimulation although follow different kinetics. In M-CLL cells no miRNA was differentially expressed between CpG-stimulated and unstimulated CLL cells. Despite this, in a hierarchical cluster driven by the UM-CLL microRNA signature, 4/8 M-CLL cases showed a microRNA profile similar to that of UM-CLL, including the increase of miR-17~92 cluster. In agreement with this data, the same 4 M-CLL clustered "near"to UM-CLL cells in a hierarchical cluster made using the GEP data of UM-CLL cells ("CpG-responder"M-CLL). Consistently, in these "CpG-responder"M-CLL, MYC gene resulted upregulated along with two (CAD and PGK1) MYC target genes. *Conclusion:* Altogether, our data suggest that TLR9 triggering is able to elicit a complete response which includes upregulation of MYC, miRNAs from the miR-17~92 cluster family and specific gene targets for these miRNAs both in UM-CLL cells and a subset of M-CLL.

0094

This abstract has been withdrawn.

0095

B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA CELLS EXPRESS FUNCTIONAL DEATH RECEPTOR 3 FOLLOWING STIMULATION OF THE B CELL RECEPTOR

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Background. In the microenvironments where B-CLL develops, complex molecular networks propagate signals that confer growth advantage to CLL. Within this context, interplaying between TNF superfamily members and their cognate receptors has been shown to play a relevant role in controlling B-CLL growth and survival. TNF-like protein 1A (TL1A) is a recently discovered member of the TNF superfamily expressed on various cell types, including macrophages, dendritic cells, and endothelial cells. The TL1A cognate receptor, death receptor 3 (DR3), is a member of the TNF receptor superfamily expressed on various cell types, including activated T cells and macrophages. TL1A-DR3 interactions have been shown to modulate immune and inflammatory functions. So far, the expression of DR3 on B-lineage cells as well as possible roles of TL1A-DR3 interplaying in CLL biology has not been explored. *Aims.* The objective of the study was to investigate DR3 expression and function(s) in B-CLL. *Methods.* B cells were purified from PBMC of 23 B-CLL patients and 13 healthy donors by negative selection with magnetic beads. DR3 surface expression was measured by flow cytometry at baseline and various time points following stimulation with F(ab')2 anti-human IgM conjugated to latex microspheres. DR3 mRNA was detected by quantitative RT-PCR. Phosphorylation states of NF-kappaB, Erk1/2, and TBK1 were measured by using phospho-specific antibodies and flow cytometry in basal condition and following DR stimulation of cells with agonistic antibody at different time points (15, 30, 60, 180 minutes). *Results.* Although both healthy and CLL B cells did not express DR3 in basal conditions, stimulation of B cell receptor (BCR) induced a statistically significant increase of DR surface expression in healthy as well as malignant B cells ($p<0.001$ for both cells). Time course analysis showed that DR3 expression peaked at 24 hour after stimulation. DR3 expression was confirmed also at the mRNA level. Time course analysis showed that DR3 mRNA in B-CLL cells peaked at 2.5 hour following anti-IgM stimulation (4-fold change with respect to basal conditions). Anti-IgM-induced DR3 expression levels were significantly higher in B-CLL cells if compared with healthy B cells ($p<0.05$). Interestingly, when B-CLL patients were stratified by IGVH mutational status, anti-IgM-induced DR3 expression levels were significantly higher in B-CLL cells harboring unmutated IGVH genes compared with cells with mutated IGVH genes. To assess whether the anti-IgM-induced DR3 molecule was functionally active in B-CLL cells, we examined the ability of DR3 to trigger phosphorylation events in biologically relevant signaling nodes (i.e. Erk1/2, NF-kappaB, and TBK1) by stimulating cells with anti-DR3 agonistic antibodies. Phospho-specific flow cytometry analysis showed that DR3 engagement induced phosphorylation of Erk1/2 but not NF-kappaB or TBK1. *Conclusions.* We described for the first time the expression of functional DR3 molecules in B-lineage cells activated by BCR stimulation in healthy and pathological cells (i.e. B-CLL cells). The findings that anti-IgM-induced DR3 expression was higher in B-CLL cells if compared with healthy B cells and that, among B-CLL patients, this expression was higher in cells with unmutated IGVH genes, suggest that DR3 stimulation may have a role in B-CLL pathogenesis.

0096

HS1 PHOSPHORYLATION, A PROGNOSTIC MARKER OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL), DEFINES A DISTINCT SIGNALING PATHWAY AND INFLUENCES THE CELL MIGRATORY CAPACITY

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Background. We previously demonstrated that the phosphorylation status of Hematopoietic cell specific Lyn substrate 1 (HS1) i) is a potential prognostic marker as CLL patients whose leukemic cells carry phosphorylated HS1 have a better prognosis than patients with the hyper-phosphorylated form; ii) in the CLL-prone Eμ-TCL1 transgenic mouse is a pivotal molecule in the signal transduction pathway triggered by the B-cell receptor (BCR), serving as a central interactor of several cytoskeletal components and being involved in tissue trafficking and homing and iii) has a profound effect on the development and progression of murine CLL suggesting that the hyper-phosphorylation of HS1 leads to its inactivation rather that activation. *Aims.* Given the role played by HS1 in BCR signalling and cell migration, we decided to dissect the signalling pathway and the migratory capacity of CLL cells presenting a differential phosphorylation status of HS1. *Methods.* We started by taking advantage of the CLL cell line MEC1 where we silenced the expression of HS1 and dissected the signalling pathway by Western Blot and Immunoprecipitation. We then utilized primary CLL cells to validate the results. Finally we investigated *in vivo* the migration of human leukemic cells labeled with different concentrations of CFSE and injected intravenously into Rag2-/-γc-/- recipient mice. *Results.* In the HS1-silenced MEC1 cell line we found that the phosphorylation status of several BCR signalling molecules, including Lyn Kinase, SHP phosphatase, ERK kinase and the cytoskeletal-related proteins Vav, Rac and HIP-55, is directly affected by the absence of HS1. We confirmed a similar pattern of modifications in primary cells from 25 patients who had a different phosphorylation status of HS1. These findings indicate that the pattern of HS1 phosphorylation is associated with a specific biochemical signature characterized by the loss of phosphorylation in several BCR downstream signalling molecules. We then investigated the homing ability of CLL cells from patients showing different HS1 levels of phosphorylation. CLL cells purified from 8 patients were paired into 4 couples according to their HS1 phosphorylation status. Each couple included a case with phosphorylated HS1 and a case with hyper-phosphorylated HS1 CLL cells. In 3/4 couples of differentially phosphorylated paired patients, CLL cells with phosphorylated HS1 had a consistent homing rate to the spleen while CLL cells with hyper-phosphorylated HS1 had a preferential homing to the BM. This observation indicates a correlation between the status of HS1 phosphorylation and the propensity of human CLL cells to accumulate in the bone marrow *in vivo*. *Conclusions.* These findings strengthen our previous observation that HS1 phosphorylation has an important role in controlling cell migration and homing of leukemic B cells, likely through its involvement in cytoskeleton organization and BCR signalling. Accordingly our future plans are: i) to increase the number of patients studied in order to detect potential correlations between signalling results, clinical behaviour and biological prognostic factors and ii) to identify novel intracellular HS1 partners to explore the possibility that the phosphorylation of HS1 may be modulated for therapeutic purposes.

0097

PROGRAMMED DEATH-1 IS A NOVEL IMMUNOTOLERANT MOLECULE EXPRESSED ON LEUKEMIC B CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Programmed death-1 (PD-1) molecule is an immunoreceptor, which through a interaction with its ligand (PD-L1), controls peripheral tolerance by limiting activation, development and effector function of T lymphocytes. PD-1/PD-L1 pathway was found to be one of the potential tumor escape mechanism from the immunosurveillance and PD-1 expression was described on exhausted T lymphocytes. *Aim.* We assessed a mRNA level and surface expression of PD-1 and PD-L1 in CLL patients since the aberrant expression of PD-1 might represent a novel target on the lymphocytic B-cells. It might also define possible prognostic markers. *Patients and Methods.* Quantitative reverse transcriptase PCR was performed for the PD-1 transcript with four splicing variants as well as for PD-L1 and one splicing variant in 43 CLL patients and 10 healthy volunteers (HVs). For characterization of the surface expression of PD-1 and PD-L1 on leukemic B-cells in a group of 33 CLL patients and 10 HVs magnetic separation followed by five parameter flow cytometric analysis was used. *Results.* The median level of PD-1 transcripts in CLL patients was higher in comparison with HVs (p=0.006). Additionally, expression of truncated PD-1 splicing variant lacing of exons 2, 3 and 4 was lower in CLL patients compared with HVs (p=0.0465). No difference of PD-L1 expression between CLL patients and HVs on mRNA level was observed. The expression of a non-functional splicing variant lacking exon 2 was elevated in CLL patients compared with HVs (p=0.008). 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. Mean fluorescence intensity (MFI) was higher among CLL patients in comparison with HVs (13.34 vs. 4.9, p<0.001). There was no difference in MFI levels of PD-L1 between CLL patients and HVs. Significantly higher MFI levels of PD-L1 were observed in early stage CLL patients (13,34 vs 4,9, in stage A and stage C according to Binet classification, respectively). There was no difference in time to progression and overall survival in groups of patients characterized by low and high PD-1 and PD-1L expression. *Conclusions.* PD-1, which is expressed both on mRNA and cell surface levels in CLL cells might represent a novel immunotolerant molecule involved in the pathomechanism of disease, as well as provide a novel target for future therapies.

0098

This abstract has been withdrawn.

Chronic lymphocytic leukemia - Clinical

0099

BENDAMUSTINE AND ALEMTUZUMAB (BEN CAM) COMBINATION IN RELAPSED AND REFRACTORY CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL): INTERIM REPORT OF THE ITALIAN TRIAL

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Background. Bendamustine (Ben) and alemtuzumab (Cam) are shown to be effective in relapsed/refractory CLL. Both agents exhibit an individual, unique mechanism of action. Therefore, a synergistic or additive effect might be expected when used in combination. *Aim.* We designed a multicentric, single arm, dose escalation study to determine maximum tolerated dose (MTD) and efficacy of Ben Cam combination in refractory/relapsed CLL. *Methods.* Previously treated CLL pts requiring therapy were enrolled. A stepwise dose escalation exploring MTD has been evaluated. Starting dosages were: Ben 50 mg/m2 d 1,2 and Cam 20 mg sc d 1-3. If MTD was not reached within the 1st cohort, the 2nd cohort received an increased dose of Cam 30 mg with a subsequent further increase of Ben 70 mg/m2 if MTD was not reached. Treatment was repeated every 28 d up to 4 cycles. Based on phase I (12 pts) results, MTD were Ben 70 mg/m2d 1,2 and Cam 30 mg sc d 1-3. *Results.* Overall, 29 pts have been enrolled in the study from July 2008 to October 2010. Median age was 69 y (range 54-82). 45% were Binet C; refractory pts represented 24% of our population. Median prior regimens were 2 (1-6), 76% received previously fludarabine based regimens, and 55% monoclonal antibodies (rituximab 34,5%, alemtuzumab 14%, both 7%). Biological characteristics are shown in the table. The 29 pts received overall 104 courses, median 4 (2-4). Response is available in 23 pts, pending in 6. Response rate was 61%, including 26% CRs and 35% PRs. Disease progression during treatment was observed in 17% of cases. Grade III-IV haematological toxicity consisted of: neutropenia 36% of courses, anemia 7%, thrombocytopenia 9%. FUO developed in 19% of cycles; 6 major infections (2 sepsis, 3 pneumonia, 1 enteritis) were observed. CMV reactivation occurred in 10 pts: no CMV disease was recorded. Extra-hematological toxicity was mild. *Conclusion.* Results from the interim analysis of this new, 4-weekly dosing Ben Cam regimen suggest that combination therapy with Bendamustine and Alemtuzumab is feasible, safe, and effective in treating patients with relapsed and refractory CLL. Major treatment toxicities were myelosuppression and manageable infections. No toxic deaths were recorded while on treatment.

Table 1.

	IGVH		ZAP70		CD38		FISH			
	mut	unmut	pos	neg	pos	neg	13q	12+	11q	17p
pts	31	55	59	34	45	38	17	14	21	31

*data not available in all pts

0100

RITUXIMAB IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ADVANCED CHRONIC LYMPHOCYTIC LEUKEMIA WITH FLUDARABINE + TBI CONDITIONING: RESULTS OF A PHASE II PROSPECTIVE MULTICENTER STUDY

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Aims and Methods. To evaluate the efficacy and toxicity of RIC regimen including fludara. and TBI with the introduction of rituximab for allo-HSCT, we conducted a prospective study for CLL patients aged < 65 years in stage B or C in response after a salvage treatment, having a HLA identical sibling donor. The conditioning included: rituximab 375 mg/m on day-5, fludarabin e 30 mg/m from day-4 to day-2, TBI 2grays on day0 and rituximab 500 mg/m on day 1 and day 8. Forty patients were included, 34 (85%) males and 6 females, median age 54 years (35-65), 38 (95%) in B stage and 2 in stage C. Among 23 explored for cytogenetics, 8 were abnormal. Before transplantation, 17 patients received 2 lines treatment, 10 three lines, 13 5 lines. At time of allograft, 7 (17%) patients were CR, 29 (73%) in PR and 4 (10%) < PR, 59% were sex-mismatched. For ABO matching, 68% were compatible, 19% major incomp. & 13% minor incopm. The median interval diagnosis-allo-HSCT was 58 months (6-177). Seven (17%) patients did not receive rituximab during conditioning because the protocol did not include it at the beginning and has been amended later. *Results.* Thirty-nine (98%) patients engrafted with a median time to neutrophils recovery of 20 days (11-70), 79% of patients reached a total donor chimerism at day 90. Seventeen patients developed aGVHD grade II (8 grII, 8 grIII & 1 grIV) with a cumulative incidence at 3 months of 44% (36-52). The cumulative incidence of cGVHD was, at 12 months: 29% (21-36) for lim. and ext.; at 18 months: 32% (24-40) lim. and 42% (34-50) ext. After a median follow-up of 28 months (3-71), the median OS was not reached with 5-years probability of 55%(41-74). The median time of EFS was 30 months (15 - 70) with a 5-years probability of 46%(33-66). The cumulative incidence of relapse at 1 and 3 years was 17% (11-23) and 22% (15-29) respectively. The cumulative incidence TRM at 1 and 3 years was 10% (5-15) and 27% (20-35) respectively. At the last follow-up, 17 patients died, 6 due to relapse and 11 due to TRM. The multivariate analysis showed a positive impact of rituximab on OS and EFS [HR=0.1 [0-0.6] p=0.02 & HR=0.1[0-0.4] p=0.035 respectively. *Conclusion.* The introduction of rituximab allowed a better outcome especially a significant reduction of incidence and severity of acute GVHD. Nevertheless there was still a high incidence of cGVHD, leading us to propose either to increase the number of rituximab injections after allo-HSCT, or to test Fludarabine/busilvex/ATG associated to rituximab.

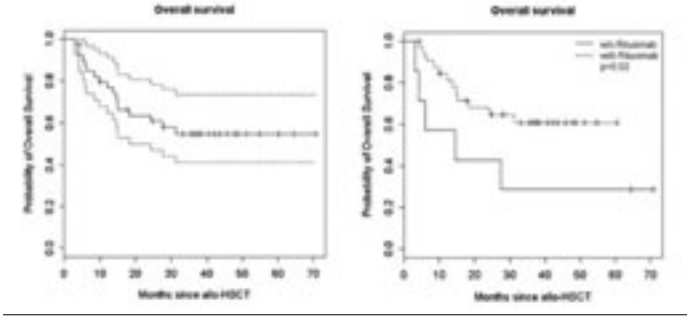

Figure 1.

0101

RESULTS FROM A PHASE II STUDY OF OBINUTUZUMAB (GA101) MONOTHERAPY IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background. Obinutuzumab (GA101) is the first type II, glycoengineered, humanized monoclonal anti-CD20 antibody to enter clinical trials. In a phase I study (BO20999), GA101 was administered as monotherapy to 13 patients (400-2000mg) with relapsed/refractory CLL, with GA101 given on Days 1, 8 and 22 and q21 (total of 9 infusions). End of treatment response (EOR) was 62% (8/13, all PR) (Cartron, EHA 2009, Morschhauser, ASH 2009). *Methods.* In the phase II part of BO20999, twenty patients with relapsed/refractory CLL received 1000mg GA101 monotherapy administered (flat dose) on Days 1, 8, 15 and 22 and q21 (total of 10 infusions). Primary endpoint was EOR with responses based on combined CT-scan and hematology results (IWCLL criteria, 2008). *Results.* GA101 was well tolerated with the most common AE being infusion related reactions (19/20 patients, grade 1,2: n=13, grade 3 n=5, grade 4 n=1), mainly during the first infusion. Thirteen patients completed all scheduled cycles. Reasons for 7 early withdrawals were: AEs [3], insufficient response [2], patient choice [1], protocol violation [1]). Most common related grade 3/4 hematological toxicities were neutropenia (n=4) and thrombocytopenia (n=2). There was one patient who developed febrile neutropenia (resolved without sequelae). Baseline characteristics and response are shown in Table 1.

Table 1. BO20999 - Phase I & II CLL baseline demog.

Patients median (range)	Phase I (n=13)	Phase II (n=20)
Age	64 (46-81)	62.5 (36-81)
Male/Female	9/4	12/8
Prior therapy	3 (1-8)	3 (1-7)
Prior rituximab	62% (8/13)	95% (19/20)
Prior stem cell transplant	15% (2/13)	20% (4/20)
Baseline SPD	2,124mm² (1,068-26,732)	3,138mm² (330-6,399)
Lymph nodes ≥5cm	31% (4/13)	35% (7/20)
Lymphocytes (x10⁹)	50.5 (5-153)	69 (0.6-134)
Platelets (x10⁹)	190 (93-356)	120 (39-246)
Hemoglobin (g/dL)	12.6 (9.4-14.9)	12.2 (9.6-15.8)
Cytogenetics		
Chromosomal aberrations	n=8: 17p (n=2), 11q (n=1), 13q (n=1), 8+12 (n=2), normal (n=3)	n=8: 13q (n=1), 11q (n=3), normal (n=1)
IgV_H status	n=8: mutated (n=2), unmutated (n=7)	n=5: mutated (n=3), unmutated (n=2)
FISH	n=8: mutated (n=1), unmutated (n=7)	n=14: mutated (n=5), unmutated (n=9)
Response		
End of Treatment	62% (8 PR, 4 SD, 1 PD)	25% (4 PR, 5 SD, 7 PD, 4 non-evaluable)
End of treatment median lymphocytes (x 10⁹/L)	1.0 (0.4-1.4)	0.8 (0.2-1.9)

Compared to the patient population in the previously reported phase I part of the study, patients had a higher tumor load with a median sum of product of diameter (SPD) of 2124 mm² (1,068-26,732mm²) for the phase I patients, compared with 3138 mm² (330-6,399 mm²) for the phase II patients. Based on 16 patients evaluable by combined CT-scan and hematology results, EOR was 25% (4 PR, 5 SD, 7 PD). Importantly, peripheral B-cell depletion for all patients was rapid following the first infusion of GA101, with all patients achieving B-cell levels below 4000/ul within one week of treatment, levels which were sustained during the treatment period, confirming the same observation reported in the phase I study. Of note, baseline tumour burden (TB) may be an important co-variate impacting patient response in the monotherapy setting in heavily pre-treated patients. Of the 4 PR patients, three had

baseline TB ranging from 1383-1989mm². Of the 6 patients with disease progression and 6 patients with stable disease, baseline TB ranged from 3004-5943 mm² and 2410-6266 mm² respectively, suggesting that those patients with baseline TB approximately >2400 mm², may not respond to the antibody when given as monotherapy, although all patients achieved impressive and sustained peripheral B-cell depletion. This is also supported in the phase I results, with 6 of the 8 responding patients having baseline TB ranging from 1068-2029 mm². This important data supports combination with chemotherapy in patients to maximize the clinical potential of GA101 in CLL to overcome lymphadenopathy. *Conclusion.* These phase II results indicate that GA101 has promising single agent activity in a heavily pre-treated patient population and will continue to be evaluated as a single-agent and in addition, the 1000mg dose is currently being investigated in combination with chemotherapy in a first line, phase III CLL trial (CLL-11).

0102

LOW DOSE ALEMTUZUMAB IS SAFE AND EFFECTIVE IN FLUDARABINE-REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Refractoriness to fludarabine-based chemo-immunotherapy is associated to poor prognosis in chronic lymphocytic leukemia (CLL). Alemtuzumab has been shown to be effective in this category of patients although associated with relevant toxicity in terms of infections. *Aim.* We evaluated efficacy and safety of alemtuzumab subcutaneously administered at lower dose in a cohort of fludarabine-refractory CLL patients. *Methods.* Thirty-nine fludarabine-refractory patients have been enrolled at our center and treated with subcutaneous alemtuzumab. Patients received 10 mg of alemtuzumab three times weekly for 18 weeks. In 18 randomly selected patients, after obtaining lymphocyte count reduction of 1 Log, the antibody was then administered once weekly at the dose of 30 mg. Biological prognostic markers were tested in 33 patients, including CD38 and ZAP70 expression by flow-cytometry, FISH panel for del(17)(p13), del(11)(q22), trisomy 12 and del(13)(q14); IGHV mutational status was available in 10 patients. *Results.* Median age was 64 years (range 48-82 years) with 31% of the patients older than 70 years. The patient population was characterized by high-risk biologic disease profile as shown by the incidence of del(17p) (18%), del(11q) (27%), unmutated IGHV (80%), CD38+ (48%) and ZAP70+ (55%). Median previous therapy lines were 3 (1-6); twenty-one were pre-treated with FC(R). Low-dose alemtuzumab yielded a 44% (95%CI 23.0-64.2%) overall response rate (ORR) whereas complete remission (CR) was obtained in 3 patients (8%; 95%CI 0-21.1%). Two of the three patients in CR resulted MRD negative. Median overall survival (OS) and progression free survival (PFS) were 29.1 months (95%CI 21.7-39.0) and 10.3 months (95%CI 8.3-16.2), respectively. Both PFS (p<.0001) and OS (p=.04) were significantly longer in responding versus non-responding patients. Treatment was well tolerated: all and 3-4 grade non-CMV infection were 46% and 7%, respectively. CMV reactivation occurred in 27% of the patients, showing only one case of disease. Moreover, no death occurred during therapy. No significant difference both in terms of safety and efficacy was observed in elderly (>70 years) patients and between the two different schedules evaluated. *Conclusion.* Our data indicate that low dose alemtuzumab is safe and effective in this setting of poor prognosis CLL patients. Efficacy results appear to be similar to those obtained with standard treatment, while toxicity is significantly lower. This data question the use of standard dose alemtuzumab in frail or elderly patients and provide evidence for an equally effective and more practical one-weekly alemtuzumab schedule.

0103

THE COEXISTENCE OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) MYELOPROLIFERATIVE NEOPLASMS. A RETROSPECTIVE MULTICENTRIC GIMEMA EXPERIENCE

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Background. The coexistence of CLL and myeloproliferative neoplasms (MPN) has been sporadically reported in the literature, but only as single cases: overall, 25 cases of CLL/chronic myeloid leukemia (CML), 18 of CLL/polycitemia vera (PV), 12 of CLL/essential thrombocythemia (ET) and only one case of CLL/primary myelofibrosis (PMF). *Aims.* We investigated the clinico-biological characteristics of CLL/monoclonal B-cell lymphocytosis (MBL), the timing of onset of the two diseases, the familiar predisposition to CLL/MBL, the role of chemotherapy for MPN on the course of CLL itself. We present data from the largest series of concomitant CLL/MBL and MPN so far reported. *Methods.* We retrospectively analyzed 46 patients affected by CLL/MBL and a concomitant MPN, diagnosed between August 1985 and May 2010. Data were collected from the CLL archives of 15 Italian centers on behalf of the GIMEMA chronic lymphoproliferative disorders Working Party: 30 were males, 16 females, with a median age at CLL/MBL diagnosis of 71 years. *Results.* Eight patients were classified as MBL, while 38 suffered from CLL. With regard to MBL, a diagnosis of MPN (2 ET, 2 PV, 2 PMF, 1 CML, 1 MPN/MDS) was made simultaneously in 5 patients and later during the follow-up in 3. None has so far progressed to an overt CLL; one patient died of an extrahematological cause. All 38 CLL patients were in Binet stage A at diagnosis. The diagnosis of MPN (16 ET, 8 PV, 8 CML, 4 PMF and 2 MPN/MDS) preceded that of CLL in 15 cases, was simultaneous in 7 and developed subsequently in 16. Six patients have so far received chemotherapy for progressive CLL (2 before, 1 simultaneous and 3 subsequent to the diagnosis of MPN). Six of the CLL patients have died after a median follow-up of 64.5 months; none of them experienced a progression of CLL requiring chemotherapy. The causes of death were never related to the MPN or to the lymphoproliferative disorder. MPN chemotherapy did not influence the course of the lymphoproliferative disorder; indeed, the lymphocyte count remained stable after 3, 6 and 12 months from MPN treatment. The biological prognostic parameters - IGHV (70% mutated), CD38 (78.5% negative), Zap-70 (71% negative) and del 17p or del11q (positive in only 7% of cases) - confirmed the clinical data indicative of a low risk CLL. Only one patient had a brother affected by CLL. *Conclusions.* Patients with a concomitant CLL/MBL and MPN usually have an indolent lymphoproliferative disorder with good clinico-biological prognostic features. None of the MBL patients have evolved into an overt CLL; only 6 stage A patients have experienced progressive disease requiring chemotherapy. Seven patients have died for causes not related to the lymphoproliferative disease nor to the MPN. Treatment for MPN or CLL did not influence the course of the other condition, suggesting that the occurrence of the MPN is not due to a leukemogenic effect of prior chemotherapy, but possibly to an immunodeficiency status associated to CLL.

0104

UPDATED RESULTS OF A SCREENING PROCESS FOR DATA QUALITY IMPROVEMENT WITHIN THE CLL10-TRIAL OF THE GERMAN CLL STUDY GROUP

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Background and Aims. The clinical course of chronic lymphocytic leukemia (CLL) is extremely variable, depending on different prognostic factors especially high risk cytogenetics. Moreover, the patient (pt) population in CLL is quite heterogenic, due to different physical conditions and the burden of comorbidity because of the broad age spectrum observed in this disease. Protocol violations are a frequent problem in clinical trials. Primarily in order to enhance patient safety, but also to describe the patient population more precisely and to increase the data quality, the GCLLSG implemented a central screening process prior to randomisation. *Patients and Methods.* The CLL10-study is an international multicenter phase III trial comparing FCR and BR in patients (pts) with low comorbidity score, normal renal function and with advanced CLL requiring treatment, that was initiated in September 2008. Pts with del(17p) were excluded and are treated in a separate protocol for very high risk pts. Central laboratory testing for immunophenotyping, cytogenetics and other prognostic parameters, as well as a medical review of all data, especially those regarding response assessments and safety data, are well established means of quality control in GCLLSG-trials. The CLL10-study is the first GCLLSG-study with a screening process prior to the randomisation. Blood samples from all patients are sent to the central laboratories for testing of cytogenetics and immunophenotyping to confirm the diagnosis of CLL and to exclude del(17p). In addition, the pt's comorbid conditions and renal function are evaluated by checking the CIRS-Score and concomitant medication and by recalculating the creatinine-clearance. Moreover, the need of treatment according to the recently published guidelines by Hallek *et al*. is reassessed by one of the GCLLSG study physicians. *Results.* Until February, 28th 2011, 582 pts were screened for participation in the CLL10-trial. The median number of included pts is 3 pts per center. 17,9% (104) of the 582 pts were not eligible for randomisation, either due to violation of the inclusion-/ exclusion-criteria (95; 92,3%) or due to physician or pts wish (9). Thirty-five pts were not eligible because of concomitant diseases or comorbid conditions, including twenty-eight pts with an impaired renal function and five pts with an active secondary neoplasia. Twenty pts had to be excluded because of a del(17p-), eight pts were pretreated, in fifteen cases the diagnosis of CLL was not confirmed by the central immunophenotyping and in twelve cases the absolute leukocyte counts were <5000/µ (small lymphocytic lymphoma). Five pts were not in need of treatment according to the recently published guidelines. At the beginning of the trial, the percentage of screening failures was around 25% in January 2009 and decreased to 18% in February 2011. *Conclusions.* The high rate of screening failures underlines the importance of quality assurance in clinical trials not only to achieve comparability and transferability of results, but also for better patient-security. The decline in the rate of screening failures could be the result of a learning process. Moreover, these data show that central laboratory diagnostic for immunophenotyping and prognostic factors is essential in CLL.

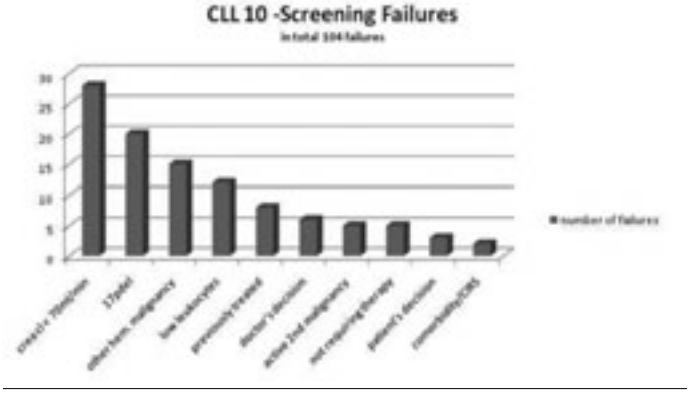

Figure 1. CLL10 Screening Failures.

0105

LOW-DOSE FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINED WITH RITUXIMAB IN THE TREATMENT OF ELDERLY/COMORBID PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: PROJECT Q-LITE OF 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 treatment because of advanced age and/or serious comorbid conditions. For these patients, chlorambucil has remained so far the standard of treatment. Low-dose fludarabine-based regimens 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. *Patients and Methods.* Between March 2009 and December 2010, we treated 93 pts with active disease (CLL, n=88, SLL, n=5, males, 59%, median age, 70 years [range, 58-83], median Cumulative Illness Rating Score 4 [range, 0-10]) by low-dose FCR at fourteen centers cooperating within Czech CLL Study Group. Dose reduction of chemotherapy was as follows: fludarabine to 50% (12 mg/m2 i.v. or 20 mg/m2 orally on Days 1-3), cyclophosphamide to 60% (150mg/m2 i.v./p.o. D1-3). The dose of rituximab was standard (375mg/m2 in 1st cycle, 500mg/m2 from 2nd cycle). Treatment was repeated every 4 weeks. Antimicrobial prophylaxis with sulfamethoxazol/trimethoprim and aciclovir or equivalents was recommended. Fifty-six per cent of pts were treated in first line, remaining 44% had relapsed/refractory disease. Advanced Rai stages (III/IV) were present in 62% pts; 40% had bulky disease. IgVH genes were unmutated in 74%; according to hierarchical model, del 11q was present in 32% and del 17p in 5%. *Results.* Based on intention-to-treat principle, the overall response/complete response rate (including clinical CR and CR with incomplete blood count recovery) was 71/39% in first-line treatment and 63/27% in relapse; 18% of pts are still on treatment. Data on PFS/OS are not available yet. Serious (CTC grade III/IV) neutropenia occurred in 54%, thrombocytopenia in 13% and anemia in 11% of pts. Serious infections were diagnosed in 13% of pts. *Conclusions.* Treatment of elderly/comorbid CLL/SLL patients with low-dose FCR demonstrated promising results. Toxicity was acceptable and manageable. Updated results will be presented. Supported by research project MZO 00179906 from Ministry of Health, Czech Republic.

0106

A RETROSPECTIVE MULTICENTER TRIAL WITH LOW-DOSE ALEMTUZUMAB IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS. ON BEHALF OF THE GIMEMA CHRONIC LYMPHOPROLIFERATIVE DISORDERS WORKING PARTY

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Background. Alemtuzumab has shown significant activity in chronic lymphocytic leukemia (CLL). However its use has been associated to profound immune suppression leading to high rates of severe infec-

tions. We have previously reported that alemtuzumab given at lower doses may be equally effective and less toxic in refractory CLL. *Aim.* We conducted a multicenter retrospective study on the routine clinical use of low-dose alemtuzumab in relapsed/refractory CLL. *Methods.* One hundred and eight patients from 11 Italian centers were included in the analysis. Low-dose alemtuzumab was defined as a total weekly dose <45 mg and a cumulative dose <600 mg up to eighteen weeks of therapy; both subcutaneous (SC) and intravenous (IV) administrations were allowed. The other inclusion criterion was that patients had to be treated with at least one previous line not containing alemtuzumab as single agent or in combination. Biological prognostic factors including CD38 and ZAP70 expression, as well as FISH analysis were available for 97 patients; the IGHV mutational status was known for 49 patients. *Results.* Median age was 68 years (range 40-84). No patients had an ECOG PS >2. Fifty patients (46%) were in Binet stage C, the median WBC count was 54.900/mmc (2.100-288.000), while bulky lymph nodes (>5 cm) were present in 17 patients. Forty-three patients (44%) showed an unfavorable cytogenetic profile, including 27 patients with del(17p) and 18 patients carrying del(11q); CD38 and ZAP70 were expressed by 47 and 48 patients, respectively; 33 of the 49 patients studied (67%) were characterized by an unmutated IGHV configuration. The median number of previous lines of therapy was 2 (1-6); 75% of patients were previously exposed to chlorambucil (60% being refractory) and 60% to fludarabine (74% being refractory). Different treatment schedules of alemtuzumab were employed; most patients (92%) received a weekly dose of 30 mg either divided into three 10 mg administrations (51%) or given as a single dose (41%). In 14 cases, alemtuzumab was given IV. The overall response rate (ORR) was 56%, including 22% of CRs. After a median follow-up of 42.2 months (2.1-91.9), the median OS and PFS were 39 months (95% CI 29.8-58.4) and 19.4 months (95% CI 15.9-23.6), respectively. In univariate analysis, response was inversely associated to lymph node (p=.01) and spleen (p=.02) size, fludarabine-refractoriness (p=.01), previous treatment lines (p=.01), presence of del(11q) (p=.009). Presence of del(17p) was not associated to a worse outcome. Response occurred regardless of the cumulative dose of alemtuzumab administered, both in the whole cohort and in the biologically-determined high risk subsets of patients. Hematologic toxicities were frequent but manageable: grade 3-4 neutropenia, thrombocytopenia and anemia were reported in 29%, 6% and 6% of patients, respectively. Severe (grade 3-4) infections occurred in 7 patients (7%) during therapy. CMV reactivation was documented in 37 patients (34%), with only one case of CMV infection. *Conclusions.* This retrospective analysis shows that alemtuzumab given at lower doses is a valid and currently used therapeutic option for the treatment of relapsed/refractory CLL, in particular in elderly and frail patients.

0107

RITUXIMAB AND SUBCUTANEOUS CLADRIBINE: CLINICAL RESULTS OF AN EXTENDED COHORT OF CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS

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Background. Rituximab in combination with purine analogue is a well established treatment for chronic lymphocytic leukaemia (CLL). Recently we published preliminary results on CLL patients using subcutaneous cladribine in combination with rituximab. *Aims.* Here we report updated results on 67 patients with active CLL or small lymphocytic lymphoma (SLL) treated with this combination treatment. *Methods.* Sixty-seven patients with active CLL or small lymphocytic lymphoma (SLL) received rituximab 375 mg/mq on day 1 and cladribine 0.1 mg/kg subcutaneously on days 2-6. The treatment was repeated every 4 weeks for a total of four cycles. Response to treatment was evaluated according to NCI-WG updated guidelines for patients with CLL and with Cheson criteria for SLL patients. *Results.* Forty-five patients were previously untreated. The median age was 63 (32-78). 2-microglobulin was abnormal in 54% of patients at baseline. 25% of patients presented multiple genetic alterations at FISH analysis and 66% of patients showed a ZAP-70 positivity. Sixty-five patients were evaluable for response. The overall response rate was 86% (45% CR and 41% PR), with 44% of untreated patients and 41% of pre-treated patients achieving a CR. Thirteen patients achieved a clinical remission in the absence of minimal residual disease. The median follow up was 27.6 months. The median time to treatment failure (TTF) was 36.7 months. There was a statistically significant difference in terms of duration of response between untreated and pre-treated patients (TTF respectively: 52 vs 23 months, p=0.065). Patients achieving a CR had a longer response duration than patients with PR (TTF respectively: not reached vs 23 months, p=0.025). Low serum lactate dehydrogenase levels and 2-microglobulin levels at baseline and a normal CT scan at the end of therapy (independent of response) did not influence TTF. Ten patients developed severe neutropenia (grade 3-4). Thrombocytopenia grade 3 was observed in one patient and anemia grade 2 in one patient. Five pts developed pneumonia, rapidly resolved without serious consequences. One patient developed febrile neutropenia with diarrhoea (Salmonella positivity). In 3 patients we observed cutaneous toxicity with resolution after the use of oral steroids. *Conclusions.* Our data confirm that combination therapy with R-cladribine is an effective and safe treatment for patients with CLL and SLL achieving results similar to those reported with more aggressive regimens.

0108

LONG-TERM FOLLOW-UP OF PATIENTS WITH HAIRY CELL LEUKAEMIA TREATED INITIALLY WITH PENTOSTATIN OR CLADRIBINE. SPANISH EXPERIENCE (BY THE SPANISH COOPERATIVE GROUP ON CLL)

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Background. Hairy cell leukaemia (HCL) is a rare indolent lymphoproliferative disorder characterised by infiltration of the bone marrow, liver and spleen by malignant B-cell with hairlike cytoplasmic projections (HC). Purine analogues are highly effective in HCL with overall responses in more than 85%. However, HCL patients are projected to progress in their life-span or, in a minority are refractory. *Aims.* To investigate the long-term outcome for patients with HCL treated with purine analogues, to compare efficacy of pentostatin and cladribine (in the absence of any randomised controlled trials) and to identify factors associated with response, or relapse. *Methods.*We reviewed clinical and laboratories dates retrospectively from 75 patients, initially treated with pentostatin or cladribine, to investigate the current long-term outlook. Patients entering the study received pentostatin 4 mgr/m2/15 days or cladribine (by different schedules) as a single course. Minimal residual disease (MRD) was studied by flow cytometric immunophenotyping in peripheral blood (PB) and bone marrow (BM). Efficacy endpoints were response to therapy, treatment free interval (TFI) and overall survival (OS). TFI was measured in all patients from treatment initiation to time of new treatment requirement. OS was measured from therapy initiation to death or last follow-up. We studied risk factors associated of response and relapse. Analysis was performed using the SPSSv15.1 software package. *Results.* Of 75 patients, 54 were males (72%). Median values and range were: age 54 years (31-82), haemoglobin 11,2 g/dL (4,1-15,8), platelets 73x109/L (13-267), WBC 2,7x109/L (0,7-20), %HC in BM 36 (5-90) and size spleen 15cm (12-33) without significant differences between two groups of treatments. Twenty one patients received pentostatin and 54 cladribine for first line therapy. The median follow-up was 156 months (4-393) for pentostatin group and 65 months (3-191) for cladribine group. The overall response rate was 100%. The complete remission (CR) rate was 95,2% with pentostatin and 90,7% with cladribine. The median numbers of pentostatin injections required to achieve CR were 7 (2-14). In 5 patients treated with cladribine who still remained in partial remission (PR), was administrated a second cycle of cladribine which led to a CR in 4 patients. MRD was positive in 52,4% of pentostatin patients and 33,3% of cladribine patients. Twenty patients relapsed: 47,6% patients treated with pentostatin and 18,5% of those who received cladribine. By univariate analysis risk factors of relapse were: Hb (P=0,001), %HC in BM (P=0,013) and size spleen (P=0,003). Cox multivariate analysis revealed Hb and splenomegaly as independent risk factors of relapse.

By Kaplan-Maier estimates the median TFI was 96 months in pentostatin, and 144 in cladribine patients; 95 months in MRD+, and 216 in MRD- patients. *Conclusions.* Although both agents are effective in HCL, the results are better for cladribine than pentostatin respect rate of relapse, TFI and MRD. Haemoglobin levels and splenomegaly are confirmed as independent risk factors of treatment failure and rapid progression. The relation between MRD+ and shorter TFI suggests the convenience to add treatment, like Rituximab, to obtain MRD negative response.

0109

SMALL LYMPHOCYTIC LYMPHOMA (SLL) AND CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): A SINGLE DISEASE WITH SIMILAR PROGNOSIS?

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Introduction. Small lymphocytic lymphoma (SLL) is a mature (peripheral) B-cell neoplasm characterized by a progressive accumulation of monoclonal B lymphocytes. It is considered to be identical to chronic lymphocytic leukemia (CLL) with similar pathologic and immunophenotypic features . Distinction is usually made based upon clinical presentation) and an arbitrary cut-off of 5x109/L lymphocytosis. *Objectives.* To perform a retrospective analysis of patients (pts) with CLL/SLL with emphasis on cytogenetic data and clinical outcome. *Patients and Methods.* We retrospectively reviewed our database of 201 SLL/CLL patients registered between 1980 and 2010. Two pts were excluded because of the diagnosis of Monoclonal Benign Lymphocytosis (MBL). All these pts fulfilled the diagnostic criteria for CLL/SLL i.e. > 5x109/L peripheral blood lymphocytes co-expressing CD5/CD19, CD23 and low levels of surface immunoglobulin by flow cytometry (B-CLL pts) or a typical immunohistochemistry on lymphnode biopsy showing diffusely effaced nodal architecture with an infiltrate composed of mostly mature-appearing, small lymphocytes with <5x109/L lymphocytes (SLL pts). A bone marrow aspirate and/or biopsy was performed in all pts. Mantle cell lymphoma, hairy cell leukemia, follicular lymphoma or Richter' syndrome were excluded. Caryotypic or FISH analysis was available in 45% of the pts. *Results.* Data are available on 196 pts (101 CLL Stage A, 41 CLL stage B, 27 CLL Stage C, and 27 SLL) 65% males with a median age of 65.4 years old. Overal survival(OS)/5 years was respectively 83.95%, 78.05%, 50.05%, 77.61% for Stage A-, B-, C- CLL and SLL. Imunophenotypic analysis (CD5, CD23, CD19, FMC7, ZAP70) was very similar in the four groups. CD38 expression appears to be lower in SLL but the size of the group does not allow any statistical comparison. This contrasts with the published data that Adherence to the stroma increases CD38 expression. Mutational status of the IgVH was rarely performed in our population of SLL but mutated and unmutated status are observed . Cytogenetic analysis was performed in 20 out of 27 SLL and only 3/20(15%) had 13q deletion whereas 7/20 (35%) pts had a poor prognostic caryotype (17p del or 11q del). Therapeutic approaches varied considerably; a majority of the SLL pts were treated by "lymphoma"regimens but fludarabine-based protocols became more frequently used in recent years. *Conclusion*. SLL and B-CLL are very similar in terms of phenotype and histology. OS of SLL is equivalent to OS of stage B CLL and should thus be managed such as progressing stage B-CLL taking into account the somewhat higher incidence of17p del.

0110

PHASE I-II STUDY OF BORTEZOMIB IN COMBINATION WITH R-HCVAD AND R-METHOTREXATE/ CYTARABINE (R-MA) IN UNTREATED MANTLE CELL LYMPHOMA (MCL)

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Although 87% of patients with advanced MCL achieve a CR with R-HCVAD/R-MA, patients still relapse over time, especially in those > 65 yrs of age, who have a median time to failure free survival of 3 years. Bortezomib (B) has 31% single agent response rate in relapsed/refractory MCL and synergizes *in vitro* with many of the drugs in the above regimen. A phase I study of BR-HCVAD/BR-MA did not show increased toxicity (Br. J. Haem. August 2010) and resulted in an elected dose of 1.3 mg/m2 bortezomib. We present preliminary data on the phase II study. Thirty-two patients have been entered, and their clinical presentation is shown in table 1.

Table 1. Patient characteristics.

Number patients 32
Median age (range) 64 (39-75)
Male 22 (69%)
Blastoid variant 1 (3%)
Ann Arbor stage IV 32 (100%)
Median ß2 microglobulin (range) 3 (1.59-9.7)
MIPI score low, int, high 37%, 44%, 19%
Ki-67 (17 pts) < 20, 21-40, > 40 46%, 35%, 18%

All 23 evaluable pts for response have responded: 12 have finished treatment (3-8 cycles, median of 6), of whom 9 have achieved a CR and 3 patients achieved a partial response. These 3 patients were responding but had delays in recovery of platelet counts and either were removed from study as per protocol guidelines (2 pts, ages 52 and 54 years, both with MIPI score of 3) or progressed while awaiting recovery (1 pt, 65 yrs old, MIPI score 5). At a median follow up of 5 months only this last patient has relapsed/progressed while on the study. When combining Phase I and II patients who received a similar dose of bortezomib of 1.3 mg/m2 and completed therapy (20 patients), 100% of the patients have responded, 89 % have achieved a CR, and only one has progressed with a median follow up of 12 months for the combined group. Grade 3-4 toxicity was mainly hematologic, as expected, and one patient died from neutropenic infection with methicillin-resistant staphylococcus aureus bacteremia. She did not have prolonged neutropenia. Seven patients went off study due to delayed recovery of counts, usually after cycles 3-5, five of them after achieved a CR (currently all five remaining in CR). There was no grade 2-4 neuropathy. In conclusion, this phase II portion of the study demonstrates continued high rates of complete remission. The study is currently accruing and updated information will be presented at the time of the symposium.

0111

SIMPLE CLINICAL AND LABORATORY FEATURES HAVE PROGNOSTIC VALUE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND 17P DELETION

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Background. Patients with chronic lymphocytic leukemia (CLL) whose tumor cells harbor a 17p deletion are considered to have a dismal prognosis. The disease is usually refractory to conventional chemotherapy and risky therapeutic approaches, such as allogeneic hematopoietic cell transplantation, are generally recommended for younger patients. However, there is a degree of clinical heterogeneity within 17p-deleted CLL patients, as a significant proportion of patients remain asymptomatic for prolonged periods of time. Furthermore, 17p deletions can be detected at diagnosis (de novo) but can also be acquired during the evolution of the disease (acquired), particularly in patients who have received chemotherapy. *Aims.* To further define the prognostic profile of CLL patients with either de novo or acquired 17p deletions. *Methods.* Clinical and laboratory data were collected from 207 CLL patients at the time the 17p deletion was detected. All patients had interphase fluorescent in-situ hybridization (FISH) tests performed using probes specific to *TP53* (17p13.1), *ATM* (11q22.3), *D13S319*

(13q14.3) and the centromeric region of chromosome 12 (12p11.1-q11). Minimum data required were age, sex, Binet stage, lymphocyte count, presence of B symptoms, date of FISH analysis, percentage of cells with 17p deletion, time to first therapy (if required) and last follow-up visit. A significant proportion of patients also had information on IGHV status, CD38 and ZAP-70 expression and beta$_2$-microglobulin serum concentration. *Results.* We identified 103 (51%) patients with de novo and 64 (32%) patients with acquired 17p deletions (i.e. not present at CLL diagnosis). In 35 (17%) patients, FISH results were only available after therapy. Sixty-four percent were male and 36% female. Median age was 68 (range 22-99) years and Binet stage was B or C in 56% of patients. Additional FISH abnormalities were detected in 49% (13q-), 17% (+12) and 13% (11q-) of patients. CD38 and ZAP-70 expression was positive in 45% and 43% of patients, respectively, while the IGHV gene was unmutated in 66% of them. Median overall survival (OS) from FISH diagnosis was 37 months for the entire cohort. By univariate analysis, OS was significantly shorter in patients older than 65 years (P = 0.008), with B symptoms (P < 0.001), an acquired 17p deletion (P = 0.023), a beta$_2$-microglobulin concentration higher than 2.5 mg/l (P = 0.002), more than 20% of cells with deletion (P < 0.001) and Binet stage B or C (P = 0.002). Cox regression analysis revealed that four variables had independent prognostic value: Binet stage (P = 0.037), B symptoms (P = 0.003), age (P = 0.017) and percentage of cells with deletion (P = 0.016). *Conclusions.* The prognosis of CLL patients with a 17p deletion is modulated by simple and widely available clinical and laboratory features such as Binet stage and the presence of B symptoms. Patients with acquired deletions had a shorter median OS compared to those with de novo deletions (32 vs 39 months, P = 0.023), but only by univariate analysis. These factors could help clinicians when deciding the most appropriate therapeutic approach for an individual patient.

0112

TP53 MUTATIONS AND OUTCOME WITHIN THE CLL2O TRIAL OF THE GCLLSG AND FCGCLL/MW: AN INTERIM ANALYSIS

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The multicenter CLL2O trial aims at achieving a higher remission rate in "ultra-high risk"CLL [17p- and/or Fludarabine(F)- or Bendamustine (Benda)-refractory] by adding high-dose dexamethasone to alemtuzumab and prolongation of remission duration and survival by alemtuzumab maintenance or allogeneic stem cell transplantation (SCT). Ninety [F-/Benda-refractory (n=40); 17p- without prior therapy (n=31) and 17p- in relapse (n=19)] enrolled pts were analysed for TP53 mutations. We used DHPLC to screen for TP53 mutations (Exons 4-10). Aberrant DHPLC profiles lead to sequencing of the respective amplicons. In addition, detailed characterization of prognostic markers and clinical features were performed. Median follow up time was 5.32 months. The incidence of the TP53 mutations was 75.5 % (68/90). For 81 of 90 patients FISH data was available: Fifty-nine of 65 patients (90.8 %) with 17p- had a TP53 mutation. The 16 F-/Benda-refractory pts without 17p- showed the following genetic features: TP53 mutation 3/16 (18.7%); 11q- 6/16 (37.5%); 13q- single 3/16 (18.7%); no abnormality 2/16 (12.5%); other chromosomal aberrations 2/16 (12.5%) and unmutated IGHV-status 12/16 (75%). In the 17p- 1stline cohort, 25 of 31 pts (80.6%) also had a TP53 mutation. All 19 relapsed pts with 17p- showed a TP53 mutation (100%). In the F-/Benda-refractory cohort 60% (24/40) had a TP53 mutation. Four of 11 pts analysed over time (2 to 6 years between analyses) acquired TP53 mutations. All of them received cytotoxic therapy before the acquisition of TP53 mutation. Five of the remaining 7 pts already had a TP53 mutation at first analysis with 3 showing increase in TP53 mutated clone size. IGHV-status was available for 82/90 patients. The vast majority 72/82 (87%) exhibited an unmutated IGHV-status with no differential distribution among TP53 mutated and TP53 wild type pts (TP53 WT). There was no significant difference between the TP53 mutated and unmutated subgroup concerning the distribution of the following markers and clinical features: gender, age, WBC, B-symptoms, ECOG performance status, ß2-microglobulin and thymidine kinase. Response data was available for 57 pts. There was no significant difference for complete and overall response rate (ORR) between TP53 WT and TP53 mutated pts. [CR: TP53 WT 0/15 (0%) vs. TP53 mutated 6/42 (14.2%) p-value: 0.3245; ORR: TP53 WT 10/15 (66.6%) vs. TP53 mutated 35/42 (83.3%) p-value: 0.2669]. For the 90 evaluated patients median progression free survival (PFS) was 13.9 months and median overall survival (OS) was 17.7 months. No significant difference in PFS between the TP53 mutated and TP53 WT subgroup was detected (TP53 WT: 8.8 months vs. TP53 mutated: 13.9 months; p-value: 0.801). OS was similar in both subgroups with 18.7 months for TP53 WT and 17.7 months for TP53 mutated cases. In conclusion we show a high incidence of TP53 mutations in this "ultra-high risk"CLL cohort. This data, showing no significant difference between TP53 mutated and unmutated cases in clinical endpoints, suggests that the CLL2O treatment algorithm might be an effective strategy for TP53 mutated cases and can overcome their refractoriness to therapy. Refractory pts without detectable TP53 alterations are of importance for further studies.

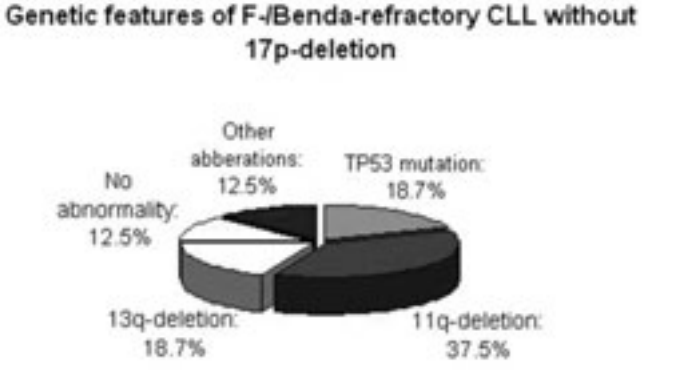

Figure 1.

0113

2SPONTANEOUS HEMATOLOGICAL REGRESSION IN CLL: LESSONS FROM THE ISRAELI CLL REGISTRY.

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Spontaneous regression in CLL is characterized by documented decrease in tumor burden not related to specific treatments. Cases of CLL regression have been occasionally documented. In the data of the Medical Research Council CLL trials, Thomas *et al.* reported a series of 10 patients who showed spontaneous remission out of 2370 (0.42%) registered cases (Br J Haematol, 2002, 116, 341). Recently Del Giudice *et al.* reported also the clinical and biological features (Blood, 2009, 114, 668) in 9 cases and found an overexpression of VH 3-30 and DICER gene by micro-RNA. An Editorial on this issue emphasized this 'unfolding CLL mystery' (Montserrat E). We analyzed the incidence of hematological remission in a large group of patients included in the Registry of the Israeli Study Group on CLL. Out of 1296 patients registered with CLL, 21 patients (1.6%) experienced spontaneous remission during the course of their disease. There were 10 women and 11 men, with median age at diagnosis of 69 years (range, 46-87). All but one were diagnosed in clinical Binet stage A, the remainder had en-

larged spleen compatible to stage Binet B. On flow cytometry, the median CD19/CD5 antigen coexpression on circulating CLL lymphocytes was 50%. Serum b2-microglobulin values were normal and mildly elevated in two patients; in these early stages no additional prognostic parameters were evaluated. The median follow-up period was 12 years (3-23 years). The regression was noted after a median of 8 years (range 1-22 years) of the follow-up. The maximal median lymphocyte count during the course of CLL was 15x109/l (9-75) and at regression dropped down to 2.3x109/l (1.2-4.7). The median CD19/CD5 antigen coexpression at regression was 28% (range, 17-37%). Distribution of T-cell subset during regression didn't reveal significant changes. A possible association of this phenomenon in CLL with infection, smallpox vaccination or development of second malignancy has been previously suggested. In order to search associated diseases or events that may explain changes in the hematological condition, we reviewed medical reports of the patients. Eight patients suffered from recurrent infections, which required multiple hospitalizations and antibiotic treatments. One another patient developed ischemic heart disease and was treated by verapamil. This drug by itself was found to induce apoptosis in CLL (Berrebi *et al.*, Leukemia, 1994, 8, 2214). One patient developed the regression three years after being treated for autoimmune hemolytic anemia. Two patients had hypothyroidism treated with eltroxin. Finally, three patients showed hematological improvement while developed solid tumors (brain, lung and nasopharynx). In 7 patients no concomitant diseases at time of regression were documented. In conclusion, spontaneous remission in CLL is a rare phenomenon developed usually in early stage with no bad biological prognostic factors. Concurrent disorders such as infections and/ or second malignancy may contribute to decrease CLL clone.

0114

ANALYSES OF PARAMETERS WITH INFLUENCE ON RATE AND DURATION OF RESPONSE AFTER THE FIRST-LINE TREATMENT OF PATIENTS WITH HAIRY CELL LEUKEMIA

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Background. For many years the first line treatment for hairy cell leukemia (HCL) were splenectomy and alpha interferon (INF-α). Major breakthrough in the treatment of HCL has appeared with introduction of purine analogs, which improved the long-term outcome. It seems that the dilema about the first line treatment was solved but there are still patients (pts) with HCL who need to receive more than one treatment for inducing the long-lasting clinical remission. *Aim.* The aim of our retrospective study was to identify the clinical and laboratory parameters which have influence on response rate after the first-line treatment of pts with HCL. *Patients and Methods.* We analysed 102 HCL pts who were diagnosed and treated in our clinic from January 1993 to December 2009, with a median follow-up of 87 months. We compared age, clinical and laboratory parameters, immunohistological characteristics of bone-marrow (BM) with response rate to therapy. Two groups of HCL pts were divided according to number of treatment-lines received to achieve remission: Group A included 48 pts who received the first and only-line treatment, Group B included 50 relapsed pts treated with 2 to 6 lines. Four pts were followed up only, without any treatment. In Group A: 38 pts received 2-chlorodeoxyadenosine (2-CDA), 4 INF-α and 6 had splenectomy, resulting in 77.1% CR, 16.7% PR and 4.2% non-response (NR). In Group B: 16 pts received 2-CDA, 1 deoxycoformycin, 12 INF-α and 21 had splenectomy resulting in 18% CR, 70% PR and 12% NR. Median duration of response in Group A was 49 months while in a Group B was 11 months ($p<0.001$). *Results.* Median age in Group A was 56 (M/F=41/7) while in Group B was 50 (M/F=42/8) ($p=0.01$). According to laboratory parameters, pts in Group B had significantly lower hemoglobin level (Hb≤120g/L) than pts in Group A (Hb>120g/l) ($p=0.01$). Number of leukocytes, platelets and the presence of lymphadenopathy and hepatomegaly were not statistically different between analysed groups. Patients in Group B had the statistically larger diameter of the spleen (≥15cm) than the pts in Group A (<15cm) ($p=0.023$). A statistically significant difference in response rate was recorded for 2 of 5 BM parameters tested: infiltration >50% hairy cells (HC) and degree of fibrosis >II/III. Patients in Group B had the statistically higher BM infiltration with HC ($p=0.027$) and larger degree of fibrosis ($p=0.045$) than pts in Group A. Patients in Group A treated with 2-CDA had the significantly higher CR rate than pts in Group B ($p<0.001$). *Conclusions.* Our data indicate that Hb <120g/L, spleen size>15cm, BM infiltration >50%HC, fibrosis >II/III have unfavorable prognostic value for the rate and duration of response. Patients with these parameters as the reflection of the larger tumor burden have to receive more than one treatment lines. Additional research of biological parameters in HCL is needed to determine the optimal treatment strategy for this disorder.

0115

SERUM HSP 70 ANTIBODIES AS AN INDICATOR FOR TREATMENT IN CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS

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Background. Chronic lymphocytic leukaemia (CLL) is the most common form of adult leukaemia in Western countries. The diagnosis is often accidental and there is no indication for early intervention. The current recommendation to start treatment includes disease-related symptoms, massive and/or progressive hepatosplenomegaly or lymphadenopathy, increasing bone marrow failure, autoimmune disease, and recurrent infections. The early treatment may be considered only in patients with unfavourable prognostic factors at the diagnosis. Heat shock proteins (HSPs) are molecular chaperones involved in a number of cellular functions in stress conditions. It is well established that leukemic cells may express HSPs including HSP70 on their surface. 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. Anti HSP70 antibodies are known to play a role in immunological and neoplastic processes. However their significance in CLL has not been well documented. *Aims.* The aim of our study was the assessment of the anti HSP70 antibody concentration in the patients with CLL. *Material and Methods.* The studied group consists of 38 newly diagnosed CLL patients (aged 46-74), males and females in equal proportion. The patients were in A-C stages of CLL according to Binet scale. Twenty two of studied individuals required chemotherapy. 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 analysis was done using Shapiro-Wilk, Mann-Whitney and Spearman's tests. *Results.* The levels of anti HSP70 antibodies were significantly lower in the group of patients that required chemotherapy in comparison to those in whom "watch and wait"strategy was applied (61.17±12.6ng/ml vs.118.86±27.8ng/ml; $p<0.04$). There was no association between the levels of antibodies and the stage of the disease. The study showed no correlations between anti HSP 70 antibody concentration and other parameters such as age, gender and some prognostic factors (LDH, ß2-microglobulin). *Conclusions.* The assessment of low anti HSP70 antibody concentrations in CLL patients may support other clinical and laboratory features that indicate starting of chemotherapy in this cohort. Further studies are required to elucidate the role of anti HSP70 antibodies as a prognostic marker in CLL patients.

Chronic myeloid leukemia - Biology

0116

EVI1-REARRANGEMENTS AND HIGH EVI1 EXPRESSION ARE FREQUENT IN CHRONIC MYELOID LEUKEMIA (CML) IN BLAST CRISIS (BC)

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Blast crisis (BC) is the terminal phase of chronic myeloid leukemia (CML). Activation processes of oncogenic factors and/or mutations leading to loss of function of tumor suppressor genes in hematopoietic stem cells are involved in disease progression. MECOM (MDS1 and EVI1 complex), located on chromosome band 3q26, functions as oncogene by transcriptional regulation binding to DNA sequences in the promoter region of target genes and further interacts with epigenetic regulators, e.g. DNMT3A. An elevated EVI1 expression is observed in myeloid malignancies with 3q26 rearrangements, some t(11q23)/MLL-rearrangements, monosomy 7/7q-, and also in a subset of acute myeloid leukemia (AML) with normal karyotype or other chromosomal abnormalities. Elevated EVI1 expression is associated with poor prognosis in CML and AML. In AML, elevated EVI1 expression has been described in the subset with 3q26/EVI1-rearrangements (1% frequency; Grimwade *et al*. 2010). Here, we studied a cohort of 34 BC-CML (21 myeloid; 8 lymphoid; 4 not specified). All patients had been thoroughly characterized by cytogenetics and FISH to identify EVI1-rearrangements. The proportion of EVI1-rearrangements (inv(3)(q21q26) n=3; t(3;21)(q26;q22) n=2; t(3;11)(q26;q23) n=1; other 3q26 rearrangements n=2) detectable in this cohort was remarkably high with 23.5% (8/34 cases). Of note, two EVI1-rearrangements were cytogenetically cryptic and therefore only detectable by FISH analysis. Next, we investigated the EVI1 expression using a quantitative real-time RT-PCR assay (LightCycler®, Roche Diagnostics, Mannheim, Germany). The expression of MDS-EVI1/EVI1 was normalized against the expression of the control gene ABL1 (values are given as % MDS-EVI1/EVI1/ABL1). In our BC-CML cohort, the median EVI1 expression was 16.5, ranging from 0-107.6 (no RNA available in 4 cases). EVI1-expression was significantly higher in cases with EVI1-rearrangement (8/34 cases) compared to patients without rearrangement; median expression 40.4 (range: 18.5-93.3) vs. 9.1 (range: 0-107.6; p=0.003). Interestingly, in both patients with lymphoid BC a high EVI1 expression with a ratio of 29.0 was detected. Subsequently, patients with detectable EVI1 expression (22/30) were further investigated for possible t(11q23)/MLL-rearrangements using FISH. However, we did not detect any t(11q23)/MLL abnormality in this subgroup. In addition, WT1 mutations were detected in 6/34 patients (17.6%). The frequency was significantly higher in patients with EVI1-rearrangement (4/8; 50%) compared to those without (2/26; 7.7% p=0.02). Of note, we sequenced ASXL1, CBL, CEBPA, IDH1, IDH2, IKZF1, KRAS, NRAS, NPM1, TET2, TP53 and investigated the BCR-ABL1 ratio for the complete cohort. Besides EVI1-rearrangement no correlation between an elevated EVI1 expression and any other molecular or cytogenetic marker was observed. Limited on cases without MLL- and EVI1-rearrangements (n=26), the median EVI1 expression in BC-CML patients was comparable to AML patients, based on a cohort of 260 AML cases we previously studied (mean 14.7 vs. 9.9, p=n.s.). Moreover, for patients with EVI1-rearrangements (n=8) the EVI1 expression in BC-CML is similar to patients harboring an AML with EVI1-rearrangement (n=57) (mean 49.1 vs. 175.3, p=n.s.). In conclusion, we demonstrated that EVI1-rearrangements occurred with a high incidence of 23.5% and both translocation and overexpression of EVI1 may play a central pathogenetic role in BC-CML. Further analyses are warranted to assess the clinical impact of these findings.

0117

THE OLIGOMERIZATION AND DC2 DOMAINS OF THE BCR PROTEIN MODULATE ITS SUBCELLULAR LOCALIZATION AND REGULATE BCR-ABL TRANSFORMING ACTIVITY

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Background. The Breakpoint Cluster Region (BCR) protein is implicated in the pathogenesis of Chronic Myeloid Leukemia (CML) as its N-terminus portion is fused in frame with most of the ABL sequence, generating the BCR-ABL chimaeric oncoprotein. To date, the intracellular localization of BCR is still controversial as previous evidence has suggested that BCR binds to heterochromatin, implying a nuclear localization. However, further results assigned BCR exclusively to the cell cytoplasm. *Aims.* To investigate the mechanisms regulating the subcellular localization of BCR and to determine if and how they modulate the localization and function of BCR-ABL. *Methods.* Computational analyses recognized two putative Nuclear Localization Signals (NLS) and a Nuclear Export Signal (NES) in BCR. The functional activity of these domains was assessed by generating GFP-fusion constructs and by determining their intracellular distribution by immunofluorescence (IF) and fractionation experiments. Multiple BCR and BCR-ABL deletion mutants were then transiently expressed in HeLa cells or in the mouse pro-B BaF3 cell line and their intracellular localization was determined by IF. BaF3 cells expressing different BCR-ABL mutants were cultured in liquid media (in the absence of IL-3) or in soft agar to investigate their transforming ability. The proliferative potential of each BCR-ABL construct was also ascertained *"ex vivo"* after lentiviral expression in CD34-positive cells. *Results.* Both NLSs directed GFP in the nuclear compartment, while the NES favored its cytoplasmic localization. The latter effect was abolished after treatment with the exportin-1 inhibitor Leptomycin B (LMB). Despite these findings, wild-type BCR was an exclusively cytoplasmic protein because of a lack of nuclear import. However, removal of the N-terminus oligomerization domain (OD), of the C-terminus Protein Kinase C conserved region 2 (DC2) and of the Rho-GAP domain generated a construct displaying strong nuclear staining that was further increased by LMB. As the OD and part of the DC2 are preserved in BCR-ABL, we wanted to establish if these domains contributed to the subcellular localization of the oncoprotein. To this end, we generated a ΔDO-ΔDC2 BCR-ABL mutant and its kinase-proficient counterpart ΔDO-ΔDC2 BCR-ABLP1124L. Each construct was transiently expressed both in HeLa and in BaF3 cells but failed to relocalize to the nucleus. Interestingly, both mutants were devoid of transforming activity as confirmed by expression in CD34-positive progenitors. *Conclusions.* Our results have identified two putative NLS and one potential NES in the BCR sequence. The NLSs are functionally/structurally inhibited by the OD, DC2 and Rho-GAP domains of BCR, causing its cytoplasmic localization. Deletion of the OD and DC2 regions in BCR-ABL fails to relocalize the oncoprotein to the nuclear compartment, regardless of its catalytic activity. However, a kinase-proficient ΔDO-ΔDC2 BCR-ABL does not display transforming potential, suggesting that the DC2 domain plays a pivotal role for BCR-ABL-dependent oncogenic activity.

0118

TRITERPENOID CDDO-ME IS HIGHLY SYNERGISTIC WITH THE HO-1 INHIBITOR SMA-ZNPP IN IMATINIB-RESISTANT CML CELLS

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Background. In chronic myeloid leukemia (CML), resistance against one or more tyrosine kinase inhibitors (TKI) may develop during therapy, often because of BCR/ABL1 mutations. Novel therapeutic strategies and drugs are required to overcome TKI-resistance and to achieve long term remission in these patients. CDDO-Me is an oleanane triterpenoid that has been described to suppress survival of neoplastic cells in various tumors and leukemias by targeting several pro-survival factors including mTOR, Akt, and STAT3, and by inducing ROS-generation and thereby apoptosis. However, not all survival pathways may be suppressed by CDDO-Me. Notably, recent data suggest that exposure of leukemic cells to CDDO-Me results in a pronounced increase in heme-oxygenase-1 (HO-1), a major survival and resistance factor in CML. *Aims.* The aim of this study was to explore whether CDDO-Me exerts effects on imatinib-resistant leukemic cells carrying BCR/ABL1 mutations, and to learn whether the HO-1 inhibitor SMA-ZnPP would synergize with CDDO-Me in producing growth inhibition and apoptosis in CML cells. *Methods and Results.* As assessed by 3H-thymidine uptake, CDDO-Me was found to inhibit the proliferation of the CML cell line K562 as well as Ba/F3 cells transfected with drug-resistant mutants of BCR/ABL (T315I, E255K, Y253F, H396P) with reasonable IC50 values (0.1-0.5 μM). Growth-inhibition was accompanied by apoptosis as assessed by combined AnnexinV/PI staining. Next, we examined the effects of CDDO-Me on primary CML cells isolated from 12 CML patients in chronic phase, including 4

patients who had developed resistance against two or more TKI. CDDO-Me was found to inhibit proliferation of leukemic cells in all patients tested, with IC50 values ranging between <0.1 and 0.5 μM. No differences in IC50 values were observed between TKI-naïve and TKI-resistant CML cells. Since BCR/ABL-targeting TKI or other drugs, when used as single agents, usually fail to induce long-term remission in advanced CML, drug combinations are currently being tested preclinically and in clinical trials. In this study, we applied the combination CDDO-Me+SMA-ZnPP and found that this combination is highly synergistic in producing growth inhibition in K562 cells and primary CML cells isolated from imatinib-naïv and imatinib-resistant patients, including one patient in whom BCR/ABL T315I was detected. We also examined whether CDDO-Me would exert synergistic effects on CML cells when combined with BCR/ABL TKI. We therefore applied the combinations CDDO-Me+dasatinib and CDDO-Me+nilotinib in K562 cells. Both combinations were found to synergize in producing growth inhibition. *Conclusions.* CDDO-Me inhibits the proliferation of imatinib-resistant BCR/ABL+ cell lines and of primary CML cells isolated from untreated and TKI-resistant patients, including cells carrying the BCR/ABL mutant T315I. Our data also show that CDDO-Me and BCR/ABL TKI synergize in producing growth inhibition in CML cells. Synergistic drug interactions were also observed with CDDO-Me+SMA-ZnPP which may be explained by the HO-1-inducing effect of CDDO-Me.

0119

IS P210 PHILADELPHIA POSITIVE-ACUTE LYMPHOBLASTIC LEUKEMIA A LYMPHOBLASTIC CRISIS OF LATENT CHRONIC MYELOID LEUKEMIA?

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Background. The Philadelphia (Ph) chromosome is a hallmark cytogenetic abnormality in chronic myeloid leukemia (CML), and is also present in ~25% of adult acute lymphoblastic leukemia (ALL). Although the p210 BCR/ABL isoform is predominant among CML patients, about 50% of adult Ph+ALL have p210 and the remainder harbor the p190 isoform. If left untreated, CML invariably progresses to a blastic phase, which is typically of myeloid lineage. However, in ~30% of CML cases the blasts are of lymphoid lineage. In the absence of documented chronic phase CML, this lymphoblastic presentation poses a diagnostic challenge to differentiate from de novo Ph+ALL. Neutrophil-FISH (N-FISH) using peripheral blood cells for CML diagnosis and/or to monitor imatinib response is widely available in Japan. Importantly, N-FISH also identifies the Ph chromosome lineage to neutrophils in some patients with ALL. *Aims and Methods.* To assess the clinical differences between p210- and p190-ALL, a retrospective chart review was performed for cases of Ph+ALL in the Tohoku Hematology Forum study group between October 2000 and September 2010. Eighty one Japanese patients with Ph+ALL were enrolled in this study. The data reviewed included diagnosis, treatment, hematologic parameters, cytogenetic characteristics, and clinical outcome. N-FISH was performed at the time of diagnosis in 24 cases (p210, n=11; p190, n=13). *Results.* No definite clinical differences were identified between p210-ALL (n=25) and p190-ALL (n=56). However, similar to what is observed in CML, N-FISH identified BCR-ABL positive neutrophils in 91% (10 of 11) of p210-ALL (average 74.4%). In contrast, BCR-ABL positive neutrophils were only found in 38% (5 of 13) of p190-ALL (average 61.3%) which was statistically different than p210-ALL (p=0.008 by Fisher's test). All Ph+ALL cases were then subdivided based on N-FISH results: "*secondary* ALL", with Ph chromosome myeloid lineage involvement (n=15); and "*de novo* ALL", without myeloid lineage involvement (n=9). Using this classification, additional features were identified that significantly differed between the two subgroups (secondary ALL vs. de novo ALL): laboratory parameters (platelets: 95x10(3)/μl vs. 26x10(3)/μl, p=0.005; basophils: 0.56% vs. 0%, p=0.041), immunophenotypes (CD13+: 12/15 vs. 2/9, p=0.005; CD33+: 11/15 vs. 1/9, p=0.003), and percentage of residual normal clones (6/15 vs. 8/8, p=0.005). *Conclusion.* N-FISH revealed myeloid lineage involvement in "*secondary* ALL", which was largely comprised of p210-ALL cases. As this group resembled CML in several other clinical features, these data suggest that "*secondary* ALL"may represent CML presenting in lymphoblastic crisis. During a latent phase prior to the clinical onset of CML, Ph chromosome patients could acquire genetic abnormalities to transform to lymphoblastic crisis. Despite the great advances in CML treatment with the development of imatinib and other tyrosine kinase inhibitors, the latent phase of CML is still poorly understood and may have the potential to progress to p210-ALL in addition to clinically overt CML.

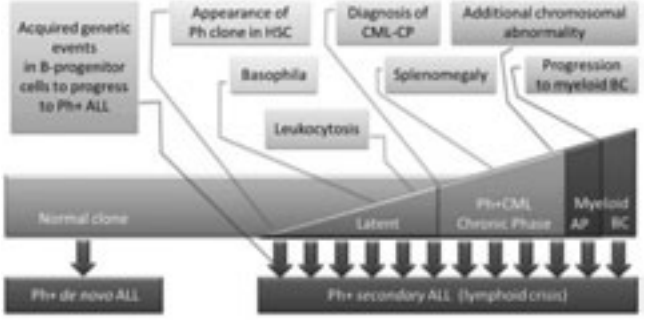

Figure 1. Ph+ secondary ALL and Ph+ latent CML.

0120

REGULATION OF CELL CYCLE IN PRIMITIVE CHRONIC MYELOID LEUKAEMIA (CML) VS NORMAL STEM CELLS

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Background. Chronic myeloid leukaemia (CML) is a clonal haemopoietic stem cell (HSC) disorder associated with the Ph chromosome and BCR-ABL oncogene, encoding a constitutively active tyrosine kinase. *Aims.* We hypothesise that the resistance of CML stem cells to chemotherapy is dependent on pathways that are deregulated in malignancy, offering the possibility of developing therapeutic approaches that are selective for leukaemic versus (vs) normal HSC. *Methods.* We have investigated the transcriptional differences between quiescent (G0) normal and CML stem cells by carrying out RNA and miRNA profiling. Biochemical analysis was carried out by flow cytometry and western blotting. *Results.* G0 CD34+ haemopoietic cells were isolated from normal controls and patient samples and gene profiling revealed differences in the expression of cell cycle genes, including CDC20, CHEK1, CDKN3, CDC27, CDC2, CDC6 and Cyclin B2. Interestingly, E2F1, the communal transcription factor for all the genes we found differentially expressed, was abnormally regulated in CML and it is likely that, together with is regulator Rb, E2F1 is a key player in modulating CML stem cell quiescence. E2F1 was upregulated in CML CD34+ cells compared to normal, at both the protein and mRNA level. Furthermore, its activation in these cells was confirmed by the high level of phosphorylated Rb. To understand if miRNAs have a role in CML stem cell survival and in particular in the regulation of the E2F1/Rb pathway, we carried out miRNA arrays. CD34+38- cells isolated from normal and patient samples were used to perform genome-wide miRNA expression profiling (LC Sciences www.lcsciences.com). Initial data analysis has provided several potential candidates which are under investigation. *Summary/Conclusions.* These data suggest that the E2F1/Rb signalling pathway may have a role CML stem/progenitor cells cycle status and contribute to the understanding of CML stem cell quiescence, suggesting new strategies to target CML stem/progenitor cells by preventing or reversing these effects. In addition, enhanced knowledge of the survival pathways that are operative within CML stem cells, which may lead to eradication of this critical population, would be of relevance to other forms of cancer.

0121

REDUCED EXPRESSION OF THE LTB4 MEMBRANE RECEPTOR BLT1 EXPLAINS ALOX5 DOWN-REGULATION IN NEWLY DIAGNOSED CML PATIENTS

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Background. Alox5 has been reported to be upregulated in mouse leukaemic stem cells (LSC), and this is not inhibited by imatinib. Furthermore, mice transplanted with Alox5 deficient BCR-ABL1+ bone marrow cells were resistant to the induction of chronic myeloid leukaemia (CML).(1) Alox5 deficiency was found to have no effect on BCR-ABL negative cells. This suggests that Alox5 is important in LSC growth and is essential for the development of CML. *Aim.* To investigate the role of Alox5 in imatinib treated CML patients. *Methods.* Functional Alox5 was assessed using an LTB4 ELISA and Alox5 mRNA determined via a TaqMan gene expression assay. BLT1 protein levels were assessed by cell surface FACS analysis. *Results.* Alox5 expression was measured in 27 chronic phase patients at diagnosis and at 3, 6 and 12 months following imatinib treatment. At diagnosis Alox5 expression in all patients was below that seen in samples from normal controls. On treatment initiation Alox5 levels increased to normal values in all patients except those who were destined to progress to blast crisis (BC) where levels remained low. All increases in Alox5 levels were statistically significant, diagnosis and 3 months (p=0.003), 6 months (p=<0.001), and 12 months (p=0.024) in patients who subsequently achieve a CCR. Plasma LTB4 levels were utilised as a measure of Alox5 function. In all CML patients LTB4 levels were higher than that measured in samples from normal controls, and all patient levels increased further on imatinib treatment. Levels in BC were always higher compared to chronic phase. These data in clinical material are in conflict with previously published work utilising a mouse model, since we find that LTB4 levels are increased despite low Alox5 expression. To investigate this further we undertook determination of LTB4 receptor (BLT1) levels. BLT1 surface expression was found to be extremely low in newly diagnosed chronic phase CML patients. BLT1 protein in newly diagnosed patients was significantly lower than the level observed in patients responding to tyrosine kinase inhibitor (TKI) treatment (defined as a BCR-ABL1 ratio 1-10%) and those patients who achieved a CCR (p=0.004 and p=<0.0001 respectively). *Conclusion.* Alox5 expression is low and LTB4 levels are high in CML patients at diagnosis. This is likely the result of increased negative regulation of Alox5 via the arachidonic acid pathway intermediates (5-HEPTE and LTA4) and a lack of positive feedback through LTB4 due to the reduced expression of the BLT1 receptor. Clearly different pathways of Alox5 regulation exists between the CML mouse model and patients which should be considered when translating findings from the mouse model to a clinical setting.

0122

DYSREGULATION OF THE TUMOUR SUPPRESSOR PTEN IN CHRONIC MYELOID LEUKAEMIA

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Background. Chronic Myeloid Leukaemia (CML) is a myeloproliferative disorder characterised by the BCR-ABL1 fusion oncogene. Despite the uniformity of causation and therapeutic modality (Imatinib), patients exhibit heterogeneity in their response. PTEN (Phosphatase and Tensin Homolog) is a tumour suppressor gene, which codes for a phosphatase antagonist of the PI3K/AKT mitogenic axis. Down-regulation or loss of PTEN, through mutations, deletions and epigenetic silencing, has been described in many malignancies and is linked to disease progression. Although PTEN is known to be down-regulated in CML, the mechanism of repression is yet to be fully elucidated, but both promoter hypermethylation and dysregulation of the pseudogene PTENP1, which acts as a decoy for suppressive microRNAs, have been implicated. *Aims.* To investigate whether CpG hypermethylation and/or changes in PTENP1 pseudogene expression contribute to the down-regulation of PTEN in diagnostic CML, and to correlate expression with patient Sokal score and 12mth clinical response. *Methods.* Peripheral blood lysates from 32 chronic-phase CML patients at diagnosis and 30 normal volunteers were used to isolate DNA and mRNA. The DNA was bisulfite treated and used for High Resolution Melt analysis (HRM) and Pyrosequencing CpG methylation studies. Two CpG island were interrogated for methylation: one residing in the 5' UTR of PTEN by HRM, and the other 1.4kb upstream of exon 1 by Pyrosequencing. The mRNA was converted to cDNA and used for qPCR gene expression studies. The qPCR employed primers specific for PTEN and PTENP1, with B2M as the endogenous control. Expression levels were given as delta-CT relative expression ratios (RER). 16 CML samples were classified by Sokal score (low, intermediate & high risk) and by 12mth response to Imatinib (400mg/day), defined by attainment of complete cytogenetic response (CCyR). *Results.* PTEN was shown to be significantly under-expressed in CML compared with Normal samples (median RER 0.31 and 1.69, respectively; p<0.001); whereas PTENP1 was conversely expressed (RER 2.06 and 0.62; p=0.0013). Patients with a high Sokal score had a significantly higher PTEN expression than those in the low risk group (1.04 and 0.52, respectively; p<0.05), but there was no difference for PTENP1. There was also no significant difference in expression for either gene between the 12mth optimal and suboptimal response groups. Neither HRM nor pyrosequencing showed any evidence of CpG hypermethylation in any of the patient samples. *Summary/Conclusions.* Although loss of PTEN is known to be a marker of disease progression in many tumour types, in CML the dynamic appears to be more complex. It was consistently down-regulated at diagnosis, but patients with high-risk Sokal scores showed a higher relative expression; although caution must be taken when drawing conclusions from such a relatively small sample number. In addition, the pseudogene PTENP1 was found to be over-expressed in CML, weakening the case for its role as a decoy for suppressive microRNA as a mechanism in PTEN dysregulation. The epigenetic assays performed found no evidence of promoter hypermethylation, suggesting that PTEN down-regulation in CML occurs via a yet to be elucidated mechanism.

0123

SHEPHERDIN-DEPENDENT SUPPRESSION OF SURVIVIN INDUCED BY A BCR- ABL/JAK2/STAT3 PATHWAY SENSITIZES IMATINIB RESISTANT CML CELLS TO DIFFERENT DRUGS

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Background. The BCR-ABL oncoprotein of Chronic Myelogenous Leukemia (CML) displays exclusive cytoplasmatic localization and constitutive tyrosine kinase activity leading to the activation of different pathways that favor cell proliferation and survival. Treatment of CML cells with Imatinib Mesylate (IM) suppresses BCR-ABL kinase activity reverting the anti-apoptotic phenotype of the leukemic cells. Survivin (SVV) is a member of the inhibitor of apoptosis proteins that is expressed in most forms of human cancer causing reduced sensibility to multiple death stimuli. *Aims.* To ascertain the signaling pathway(s) triggered by the BCR-ABL kinase to induce SVV expression and determine whether reduced SVV levels increase the anti-proliferative activity of different cytotoxic compounds on IM-resistant CML cells. *Methods*. Lysates from K562, KCL22, KYO-1 and LAMA 84 CML cell lines exposed to IM, PD98059, LY294002, AG490 or incubated with siRNAs against JAK2 or antisense oligonucleotides against STAT3 were employed to identify BCR-ABL-dependent signaling pathways responsible for SVV induction. Viability of IM-resistant cells was assessed using the ATPlite luminescence assay and/or Annexin V-FITC/PI staining after SVV silencing or incubation with the peptidomimetic Shepherdin in association with IM, hydroxyurea (HU), Cytosine Arabinoside (Ara-C) or Doxorubicin. *Results.* Inactivation of BCR-ABL catalytic activity decreased SVV levels confirming that, in CML cells, SVV expression relies on BCR-ABL constitutive tyrosine kinase activity. BCR-ABL-mediated up-regulation of SVV involved the JAK2/STAT3 pathway since silencing of either JAK2 or STAT3 caused a consistent reduction of SVV. In cells unresponsive to IM, SVV silencing failed to restore sensitivity to the drug, indicating that SVV is not directly involved in the development of IM resistance. Cell lines unresponsive to IM because of point mutations in the BCR-ABL kinase domain were highly responsive to HU after down-regulation of SVV. However, this was not the case in cells failing IM because of BCR-ABL gene amplification. Finally, incubation of IM-resistant cells with cell-permeable Shepherdin, a peptidomimetic compound that down-regulates SVV expression by preventing its interaction with heat shock protein 90, enhanced cell death induced by IM, HU, Ara-C and Doxorubicin. *Conclu-*

sions. BCR-ABL kinase activity induces SVV expression in CML cells via the JAK2/STAT3 signaling pathway. Suppression of SVV fails to resensitize resistant cells to IM. However, the combination of Shepherdin with different cytotoxic drugs significantly increases the activity of these compounds on cells unresponsive to IM because of point mutations (including T315I) in the BCR-ABL catalytic domain.

0124

PROTEIN SIGNALLING TRIGGERED BY IMATINIB AND DASATINIB IN THE MYELOID BLASTIC CRISIS OF CML: AN IN VITRO PROTEOMIC AND PHOSPHOPROTEOMIC STUDY

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Background. Imatinib resistance (IM-R) developing in approximately 30% of Chronic Myeloid Leukemia (CML) patients (pts) recognizes both BCR-ABL-dependent and BCR-ABL-independent mechanisms. The former include point mutations in the BCR-ABL kinase domain and amplification of the BCR-ABL gene locus while the latter are still poorly understood, probably involving improper activation/inactivation of BCR-ABL-independent signaling pathways. *Aims.* We describe preliminary results of a study employing immortalized CML cells - either sensitive or resistant to IM - to perform Reverse Phase Protein Micro-Arrays (RPMAs) aimed at characterizing the proteomic profiles of these cell lines and at identifying BCR-ABL-independent pathways that contribute to the development of IM-resistant CML. *Methods.* RPMA is a reproducible, high-throughput system for protein signaling pathway profiling. The phosphorylation state of kinase-associated therapeutic targets provides direct information regarding the target and off-target effects of different drug treatments. Human CML cell lines, sensitive (K562S, LAMA84S) and resistant (K562R, LAMA84R) to Imatinib, were incubated with IM 1uM, Dasatinib (DAS) 1uM or LY-294002 10uM (LY) used as a control. After 2 or 12 hours, cells were placed in a preservative that suppresses fluctuations in kinase pathway proteins. RPMAs were used to quantitatively map 45 cell signaling pathway endpoints, including autophagy, DNA repair systems, DNA damage and transcriptional factors crucial for CML. *Results.* Previous evidence has demonstrated that K562R are unresponsive to IM because of unknown BCR-ABL-independent mechanisms, while LAMA84R display BCR-ABL genomic amplification. Compared to their sensitive counterpart, K562R exhibited a paradoxical reduction in BCR-ABL signaling at BCR(Y177), CRKL(Y207), ERK(T202/Y204) and Cofilin(D59), associated with over-expression of autophagy markers (ATG5 and LC3B). Conversely, LAMA84R presented increased BCR-ABL signaling at BCR(Y177), CRKL(Y207) and ERK(T202/Y204). In drug treatment endpoints, DAS was confirmed stronger than IM in abrogating BCR-ABL auto-phosphorylation on BCR(Y177). We found HDAC3 induction in both K562R and LAMA84R and this event was positively associated with increased expression of c-MYC and NUMB and higher phosphorylation on mTOR(S2445), pRb(S608), CHK1(S345), FOXO1(T24), FOXO3a(T32), but not on BCR(Y177) or c-ABL(Y245). *Conclusions.* Taken together, our data confirm the value of the RPMA assay to investigate improperly activated pathways that could identify one or more potential targets for future treatment strategies of IM-resistant pts.

0125

INHIBITION OF THE PTP1B PHOSPHATASE MEDIATES UBIQUITINATION AND DEGRADATION OF BCR-ABL

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Background. Chronic myelogenous leukemia (CML) is a myeloproliferative disorder characterized at the molecular level by the expression of Bcr-abl, a chimeric protein with deregulated tyrosine kinase activity. Expression of this oncogene is associated with an increase in activity of the PI3k/Akt kinase driven pathway which in turn contributes to tumour cell survival. The protein tyrosine phosphatase 1B (PTP1B) is upregulated in Bcr-abl expressing cells suggesting a regulatory link between the two proteins. In addition a direct interaction between these two proteins has been demonstrated, but the function of this interaction is unknown. *Aims.* The aim of this piece of research work is to investigate at a molecular level why and how Bcr-abl interacts with PTP1B and how this might affect tumour cell survival. *Methods.* TonB.210 cells kindly provided by Dr. George Daley (MIT, Cambridge, MA, USA) contain a Doxycycline responsive promoter whereby Bcr-abl p210 can be conditionally induced when incubated with the antibiotic. For PTP1B inhibition, cells were treated with 1 g/ml DOX for 48 hours and 35 M 3-(3,5-Dibromo-4-hydroxy-benzoyl)-2-ethyl-benzofuran-6-sulfonicacid-(4-(thiazol-2-ylsulfanyl)-phenyl)-amide (PTP1B inhibitor or PTP1B). Western blotting was carried out to look at Bcr-abl and PTP1B protein expression levels. siRNA, Negative Control #1 siRNA and two Silencer® pre-designed siRNA (Ambion, Warrington, UK) were used for silencing PTP1B over a 24/48 h incubation period. The siRNA chosen for PTP1B were #1 siRNA ID:s72430 and #2 siRNA ID:s72431. The sequences are available from the manufacturer website. Confocal microscopy was used to look at where in the cell Bcr-abl degradation occurred. 2D-SDS-PAGE was used to look at the phosphorylation status of Bcr-abl. *Results.* We describe a novel mechanism by which the phosphatase PTP1B is required for Brc-abl protein stability. When TonB.210 cells were treated with increasing concentrations of the PTP1B inhibitor or siRNA to PTP1B there was almost a complete loss of Bcr-abl expression at the protein level. A dose of 35 µM of the PTP1B inhibitor for 2 hours was optimal for reducing Bcr-abl protein levels. A similar result was demonstrated when siRNA to PTP1B was used over a 24/48 hour period. RT-PCR analysis demonstrated that there was no effect at the RNA level indicting that the loss of Bcr-abl was the result of protein degradation. Subsequent western blotting demonstrated that this loss is the result of Bcr-abl ubiquitination. Degradation occurs via a lysosomal pathway rather than the proteosome since the proteosomal inhibitor Lactacystin had no effect. Confocal microscopy confirmed this. 2D-SDS-PAGE demonstrated that the constant removal of a phospho group from Bcr-abl by an active PTP1B was necessary to maintain its stability. When PTP1B was inhibited or knocked down by siRNA Bcr-abl remained phosphorylated and this triggered its ubquitination and degradation. *Conclusions.* In this work, we describe a novel mechanism by which Bcr-Abl is stabilized by PTP1B. These results suggest that inhibition of PTP1B may be a useful strategy to explore in the development of novel therapeutic agents for the treatment of CML.

0126

STATINS CAN POTENTIATE ANTILEUKEMIC EFFECT OF IMATINIB THROUGH THE INHIBITION OF ABCB1 AND ABCG2 ACTIVITY

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Background. Despite clinical success of imatinib in the majority of patients, development of resistance becomes relevant clinical problem. Variations of intracellular concentration of tyrosine kinase inhibitors, dependable on the activity of drug transporters, are among major mechanisms responsible for development of resistance in the absence of BCR/ABL mutations. Intracellular concentration of imatinib results from the balance of drug influx mediated mainly by OCT-1 transporter and drug efflux mediated by ABC transporters. According to the recent knowledge, in clinical settings imatinib is a substrate of ABCB1 and ABCG2 transporters. Strong evidence suggest that agents modulating membrane cholesterol level, e.g. statins, are able to change conformation of membrane-bound drug transporters and increase intracellular imatinib concentration. *Aim of the study.* Potentiation of antileukemic activity of imatinib in cell lines transformed with BCR/ABL-1 oncoprotein and primary human CML CD34+ cells employing statins. *Methods.* The following cells were used in the study: primary CD34+ cells obtained after informed consent from the patients with CML as well as from healthy blood donors; cell lines: K-562, 32Dcl3 wild type and BCR/ABL-transformed. ABC transporters activity was evaluated using specific fluorescent substrates: BODIPY-prazosin (for ABCG2) and rhodamine 123 (for ABCB1) while direct imatinib efflux studies were performed using 14C-labelled drug. Imatinib-mediated cytotoxicity was analyzed using cell viability and apoptosis assays (XTT and propidium iodide staining). Clonogenic assay after drug treatment was performed. Expression of BCR/ABL-dependent proteins was assessed . *Results.* Cytometric analysis of ABC transporters activity has shown that lovastatin significantly decreased the activity of ABCB1 and ABCG2-mediated efflux capacity. The effect was completely reversed by the addition of cholesterol. It was observed both for stable cell lines and primary CD34+ CML cells. These results were confirmed using radiolabeled 14C-imatinib concentration measurement after incubations with

statins. Statins caused at least 2-fold increase in intracellular imatinib concentration in cell lines and primary CML CD34+ cells, including cells obtained from patients clinically resistant to imatinib with no detectable ABL kinase domain mutations (Figure 1). We did not observe changes in initial influx of the drug between statin-treated and control cells. Interestingly, statins did not influence the expression of ABC transporters. Lovastatin enhanced cytotoxicity of imatinib in BCR/ABL-positive cell lines and CML CD34+ primary cells as compared to wild-type counterparts and cells obtained from the healthy donors respectively. Similar effects were observed for other members of statin family. Flow cytometry analysis after propidium iodide staining revealed that co-treatment induced cell cycle arrest and increased percentage of apoptotic leukemic cells in comparison to imatinib alone. Lovastatin also increased imatinib-induced reduction of the phosphorylated form of the adaptor protein CrkL, which serves as a marker for efficient BCR/ABL kinase inhibition in both stable cell lines and primary CML CD34+ cells. *Conclusions.* Our results indicate that statins may enhance therapeutic efficacy of imatinib through the inhibition of drug efflux mediated by ABCB1 and ABCG2 transporters and may become potent treatment modality for the patients resistant to imatinib without mutations in BCR/ABL kinase domain.

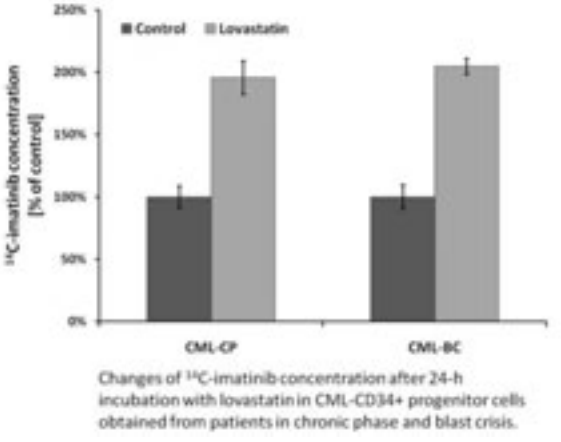

Figure 1. 14C-imatinib concentration after statins.

0127

OVER-EXPRESSED NUCLEOPHOSMIN/ B23 AND NUCLEOLIN C23 PREDICTED DISEASE PROGRESSION IN CHRONIC MYELOID LEUKEMIA

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Background and Aims. Nucleophosmin/ B23 NPM B23), or NPM1,also termed NO38 or numatrin, is an acidic nucleolar protein which belongs to the nucleoplasmin/nucleophosmin family of nuclear chaperones. B23 interacts with nucleolin C23, both are multifunctional nucleolar proteins and play important role in various aspects of ribosome biogenesis from transcription regulation to the assembly of pre-ribosomal particles. Compared to C23, NPM is much more investigated for its relation to cancer. The deletion and translocation of NPM gene have been observed in hematological malignancies. NPM1 mutations occur specifically in about 30% of adult de novo AML. However, NPM1 mutation and B23 protein expression were rarely reported in CML. Our previous study showed that proteins B23 and C23 were over-expressed in relapased/refractory acute leukemia patients. *Methods.* In order to investigate their clinical significances in CML, B23 and C23 expressions were detected by western blot and RQ-PCR in leukemia cell lines K562, K562/ADR (adriamycin-resistant K562), KG01 (imatinib-resistant K562) and 139 CML patients, including 106 chronic phase(CP), 13 accelerated phase(AP) and 20 blast crisis(BC). *Results.* Compared to healthy control samples, which showed no expressions of these 2 proteins, leukemic cell lines revealed an obvious up-regulation of B23 and C23. Moreover, significantly higher expressions of B23 and C23 were found in 2 resistant cell lines compared to the parent K562 cells. In primary CML samples, much higher expressions of B23 and C23 were noted in CML-BC than in CML-AP and CML-CP, which were 75%(15/20), 7.7%(1/13) and 12.3%(13/106) respectively. The concomitant expression of B23 and C23, both positive or negative, was noted in 96%(133/139) patients. No NPM1 mutations were found from 20 CML samples (10 B23+ and 10 B23-), which were not consistent to B23 protein expressions. B23 and C23 positivity significantly correlated with shorter OS and poorer prognosis. One patient became B23 positive as disease progressed from CP to BC. *Conclusions.* It could be concluded that B23 and C23 may be prognostic indicators for CML and be useful in predicting progression of CML. Further investigation is needed to explore their involved mechanisms in progression of CML.

0128

THE ROLE OF SPARC IN CHRONIC MYELOID LEUKEMIA

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Background. Secreted protein acid and rich in cysteine (SPARC/osteonectin/BM-40) is a multifunctional matricellular glycoprotein with counter-adesive properties and effects on cell shape, proliferation, cell cycle and angiogenesis. In some types of cancer, SPARC correlates with poor prognosis (melanoma, glioma, prostate and breast cancer), while in others the protein functions as a tumor suppressor (ovarian, pancreatic and colorectal cancers). Studies of SPARC in hematopoietic malignancies resulted in conflicting reports about its role as a tumor suppressor (5q¬-MDS and AML with rearrangement of the MLL gene) or promoter (multiple myeloma and plasmacytoma). In chronic myeloid leukemia (CML) a recent study indicates that intracellular SPARC may be involved in resistance to imatinib (IM) *Aims.* Inhibition of BCR/ABL by IM results in a G1 cell cycle arrest mediated by PI3K pathway. It has been reported that PI3K inhibitors synergize with IM, increasing apoptosis of CML cells. Because SPARC affects PI3K/AKT, we focused on its potential role as tumor suppressor protein in CML cells. *Methods and Results*. By using qRT-PCR and western blot analysis, we demonstrated that SPARC was downregulated in CML cells from peripheral blood of 35 patients at diagnosis in respect to healthy controls ($p<0.001$ as mRNA and $p<0.05$ as protein). Ten patients were evaluated during IM treatment and in all patients a significant increase in SPARC mRNA and protein expression was recorded already at 3 months of treatment and this increase was maintained at least through the 12th month of therapy, when all patients were in complete cytogenetic remission ($p<0.01$ as mRNA and $p<0.001$ as protein). In one patient, discontinuation of IM at the 12th month, resulted in a significant reductionof SPARC protein level that increased again after IM was restarted. Analysis of SPARC serum levels by ELISA showed that, in CML patients, SPARC resulted decreased compared to healthy donors at diagnosis ($p<0.01$) but it progressively increased, reaching its peak at 18th months of IM treatment ($p<0.001$). We also incubated K562 with human recombinant SPARC for 2 days and then with IM for 24 h. Cell proliferation was evaluated after 72 h by ATP-lite: a cytotoxicity of 18±3,2% and 29±1,6% vs untreated cells was recorded with SPARC and IM alone respectively; their association induced a reduction of cell viability of 37,5±3,7%. After 96 h the combination SPARC/IM resulted more effective than IM alone with an increase of cytotoxicity of 16,5±3% ($p<0.01$ vs IM alone). Flow cytometry analysis revealed an accumulation of K562 cells in G0/G1 after 24 h exposition to IM alone (15±1,7% vs untreated cells; $p<0.01$) or SPARC alone (14,5±4,1%; $p<0.01$) while SPARC/IM combination showed an additive effect (26,5±3,3%; $p<0.001$). *Conclusion.* SPARC production is downregulated in CML cells. Treatment of IM induces overproduction of SPARC by normal PBMC. *In vitro*, SPARC induces a G0/G1 cell cycle arrest thus reducing the growth rate of K562 cells and increasing their sensitivity to IM. In conclusion, we found that exogenous SPARC has an antiproliferative effect on BCR/ABL positive cells.

0129

IMATINIB HAS THE POTENTIAL TO EXERT ITS ANTILEUKEMIA EFFECTS BY DOWN-REGULATING HERG1 K+ CHANNELS IN CHRONIC MYLEOGENOUS LEUKEMIA

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Background. Imatinib (STI571, Gleevec), a powerful protein tyrosine kinase (PTK) inhibitor, is the current first-line therapy for all newly diagnosed chronic myeloid leukemia (CML). Studies aimed at deci-

phering the mechanism of imatinib anti-leukemic activity have focused on inhibited PTKs activity, such as Abl, c-Kit, and the platelet-derived growth factor receptors. Indeed, in addition to the mediation of cellular events such as cell proliferation, differentiation, embryonic development, metabolism, and oncogenesis, PTKs also regulate a large number of ion channels. Increasing evidence suggests that PTK inhibitors alter the activity of a large number of voltage-dependent ion channels. *Aims.* hERG1 K+ channels are highly expressed in leukemia cells and appear of exceptional importance in favoring leukemogenesis, while the effect of imatinib on hERG1 K+ channels in CML has not been well characterized. The present study explored a possible regulatory effect of imatinib upon hERG1 K+ channels as a mean to uncover new molecular events involved in the antileukemia activity of this PTK inhibitor in CML. *Methods.* Real-time PCR, flow cytometry analysis and the whole-cell patch clamp technique were used to analyze effects of imatinib on hERG1 K+ channels. The hERG1 K+ channels inhibitor was used to detect whether hERG1 K+ channels act a potential target for imatinib-induced antileukemia effects in primary CML cells and Bcr-Abl-positive K562 cells. *Results.* Present data demonstrated that hERG1 was highly detected in K562 cells and primary CML cells. K562 cells cultured with imatinib (5 μM), the expression of hERG1 mRNA and protein were down-regulated by (67.9 ± 19.2) % and (64.3 ± 12.4) %, separately. Furthermore, current recordings of HEK293T cells transiently transfected with herg showed that imatinib markedly reduced the step hERG currents amplitude to (45.7 ± 2.5) % (n = 6) of control at 10 mV, and peak tail currents amplitude to (25.8 ± 3.1) % (n = 6) of control at 20 mV. Unexpected, imatinib failed to alter the voltage-dependence of hERG channel activation. The consequent pathophysiological effects of suppressing hERG1 K+ channels by imatinib illustrated that cells pretreated with imatinib in the present of 1 μM E-4031, a specific hERG1 K+ channels inhibitor, which achieved 1.9-fold higher the inhibition of proliferation and 1.3-fold stronger induction of apoptosis than that of cells treated with imatinib. Moreover, the synergistic effects of E-4031 and imatinib on the suppression VEGF secretion and NF-κB activation were observed. When treated with imatinib in the present of E-4031, the expression of VEGF mRNA was significantly reduced by 60%. The mean VEGF concentration in culture supernatants from cells treated with imatinib combined with E-4031 was 199.9 ± 101.5 ng/L per 10*6* cells, which was 1.6-fold higher inhibition efficiency than that of cells treated with imatinib. *Conclusions.* Our data demonstrate that imatinib down-regulates hERG1 K+ channels currents, which involves in imatinib-induced antileukemia effects in CML. These findings reveal a novel potential molecular mechanism of antileukemic activities by imatinib, which independent of targeting on the Bcr-Abl pathway.

0130

OVEREXPRESSION OF FBP1 IS ASSOCIATED TO HIGH SOKAL RISK IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background. The oncogenic transformation of Chronic Myeloid Leukemia (CML) cell of origin has been associated to an increased glucose metabolism, where the glycolytic pyruvate is directed away from the mitocondria, converted into lactate and secreted from the cell. This metabolic conversion is termed aerobic glycolysis, or Warburg effect and very essentially, it serves to support the tumor cell proliferation, by providing nucleotides, aminoacids and lipids enough to replicate all of cellular content. CML patients are historically stratified according to their Sokal risk score, which for more than 30 years has been regarded as the most significant prognostic factor in this hematological malignancies. The putative genetic and/or genomic basis driving this stratification are still not known. *Aim.* Here we present data obtained from GEP experiments aimed at the identification of genes and pathways able to predict and/or to elucidate the onset of the disease and the disease course in CP-CML pts by analyzing the transcriptome of the CD34+ cell fractions obtained at diagnosis from a cohort of high and non-high Sokal risk CML patients. *Patients and Methods.* Overall, 67 patients with previously untreated CML in chronic phase (CP) entered the study; all of them have been enrolled in GIMEMA CML protocols and provided highly enriched CD34+ cell fractions from peripheral blood. Gene expression profiling (GEP) was performed, in order to identify genes most significantly and most differentially expressed between high and non-high Sokal risk patients. All other cases were used in Real-time experiments, in order to validate the GEP data. *Results.* By GEP, 82 probe-sets, corresponding to 78 genes resulted significantly differentially expressed between high and non-high Sokal risk patients in a supervised analysis of gene profiles. A gene enrichment analysis of this profile showed that genes involved in hypoxia and oxidative stress resulted significantly overexpressed in the comparison between high and non-high risk patients. Particularly interesting resulted a significantly higher expression in high Sokal risk patients of FBP1 (fructose 1,6 bisphosphatase), a key-enzyme of gluconeogenesis, together with a significant over-expression of genes coding for enzyme involved in gluthatione biosynthesis (FBP1,GSTM3,SEPP1). These data suggest that CD34+ cells obtained from high Sokal risk patients might exhibit an unexpected moderation of the glycolytic flux, mainly due to the over-expression of FBP1, which might cause a re-direction of the pathway into the pentose phosphate shunt. Moreover, we validated by Real-time the de-regulated expression of these genes in a different set of newly diagnosed CP-CML patients, thus confirming that they are differentially expressed between high and non-high risk patients and we found that HIF1α, which is a gene that may be involved in the metabolic reprogramming, is indeed deregulated in our system. Overall, our data demonstrate for the first time, that the expression at diagnosis of sugar metabolic enzymes, might drive the evolutive Sokal risk of CML patients.

0131

EVI-1 GENE OVER-EXPRESSION: A POSSIBLE MECHANISM OF THROMBOCYTOSIS AT CHRONIC MYELOID LEUKEMIA ONSET

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Background. A considerable proportion of chronic myelogenous leukemia (CML) patients are characterized by elevated platelet counts at diagnosis. The underlying mechanisms are still poorly understood. Besides, thrombocytosis in acute leukemias has been recently linked with structural abnormalities of the short arm of chromosome 3, where EVI-1 gene maps. Activation of the EVI-1 oncogene has been reported in acute myeloid leukemia, CML in blast crisis, and less commonly, in chronic-phase CML patients. *Aim.* To investigate EVI-1 overexpression [EVI-1(+)] in chronic phase (CP) CML patients with extreme thrombocytosis in comparison to patients with normal or moderately elevated values as well as to patients with essential thrombocytemia (ET). *Materials and Methods.* In total, 21 patients with BCR-ABL-positive CP-CML and platelet counts above 1000x10^9/L (ranging 1000-2644x10^9/L) were studied for EVI-1 over-expression by reverse transcription polymerase chain reaction (RT-PCR) and the results were compared to a group of 22 BCR-ABL-positive CP-CML patients with platelet counts ranging 209-833x10^9/L. A group of 15 patients with ET, including 6 cases positive for JAK2 V617F mutation, were also included. Major clinical and laboratory variables, as well as the BCR-ABL type of transcripts were analysed. *Results.* In total, 21 patients with CP-CML were found to be EVI-1(+). The incidence of EVI-1 overexpression was significantly higher in CML patients with thrombocytosis >1000x10^9/l where it was found in 16/21 cases (76,2%) compared to 5/22 (22,7%) in CML patients with lower platelet counts (Fisher's Exact Test, p=0.001). No association between EVI-1 gene status with basic laboratory features such as leukocyte counts, hemoglobin levels, and age nor with the BCR-ABL type of transcripts was found. However, even in the whole CP-CML group, the platelet counts in EVI-1(+) patients was significantly higher compared to the EVI-1(-) cases (mean 1192.9±542.4x10^9/L vs 708.1±551.9x10^9/L; Pearson Chi square, p=0.006). Interestingly, none of the 15 ET patients (0%) had detectable EVI-1 mRNA levels. *Conclusions.* Until recently the EVI-1 gene over-expression was considered as a marker of disease progression. Up to our knowledge the present study provides for the first time additional evidence that the oncogene might play a role in the pathogenetic mechanisms of thrombocytosis in CML patients that differ from the involved pathways in ET.

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0132

DISTINCTIVE MIRNA EXPRESSION PROFILE AND FUNCTIONAL ROLE IN K562 CELLS

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Background/Aims. MicroRNAs (miRNAs) are short non-coding regulatory RNAs that control gene expression at the post-transcriptional level. Deregulation of miRNA expression has been discovered in a variety of tumors and it is now clear that they contribute to cancer development and progression. Chronic myeloid leukemia (CML) is an extensively studied neoplasms. The hallmark of CML is the Philadelphia chromosome, created by the t(9;22)(q34;q11) translocation, resulting in the formation of the bcr-abl oncogene, which codes for a deregulated tyrosine kinase. Imatinib, a Bcr-Abl inhibitor, selectively induces apoptosis of Bcr-Abl+ cells and is successful in treating CML patients. One major obstacle to imatinib-based therapies is imatinib resistance. In an attempt to override resistance, second-generation inhibitors, such as dasatinib were developed. Dasatinib is a multikinase inhibitor targeting Bcr-Abl and Src kinases and inhibiting most imatinib-resistant mutants. Our objective is to decipher an miRNA expression signature associated with CML and to determine potential target genes and signaling pathways affected by these signature miRNAs. *Methods.* Global miRNA expression profiling was performed with miRNA-based microarrays. Analysis was performed using Partek Genomics Suite and validation was done by Taqman-miRNA. Bioinformatics was performed using: Target Scan, Ingenuity Pathway Analysis, KEGG pathway database. *Results.* We analyzed the miRNAs expression profile of K562 cells in reference to a pool of healthy blood. We also looked into the expression profile of K562 cells treated with imatinib or dasatinib. With the aid of unsupervised hierarchical clustering we found that healthy blood samples were clustered separately from K562 cells. Untreated K562 cells were clustered separately from treated ones and imatinib treated cells were clustered closely to those treated with dasatinib. Seventy-five miRNAs were downregulated and 35 upregulated in K562 cells as compared to healthy blood. Seventeen miRNAs were upregulated and 21 were downregulated following imatinib or dasatinib treatments. Following miRNA real-time PCR validation, we focused on 8 statistically significant differentially expressed miRNAs: 7 were downregulated (miR-31, miR-34a, miR-143, miR-145, miR-155, miR-196b, miR-564) and one was upregulated (miR-128) in K562 cells. MiR-128 was downregulated following imatinib and dasatinib treatments. MiR-564 was upregulation following imatinib treatment. The expression of the remaining 6 miRNAs was not altered following either drug treatment. We next analyzed predicted targets and affected pathways of the 8 deregulated miRNAs using Target Scan and Ingenuity Pathway Analysis. Reassuringly, the analysis identified cancer, and specifically CML, as the main disease associated with these 8 miRNAs. MAPK, TGF-β and Wnt were the main molecular signaling pathways related with these expression patterns. Utilizing Venn diagrams we found appreciable overlap between the CML-related miRNAs and the MAPK and TGF-β-related miRNAs. *Conclusions.* Our data suggest that our identified miRNAs might offer a pivotal role in CML. Nevertheless, while these data point to a central disease, the precise molecular pathway/s targeted by these miRNAs is variable implying a high level of complexity of miRNA target selection and regulation. These deregulated miRNAs highlight new candidate gene targets allowing for a better understanding of the molecular mechanism underlying the development of CML, and propose possible new avenues for therapeutic treatment.

0133

IMPROVEMENT OF INTER-LABORATORY STANDARDISATION FOR BCR-ABL QUANTIFICATION WITH SECONDARY REFERENCE MATERIAL CALIBRATED ON THE WHO REFERENCE PANEL

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Background. Real-time quantitative PCR (RQ-PCR) measurement of BCR-ABL transcripts in blood is a key tool for disease monitoring in Chronic Myeloid Leukemia (CML). Achieving major molecular response (MMR), i.e. a BCR-ABL load below 0.1% on the International Scale (IS), is now the main treatment objective with tyrosine kinase inhibitors, highlighting the need for more accurate and precise measurement methods. Recently, calibrated reference materials (CRM) have been developed and validated under the WHO umbrella, allowing for a simpler international standardization of BCR-ABL quantification. However, these CRM are a limited resource and their availability is restricted to manufacturers of secondary reference materials. In this context, Ipsogen developed an IS-MMR RNA strictly calibrated on the CRM, intended to be used as an intra-run calibrator for BCR-ABL/ABL conversion on IS. *Aims.* This study aimed at evaluating inter-laboratory variability when using a kit containing the IS-MMR RNA sample compared to in-house methods before and after conversion to the IS with laboratory-specific conversion factors (CF). *Methods.* Fourteen laboratories participated in the study. Analyses were run blindly on 8 test RNA samples provided by Ipsogen, previously calibrated on the IS, and corresponding to 7 BCR-ABL levels (5 below 1%, one above 10%) and a negative sample.

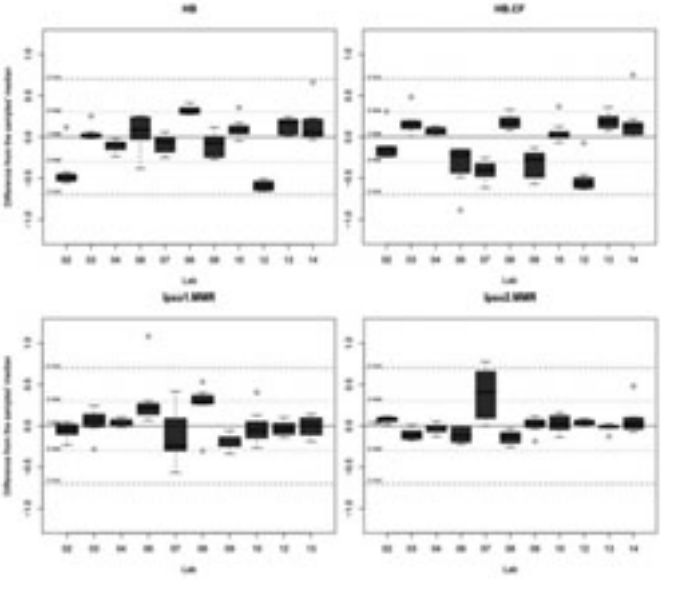

Figure 1.

A high positive control (HC) was also provided and included in each run. Each laboratory performed 2 runs with the BCR-ABL IS-MMR kit (Ipsogen) following the kit package insert instructions, and 1 run with their validated in-house method. Eleven laboratories had a CF, and were able to report home-brew results before (HB) or after (HB-CF) conversion to the IS. Four different real-time instruments were used. *Results.* Technical failure rate with the kit was low: 18 missing measures (7.1%), corresponding to two runs 1 failed in lab 5 and 14. Lab 7 reported a technical problem on its real-time instrument during the kit experiments, but results were not discarded. Inter-laboratory comparison was evaluated on fold-changes, taking the median values observed among laboratories as reference (see figure). On 81 common interpretable measures assessed in 10 laboratories on BCR-ABL positive samples, 85% and 94% of BCR-ABL/ABL% IS obtained with the kit (for run 1 and 2, respectively) were within the 2-fold interval, compared to 74% with HB and only 69% with HB-CF (p=0.00026). Values obtained with the kit were consistent with expected values and HB results, whereas HB-CF median values were systematically below the BCR-ABL/ABL% IS median values obtained with the kit. Kit results were consistent between runs, with 97% overall agreement on MMR prediction. *Conclusions.* In this study, the IS-MMR kit significantly improved inter-laboratory variability compared to HB-CF. With HB methods, laboratory-specific CF did not reduce inter-laboratory variability and even led to an underestimation of BCR-ABL values. Routine practice use of secondary reference materials calibrated on the WHO CRM should therefore be a simple and effective approach to standardise BCR-ABL quantification.

Chronic myeloid leukemia - Clinical 1

0134

HARMONIZED TESTING FOR BCR-ABL1 KINASE DOMAIN MUTATIONS IN CML: FINAL RESULTS OF A SURVEY AND FIRST CONTROL ROUND WITHIN 24 NATIONAL REFERENCE LABORATORIES IN EUROPE

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Various techniques have been employed to detect BCR-ABL1 kinase domain (KD) mutations in patients with chronic myeloid leukemia (CML) resistant to imatinib or second generation tyrosine kinase inhibitors (TKI). This variation may at least partially explain the different frequencies of mutations that have been reported. Standardized techniques and protocols for the detection of BCR-ABL1 KD mutations will be necessary in the future to obtain comparable mutation results within clinical studies. The first objective of this study conducted within the EUTOS (European Treatment and Outcome Study) for CML program was to record the mutation analysis techniques and protocols that are used for routine diagnostics by 24 national reference laboratories in 20 European countries, 9 of whom perform regular mutation analyses as a central laboratory for national or international clinical trials. Most laboratories (n=13; 54%) perform 1-10 mutation tests per month (range <1 to >100) and use published protocols for nested RT-PCR from peripheral blood or bone marrow leukocytes. Failure to amplify BCR-ABL1 mRNA at low BCR-ABL1 transcript levels was reported to be the most common PCR related difficulty in 16 (67%) laboratories. Sanger sequencing is applied for routine BCR-ABL1 KD mutation analysis in 23 (96%) laboratories. Additional screening techniques (e.g. D-HPLC, HRM) or more sensitive detection methods (e.g. ASO-PCR, pyrosequencing) are used routinely by eight laboratories each, respectively. Quantitative mutation analysis is performed routinely in 9 (38%) laboratories. The second objective of this study was to evaluate the techniques by analysis of blinded samples containing various BCR-ABL1 KD mutations. Twenty blinded cDNA samples were sent out on dry ice to each participating laboratory (total of 480 samples). Seventeen Ba/F3BCR-ABL cell lines harboring various BCR-ABL1 KD mutations were mixed with non-mutated Ba/F3BCR-ABL to produce dilutions ranging from 1% to 100% of mutant alleles. Three samples were non-mutated Ba/F3BCR-ABL only and were used as negative controls. The three non-mutated samples were identified cor-

rectly in 71/72 (99%) tests. For the 17 mutated samples, 240/408 (59%) samples were identified correctly. For further analysis, those samples with high (≥20%; n=11) and low (≤10%; n=6) levels of mutation were distinguished. For the high level samples 224/264 (85%) tests were reported correctly. Of the incorrect results, 28 were scored as negative and 12 were false positives. For the low level samples, 16/144 (11%) tests correctly identified the mutation. Most (127/144; 88%) were reported as undetectable and a false positive result was reported in one case. We conclude that Sanger sequencing is the most frequently applied technique for routine analysis of BCR-ABL1 KD mutations in CML in Europe. In general it reliably identifies mutations when the proportion of mutant alleles comprise 20% or more. Nevertheless, false negative and false positive results were reported in a substantial proportion of samples with ≥20% mutation level (40/264, 15%). For mutations that are present at 10% or less mutant alleles, routine methods mainly failed to identify mutations. This study indicates that further work is needed to establish reliable tests to identify BCR-ABL1 KD mutations in CML on TKI therapy.

0135

NILOTINIB 400 MG BID FRONTLINE: WITH A FOLLOW-UP OF 3 YEARS, RESULTS REMAIN EXCELLENT AND STABLE. (A GIMEMA CML WP PHASE 2 TRIAL)

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Background. Nilotinib is a potent and selective BCR-ABL inhibitor. The phase 3 ENESTnd trial demonstrated superior efficacy of nilotinib vs imatinib, with higher and faster molecular responses. After a median of 24 months, the rates of progression to accelerated-blastic phase (ABP) were 0.7% and 1.1% with nilotinib 300mg and 400mg BID, respectively, significantly lower compared to imatinib (4.2%). Nilotinib has been approved for the frontline treatment of Ph+ CML. With imatinib 400mg (IRIS trial), the rate of any event and of progression to ABP were higher during the first 3 years. Consequently, a confirmation of the durability of responses to nilotinib beyond 3 years is extremely important. *Aims.* To evaluate the response and the outcome of patients treated for 3 years with nilotinib 400mg BID as frontline therapy. *Methods.* A multicentre phase 2 trial was conducted by the GIMEMA CML WP (ClinicalTrials.gov.NCT00481052). Minimum 36-month follow-up data for all patients will be presented. Definitions: Major Molecular Response (MMR): BCR-ABL/ABL ratio <0,1%IS; Complete Molecular Response (CMR): 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 by 12 months was 100%. CCgR at each milestone: 78%, 96%, 96%, 95%, 92% at 3, 6, 12, 18 and 24 months, respectively. The overall estimated probability of MMR was 97%, while the rates of MMR at 3, 6, 12, 18 and 24 months were 52%, 66%, 85%, 81% and 82%, respectively. The overall estimated probability of CMR was 79% at 30 months, while the rates of CMR at 12 and 24 months were 12% and 27%, respectively. No patient achieving a MMR progressed to ABP. Over 3 years of observation, only one patient progressed to ABP, at 6 months, and subsequently died (high Sokal risk, T315I mutation). AEs were mostly grade 1 or 2 and manageable with appropriate dose adaptations. During the first 12 months, the mean daily dose was 600-800mg in 74% of patients. Nilotinib last daily dose was as follows: 800mg in 46 (63%) patients, 600mg in 3 (4%) patients and 400mg in 18 (25%). Six permanent discontinuations: 2/6 deaths (1:ABP, 1: mental deterioration and starvation, unrelated to study drug). 3/6, recurrent episodes of amylase and/or lipase increase (no pancreatitis) and 1/6, atrial fibrillation (other epysodes before nilotinib): 2 patients are currently on imatinib second-line and 2 on dasatinib third-line. With a median follow-up of 39 months, 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.* The rate of failures was very low during the first 3 years. Responses remain stable. The high rates of responses achieved during the first 12 months are being translated into optimal outcome for most of patients.

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0136

LONG-TERM EFFICACY AND SAFETY OF DASATINIB 100 MG ONCE-DAILY (QD) IN PATIENTS WITH IMATINIB-RESISTANT/INTOLERANT CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CML-CP): 5-YEAR FOLLOW-UP FROM CA180-034

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Background. For almost 10 years, the BCR-ABL inhibitor imatinib was the standard first-line treatment for CML-CP. Dasatinib, a more potent BCR-ABL inhibitor, is an approved therapy for patients with imatinib-resistant or imatinib-intolerant CML or newly diagnosed CML-CP. The recommended dosing regimen is dasatinib 100 mg once daily (QD), based on results from the CA180-034 dose-optimization study in patients with CML-CP and resistance or intolerance to prior imatinib therapy. The CA180-034 trial provides the longest follow-up of patients with CML-CP treated with a newer BCR-ABL inhibitor. *Aims.* To investigate the long-term efficacy and safety/tolerability of dasatinib 100 mg QD in patients with CML-CP and resistance or intolerance to prior imatinib therapy. *Methods.* Details of the study design and endpoints have been published (Shah *et al*. J Clin Oncol, 2008; 26: 3204-12). Patients (n=670) provided informed consent and were randomized using a 2x2 factorial design to one of four dasatinib dosing regimens: 100 mg QD, 50 mg twice daily (BID), 140 mg QD, or 70 mg BID. *Results.* After a minimum follow-up of 4 years, 251 (37%) patients remained on dasatinib treatment. More patients in the dasatinib 100 mg QD arm (45%) remained on treatment compared with the other three arms (33-37%). For patients randomized to dasatinib 100 mg QD (n=167), the 4-year rate of progression free survival (PFS) was 66%, overall survival (OS) was 82%, and 4% of patients had transformed to accelerated or blast phase while on treatment. In the dasatinib 100 mg QD arm, 4-year PFS rates were 93% and 87% in patients who had achieved a complete cytogenetic response (with or without a major molecular response) at 6 and 12 months, respectively. For dasatinib 100 mg QD, 4-year cumulative rates of nonhematologic adverse events (AEs) of any grade included headache (33%), diarrhea (28%), fatigue (25%), and pleural effusion (24%). Most nonhematologic AEs first occurred within 24 months of starting treatment. Grade 3/4 hematologic AEs usually first occurred within the first 12 months of treatment and included neutropenia (36%) and thrombocytopenia (24%). Dose modifications or changes to the dosing schedule were permitted for managing AEs. At the last available follow-up across the four treatment arms, 178 (71%) patients were on a QD dosing schedule, of which 107 (60%) patients were taking ≥100 mg QD. 44% of patients switched

from BID to QD dosing. Five-year follow-up will be presented. *Conclusions.* In the CA180-034 study, the majority of patients who remain on study are on a QD dosing schedule after 4 years of dasatinib treatment. Continued follow-up of patients with CML-CP receiving dasatinib following prior imatinib therapy further demonstrates the durable efficacy and acceptable tolerability and safety profile of long-term dasatinib 100 mg QD treatment.

0137

CML PATIENTS FAILING TO ACHIEVE MMR BY 12 MONTHS MAY BENEFIT FROM DOSE ESCALATION OR SWITCHING TO NILOTINIB: A 24 MONTH UPDATE OF RESULTS FROM THE TIDEL-II TRIAL

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Background. We have previously demonstrated excellent results from the TIDEL-II trial, using sequential therapy (imatinib [IM] then nilotinib [NIL]) in de novo chronic phase CML (CML-CP) patients. Here, we present longer term results (median follow-up of 24 months). *Aim.* To optimise molecular outcome and survival in treatment naïve CML-CP patients by selective dose escalation of imatinib and early switching to nilotinib based on imatinib blood level and early treatment response. *Method.* 105 patients entered the first cohort of this open label multicentre prospective trial, conducted through the ALLG. Therapy starts with IM at 600mg/d. Patients with IM trough level <1000ng/mL on day 22 and those failing to achieve molecular goals (BCR-ABL RQ-PCR of 10%, 1% and 0.1% IS at 3, 6 and 12 months respectively) had their IM dose escalated to 800mg/d. Patients were switched pre-emptively to nilotinib for a) failure to achieve molecular goals 3 months after dose escalation; b) loss of response or c) IM intolerance (Grade III/IV or persistent Grade II non-haematological toxicity). *Results.* The median follow-up is 24 months (range: 15-29). The confirmed MMR & CMR rate was 65% and 12% at 12 months respectively (ITT). Two patients progressed to AP/BC, both had kinase domain mutations (one T315I amongst others, the other H396P). There were two deaths, one from disease progression and one following a myocardial infarct. Seventeen patients withdrew from the study (16%). In total, six patients developed mutations (6%), two with T315I (5 patients on IM at the time mutations were detected). All three patients who experienced disease progression or T315I mutation had PCR >1% at 3 months. One, six and 11 patients dose escalated from IM600mg/d to IM800mg/d for suboptimal responses at 3, 6 and 12 months respectively; 5/7 patients who dose escalated at 3 and 6 months met their goals after 3 months, although two of these patients subsequently failed their 12 month time point. In all, 11/18 (61%) met treatment goals within 3 months of dose escalation. Thirty-seven patients failed to achieve confirmed MMR by 12 months, (5 had withdrawn before that time point). Thirty-two remained on trial with a median additional follow up of 12 months, 10 of whom achieved confirmed MMR (31%) subsequently. Of the 22 patients who did not achieve MMR, seven had withdrawn (1 BC transformation, 1 T315I mutation, 5 for other reasons); the remaining 15 all have RQ-PCR between 0.1% and 1%. *Conclusions.* Selective dose escalation of IM for patients who failed to achieve adequate drug levels or treatment milestones, with pre-emptive switching to nilotinib, is a successful therapeutic strategy in treatment naïve CML-CP patients, with excellent 12 month MMR rates. For those who failed to reach MMR at 12 months, even though a majority have sustained RQ-PCR between 0.1-1%, achieving MMR in the subsequent 12 months is difficult. The TIDEL-II approach has highlighted the problems in achieving a deeper response in this group of patients and the importance of early disease control.

0138

THE PROGNOSTIC SIGNIFICANCE OF MOLECULAR, CYTOGENETIC AND HEMATOLOGIC RESPONSE LANDMARKS AFTER 3 MONTHS OF IMATINIB IN THE UPFRONT TREATMENT OF CHRONIC MYELOID LEUKEMIA

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Background. The achievement of a molecular response level of 10% BCR-ABL IS (International Scale) or deeper after three months of imatinib treatment has been shown to be associated with a favorable outcome in terms of failure free survival according to European LeukemiaNet (ELN) definitions and progression free survival (Hanfstein *et al.*, Blood. ASH Annual Meeting Abstracts;116:360). With the availability of potent second generation tyrosine kinase inhibitors (TKI) for upfront treatment of CML, the early identification of patients at risk under imatinib is crucial for clinical decision making and patients' outcome. According to ELN recommendations (Baccarani *et al.,* JCO 2009) patients are considered treatment failures if they lack a complete hematologic remission (CHR) after three months of imatinib treatment. If no cytogenetic response is observed after three months (No CgR, Ph+ metaphases >95%) patients are considered suboptimal responders. Molecular landmarks offering guidance in the interpretation of three month BCR-ABL levels are lacking up to now. *Aims.* We sought to compare the predictive significance of early molecular, cytogenetic and hematologic landmarks to evaluate new and established response criteria in a large data set. METHODS: In the randomized German CML Study IV n=949 patients were treated with an imatinib based therapy consisting of standard dose imatinib (400 mg/d, n=265), high dose imatinib (800 mg/d, n=260) and combinations of standard dose imatinib with low dose cytarabine (n=138) or interferon alpha (n=286). BCR-ABL IS was determined by quantitative RT-PCR. The type of BCR-ABL transcript (b2a2, n=424; b3a2, n=464; b2a2 and b3a2, n=148) was defined by multiplex PCR. Patients with atypical BCR-ABL transcripts were excluded from the analysis. Cytogenetic response was determined by G-banding metaphase analyses. Disease progression was defined by the incidence of accelerated phase, blastic phase or death on an intention-to-treat basis. In total 49 progressions were observed after a median of 22 months (range 1-70), 37 patients died. 441 patients were evaluable for hematologic, 423 for cytogenetic and 570 for molecular prediction after three months. Log-rank tests were used to analyze Kaplan-Meier plots. *Results.* Treatment failure after three months defined by the lack of CHR (n=94) did not prove to be predictive for disease progression (p>0.5). Suboptimal response defined as no CgR (n=23) showed a trend to significance (p=0.0558). In contrast, the 10% BCR-ABL IS landmark separated a high risk group (n=161) with a 17% risk for disease progression after 7 years from a low risk group with a 4% risk (Kaplan-Meier estimate, p=0.0156). The lack of a partial cytogenetic remission (Lack of PCgR, Ph+ metaphases >35%) is suited as a cutoff level as well (p=0.0367). *Conclusions.* After three months of imatinib treatment high risk patients can be identified by a BCR-ABL of 10% or more, whereas the lack of a complete hematologic remission is of no prognostic relevance.

0139

NILOTINIB EXPOSURE-RESPONSE ANALYSIS IN PATIENTS WITH IMATINIB-RESISTANT OR -INTOLERANT CHRONIC MYELOID LEUKEMIA (CML)

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Background. Nilotinib is approved for the treatment of patients with newly diagnosed Ph+ CML in chronic phase (CML-CP), and Ph+ CML-CP and accelerated phase (AP) resistant or intolerant to imatinib. *Aims.* To evaluate the population pharmacokinetics (PK) of nilotinib and its relationship with safety and efficacy in patients with imatinib-resistant or -intolerant CML. *Methods*. Population PK was assessed by non-linear mixed effects modeling, including 495 CML patients in CP (n = 235), AP (n = 135), or blast crisis (BC, n = 125) who had nilotinib PK data available. Exposure-efficacy analysis was performed in CML-CP patients, where the efficacy measures included complete cytogenetic response (CCyR) at 12 and 24 months, major molecular response (MMR) at 12 and 24 months, time to CCyR, time to MMR, and time to progression (TTP, defined as time from study entry to discontinuation due to disease progression or death). Baseline prognostic risk score (0 to 2) was investigated as a covariate in the exposure-efficacy analysis: 0 if hemoglobin > 120 g/L, basophils < 4%, and no insensitive mutation; 1 if missing 1 criterion; 2 if missing 2 or 3 criteria (Kantarjian *et al.*, ASH 2009). The relationship between nilotinib C_{min}, UGT genotype, and total bilirubin levels over 24 months was assessed in all patients. *Results.* Nilotinib dose intensity and PK were similar in all phases of CML. Baseline demographics did not significantly affect nilotinib PK. Patients with lower nilotinib dose and C_{min} (Q1) tended to have lower CCyR at 12 months, lower MMR at 12 and 24 months, longer time to CCyR and MMR, and shorter TTP than patients with higher nilotinib dose and C_{min} (Q2-Q4, Table).

Table 1.

	Nilotinib Response According to C_{min}* With Corresponding Dose†				P	
	Q1	Q2	Q3	Q4	Overall	Q1 vs Q2-Q4
Response **Median Dose, mg**						
CCyR at 12 mo, % Dose n = 89	39 469	68 717	68 780	59 800	.15‡	.053‡
CCyR at 24 mo, % Dose n = 56	71 426	83 735	71 799	75 800	.89‡	1.0‡
MMR at 12 mo, % Dose n = 92	28 486	37 721	56 798	39 800	.24‡	.22‡
MMR at 24 mo, % Dose n = 52	50 607	50 753	60 789	77 800	.47‡	.66‡
Time to CCyR, months Dose n = 163	26 429	20 754	18 798	19 800	.079§	.010§
Time to MMR, months Dose n = 158	31 413	24 697	23 799	24 800	.089§	.012§
TTP, months Dose n = 157	23 408	28 764	31 788	27 800	.037§	.009§

*Model-based daily C_{min} averaged to time of assessment.
†Total daily dose averaged to time of assessment.
‡Chi-square.
§Log-rank.

Baseline prognostic risk score also significantly affected TTP (27.9 and 18.7 months for scores 0-1 vs 2). Nilotinib C_{min} and UGT genotype were significantly associated with the occurrence of bilirubin abnormalities (both $P < .1$). The occurrence of grade 3/4 bilirubin abnormalities was 6%, 10%, 10%, and 14% in patients with nilotinib C_{min} in Q1 (< 429 ng/ml, n = 124), Q2 (429 - < 615 ng/mL, n = 123), Q3 (615 - < 850 ng/mL, n = 123), and Q4 (≥ 850 ng/mL, n = 123), respectively, and 6%, 12%, and 48% for patients with TA(6)/TA(6), TA(6)/TA(7), and TA(7)/TA(7) UGT genotypes, respectively. *Conclusions.* Patients with lower nilotinib dose, lower C_{min}, and higher baseline prognostic risk showed a higher risk of progression and a trend of poorer response. Nilotinib C_{min} was also significantly associated with total bilirubin elevation. However, the ultimate impact of such an association on patient outcomes might be limited, since bilirubin elevations were reversible and the observed hyperbilirubinemia was clinically manageable in patients receiving nilotinib therapy. The present analysis suggests that adherence to nilotinib dose in order to maintain sufficient C_{min} is important in maximizing the clinical efficacy of nilotinib.

0140

LYMPHOCYTE MOBILIZATION AFTER DASATINIB INTAKE IS CORRELATED WITH PLASMATIC LEVEL OF DASATINIB AND MAY INFLUENCE MOLECULAR RESPONSE IN CP-CML PATIENTS RECEIVING DASATINIB AS SECOND LINE THERAPY

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Background. Lymphocytes mobilization after dasatinib intake has been recently reported (S Mustjoki at al., ASH Annual Meeting Abstracts; 116: 1204). The clinical significance of this phenomenon is not clearly established. We aimed to describe lymphocytes mobilization in Chronic Phase Chronic Myelogenous Leukemia (CP-CML) patients receiving dasatinib as a second line therapy. *Patients and Methods.* CP-CML patients from two institutions and receiving dasatinib were prospectively proposed to perform a WBC and a platelets analysis before (H0) and 2 hours (H2) after dasatinib intake. Dasatinib plasmatic levels were determined on the same time points (Cmin and C2hours). BCR-ABL/ABL IS ratio was determined during the same period of time. *Results.* 40 CP-CML patients were analysed and we report here the data on the first 28 patients studied. Median age was 59.6y (27-90) and sex ratio (M/F) was 1.5. The Sokal risk group was low in 45%, intermediate in 25% and high in 30% of the patients. 48% of the patients received dasatinib after imatinib failure and 52% after imatinib intolerance. The median duration of dasatinib before the lymphocytes analysis was 29 months (0.4-68). The median lymphocytes count values at H0 was 1.27 G/L and 2.6 G/L at H2 (paired t-test $p<0.001$). Surprisingly, a transient decrease in the platelets counts was observed between H0 and H2 (195.5 G/L versus 167.5 G/L, paired t-test $p<0.001$). This was associated with a transient increased of the occlusion time. The magnitude of lymphocytes mobilization (H2/H0) was 1.82 fold in median (1-3.56). We next analyzed if the lymphocytes increment was linked to pharmacokinetic (PK) values. No correlation was found with the Cmin. However, a positive correlation was demonstrated with the 2 hours plasmatic level of dasatinib ($r^2=0.5736$, $p=0.0011$). We then compared the magnitude of lymphocytes mobilization in patients with or without major molecular response (MMR). A significant association was found between high levels of mobilization and MMR ($p=0.047$). A phenotypic analysis was conducted in 7 patients. Despite a pan lymphocytes mobilization, a preferential increased in the CD3-/CD16/56+ and in the CD3-/CD57+ lymphocytes populations was observed (1.83 and 2.94 fold in median respectively). Of note, large granular lymphocytes was reported in only 2 patients and no patient had an absolute lymphocytosis at H0, suggesting that lymphocytes mobilization could be an independent phenomenon. *Conclusion.* We have demonstrated that lymphocyte mobilization could be observed in virtually all patients receiving dasatinib in the absence of an absolute lymphocytosis. We also confirm that lymphocytes mobilization is driven by dasatinib PK and report for the first time a link between lymphocytes mobilization and MMR. More patients will be presented and we are currently analysing lymphocytes mobilization in our first line patients included in the OPTIM dasatinib French trial.

0141

MOLECULAR RESPONSE <1% BCR-ABL (IS) AT 12 MONTHS IS ASSOCIATED WITH IMPROVED OVERALL AND PROGRESSION-FREE SURVIVAL AND REPRESENTS A SUPERIOR PREDICTOR THAN COMPLETE CYTOGENETIC REMISSION

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Background. The prognostic relevance of major molecular remission (MMR, <=0.1% BCR-ABL according International Scale, IS) for survival on imatinib-based treatment has remained uncertain. Gold standard is complete cytogenetic remission in spite of its limited sensitivity, the need for bone marrow. Standardization of PCR, definition of a uniform reporting system, and harmonization of laboratories using conversion factors to account for differences of methods and reagents have changed the situation in Europe. *Aims.* To determine the degree of molecular response after 12 months of imatinib-based treatment and its impact on overall and progression-free (PFS) survival. *Methods.* 848 out of 1014 patients within the CML-Study IV (randomized comparison of imatinib 800 mg vs 400 mg vs 400 mg + IFN) were eligible for evaluation since 12 month molecular data was available. Patients (61% male) have been recruited between July 2002 and April 2009, the median age was 54 years (range 16-84), and the median observation time was 40 months (minimum 12). Landmark analyses at 12 months and log-rank tests were performed. *Results.* 341 patients (40%) achieved a BCR-ABL expression <=0.1% (MMR), 240 patients (28%) between 0.1% and 1% and 267 patients (31%) >1% by 12 months. Independent of treatment approach, the groups of patients achieving MMR and 0.1%-<1% at 12 months showed significantly higher PFS (97% vs 95% vs 87% at 5 years, p=0.0023) and better overall survival (97% vs 96% vs 88% at 5 years, p=0.0011) compared to the group with >1% BCR-ABL by 12 months. Applying a landmark analysis at 12 months depending on the achievement of complete cytogenetic remission (CCyR) revealed less pronounced differences in overall survival (96% vs 91% at 5 years, p=0.015) than using the molecular predictor. *Conclusions.* Faster and deeper response to imatinib-based treatment by 12 months revealed to be associated with improved PFS and overall survival. The critical cutoff level seems to be 1% BCR-ABL IS which is supposed to closely correlate with complete cytogenetic remission. Furthermore, RQ-PCR from peripheral blood is more precise and better tolerable than cytogenetics from bone marrow.

0142

DETECTION AND PROLIFERATION KINETICS OF SUBCLONES CARRYING POINT MUTATIONS IN THE BCR-ABL1 TYROSINE KINASE DOMAIN IN CML PATIENTS TREATED WITH TYROSINE KINASE INHIBITORS

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Background. Point mutations in the BCR-ABL1 tyrosine kinase domain (TKD) are an important mechanism of resistance to treatment with tyrosine kinase inhibitors (TKIs). However, detection of mutant subclones may not always permit reliable assessment of imminent resistant disease because various mutations may be biologically neutral, and even subclones carrying mutations known to confer TKI resistance can disappear spontaneously. *Aims.* We have addressed the question whether quantitative monitoring of mutant subclones during treatment would facilitate timely identification and surveillance of resistant disease. *Methods.* A ligase-dependent (LD) PCR technique permitting sensitive detection and quantitative monitoring of point-mutated subclones was employed (Preuner *et al*. Leukemia 2008). The LD-PCR approach displays a detection limit ≥1%, and permits reproducible assessment of changes in the size of mutant subclones exceeding ±5%. We have investigated prospectively collected, serial PB specimens derived from CML patients displaying a variety of mutations including M244V, L248V, G250E, E255K, T315I, F317L-A/G, M351T, and F359V, and monitored the proliferation kinetics during the course of treatment. *Results.* Different mutant subclones appeared sequentially in individual patients upon changes of treatment. The appearance of new mutant subclones at a level detectable by LD-PCR has occasionally occurred within a few weeks after initiation of treatment with a TKI, but the time span until mutant leukemic cells became the dominant BCR-ABL1 positive clone was variable, ranging between 1-18 months after first detection. Expanding mutant clones could be documented even in the presence of decreasing BCR-ABL1 transcripts, and occasionally preceded the subsequent rise in BCR-ABL1 transcript levels by several weeks. Therapy-independent disappearance of a subclone carrying the T315I mutation, and the response of other mutant subclones to treatment could be documented by the quantitative LD-PCR approach. *Conclusions.* Our observations of multiple expanding mutant subclones in individual patients support the notion of non-linear, branching clonal architecture. The biological behavior of mutant subclones *in vivo* does not necessarily reflect the observations of sensitivity and resistance to TKIs made *in vitro*. Point mutations in the BCR-ABL1 TKD with expected sensitivity to certain TKIs may be markers of subclones displaying independent mechanisms of resistance. Quantitative monitoring of mutant subclones during treatment with TKIs provides information on their actual responsiveness to therapy and the imminent onset of resistant disease. Judicious implementation of quantitative diagnostic approaches such as the LD-PCR technique in the surveillance of CML patients can improve our current options for timely treatment decisions, and may help optimizing disease management in patients displaying point mutations in the BCR-ABL1 TKD or other sites of potential relevance.

0143

BOSUTINIB AS THIRD-LINE THERAPY FOR CHRONIC PHASE CHRONIC MYELOID LEUKEMIA FOLLOWING FAILURE WITH IMATINIB AND DASATINIB OR NILOTINIB

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Background. Bosutinib is an orally active, dual Src/Abl tyrosine kinase inhibitor (TKI) with minimal inhibitory activity against PDGFR or c-kit. *Aim.* This open-label, phase 1/2 trial evaluated the safety and efficacy of bosutinib as third-line therapy in patients with Philadelphia chromosome-positive chronic phase chronic myeloid leukemia (CP CML). *Methods.* After informed consent was obtained, adults (aged ≥18 years) with prior imatinib failure who were dasatinib-resistant (n = 37), dasatinib-intolerant (n = 50), or nilotinib-resistant (n = 27) received daily oral bosutinib starting from 400 to 600 mg/day. *Results.* Of the 114 third-line CP CML patients enrolled, 45% were male, the median age was 56 years (range, 20-79 years), and the median time from CML diagnosis was 6.3 years (range, 0.6-18.3 years). Median follow-up was 26.4 months (range, 0.3-54.0 months), and the median bosutinib dose was 476 mg/day (range, 185-563 mg/day). Non-hematologic treatment-emergent adverse events (TEAEs) seen in ≥20% of patients (all grades/grade ≥3) included diarrhea (83%/9%), nausea (46%/1%), vomiting (40%/1%), rash (27%/4%), headache (25%3%), fatigue (21%/1%), and abdominal pain (20%/1%). The incidence of TEAEs was similar for dasatinib-resistant, dasatinib-intolerant, and nilotinib-resistant patients. Gastrointestinal events were predominantly grade

1/2, early in onset, and usually subsided within a month. Two dasatinib-intolerant patients had grade 3 pleural effusions; both patients had these events on prior dasatinib and were grade 1 on study entry. No grade 3/4 QTc prolongation was observed. Grade ≥3 laboratory abnormalities (≥10% of patients) included thrombocytopenia (26%), neutropenia (18%), and hypermagnesemia (12%). Of 82 (73%) evaluable patients achieving complete hematologic response (CHR; Table), 34 (41%) retained their response and are still on therapy. Of 31 (32%) evaluable patients achieving major cytogenetic response (MCyR), 12 (39%) retained their response and are still on therapy; 21 (22%) patients achieved complete response (CCyR). Major molecular response (MMR) was achieved in 21 (22%) of evaluable patients. MCyR and CHR were observed across 11 different Bcr-Abl kinase domain mutations, but not T315I. *Summary/Conclusions.* Third-line bosutinib therapy demonstrated acceptable toxicity and promising activity, emphasizing the therapeutic potential of bosutinib in CP CML patients with resistance or intolerance to other second-generation TKIs.

Table 1.

n/n evaluable (%)	Imatinib failure+ dasatinib resistance	Imatinib failure+ dasatinib intolerance	Imatinib failure+ nilotinib resistance
CHR	23/37 (62)	39/49 (80)	20/26 (77)
MCyR	11/35 (31)	13/37 (35)	7/24 (29)
CCyR	4/35 (11)	12/37 (32)	5/24 (21)
MMR	3/32 (9)	17/46 (37)	1/19 (5)

0144

LONG TERM OUTCOME OF CHRONIC MYELOID LEUKEMIA ELDERLY PATIENTS TREATED FRONTLINE WITH IMATINIB. A SURVEY BY THE GIMEMA CML WP

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Background. The median age of Chronic Myeloid Leukemia (CML) patients is around 60 years and age is still considered an important prognostic factor, included in Sokal and EURO risk scores. With IM, this negative impact of age has been partially reappraised, at least in late chronic phase (CP). However, only limited data about the long term outcome are available for elderly patients treated in early CP; moreover, the allocation of elderly patients to IM, in particular within clinical trials, is still an important issue. *Aim*. To evaluate the long term outcome of elderly patients treated frontline with IM. *Methods*. We analyzed the relationship between age and outcome in 559 early CP CML patients, enrolled in 3 prospective clinical trials of GIMEMA CML-WP (Clin. Trials Gov. NCT00514488 and NCT00510926), treated with IM. The median follow-up was 60 months (range 1-83 months). Older patients (≥ 65 years old) were 115 (21%). Events were defined as: treatment failure or permanent discontinuation of IM for any reason; treatment failures were defined according to the updated European LeukemiaNet (ELN) recommendations; progression to accelerated/blast phase was defined according to ELN criteria. *Results*. The hematologic, cytogenetic, and molecular response rates observed in the 2 age groups were similar. The cumulative incidence of complete cytogenetic response (CCyR) and major molecular response (MMR) were 87% (100/115) and 85% (98/115) vs. 88% (391/444) and 85% (377/444), in older and younger patients, respectively. CCyR at 6, 12, and 18 months were 79/115 (69%), 90/115 (78%), and 85/115 (74%) in older patients, respectively, and 299/444 (67%), 343/444 (77%), and 346/444 (78%) in younger ones, respectively; MMR at 6, 12, and 18 months were 54/115 (47%), 67/115 (58%), and 65/115 (57%) in older patients, respectively, and 215/444 (48%), 262/444 (59%), and 278/444 (63%) in younger ones, respectively; all the differences were not significant. Regarding the long term outcome, the estimated 6-year EFS (55% vs. 67%, p = 0.006), FFS (62% vs. 78%, p=0.009), PFS (75% vs. 90%, p=0.0001) and OS (78% vs. 92%, p<0.0001) were all significantly worse in the older age group. Importantly, the analysis of the causes of death showed that in older patients more deaths in complete hematologic response (CHR) (unrelated to CML progression) have been recorded: 17/115 (15%) and 15/444 (3%) for older and younger patients, respectively (p<0.0001). On the other hand, deaths due to progression of CML were 6/115 (5%) and 15/444 (3%), for older and younger patients, respectively (p = 0.4). The analysis of the estimated 6-year EFS, FFS, PFS, and OS, censoring deaths unrelated to CML progression (all in CHR), showed that there was no longer any significant difference between the 2 age groups. *Conclusion*. These data show that response to IM was not affected by age and that the mortality rate linked to CML is similar in both age groups. Therefore older age must not be a limitation for treating patients with IM and for enrolling them in clinical trials.

0145

NILOTINIB IS ASSOCIATED WITH FEWER TREATMENT FAILURES AND SUBOPTIMAL RESPONSES VS IMATINIB IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): RESULTS FROM ENESTND

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Background. The ENESTnd trial established the superiority of the tyrosine kinase inhibitor (TKI) nilotinib over imatinib in newly diagnosed patients with Philadelphia chromosome-positive (Ph+) CML-CP. *Aims.* We sought to investigate the frequency of suboptimal response (SoR) and treatment failure (TF) on ENESTnd with 24 months' follow-up. Also, the outcome of the imatinib-treated patients after dose escalation following SoR/TF is presented. *Methods.* 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). An escalation in dose to 400 mg BID was permitted in patients on the imatinib arm who had a SoR or TF, using the European LeukemiaNet (ELN) criteria originally defined for patients treated with imatinib 400 mg QD. Dose escalation of nilotinib or crossover was not permitted. *Results.* Patients on both nilotinib doses had lower rates of SoR and TF by each ELN milestone, and higher rates of optimal response (Table). The rates of SoR observed on all arms at 18 months were primarily due to lack of major molecular response (MMR) at 18 months. Among patients randomized to imatinib, 82 (29%) patients received dose escala-

tion to 400 mg BID for SoR or TF after a median of 14 months of treatment with imatinib 400 mg QD. Median time on treatment after dose escalation among these patients was 9 months (range 0.2 - 28.2). Subsequent to dose escalation, 21 (26%) patients achieved MMR; 14 (17%) patients achieved CCyR without MMR, and 47 (57%) patients had no improvement in responses after dose escalation. After dose escalation, 36 (44%) patients had dose reductions / interruptions. A total of 37 (45%) patients discontinued treatment subsequent to imatinib dose escalation: 23 (28%) due to SoR or TF, 6 (7%) due to intolerance, 3 (4%) due to disease progression, and 5 (6%) due to other reasons. *Conclusions.* Frontline therapy with nilotinib offers improvement over the standard of care imatinib 400 mg QD in terms of less frequent SoR and TF. Favorable long-term outcomes are unlikely in patients with TF, and may be less likely in patients with SoR than in optimal responders. Dose escalation was not an effective strategy to overcome SoR/TF on imatinib, as approximately three quarters of these patients failed to achieve MMR after dose escalation, and nearly half had dose reductions/interruptions and discontinued treatment. Using 2009 ELN criteria, nilotinib frontline therapy (especially 300 mg BID) appears to be more effective than imatinib frontline therapy, even with imatinib optimization by dose escalation.

Table 1.

Response, %	Nilotinib 300 mg BID (n = 282)	Nilotinib 400 mg BID (n = 281)	Imatinib 400 mg QD (n = 283)
By 6 months			
Optimal	71	68	62
Suboptimal	2	2	6
Treatment Failure	1	1	6
Discontinued	1	2	2
Missing	25	26	24
By 12 months			
Optimal	80	77	63
Suboptimal	4	4	13
Treatment Failure	3	3	11
Discontinued	6	9	7
Missing	7	7	6
By 18 months			
Optimal	65	57	31
Suboptimal*	24	30	45
Treatment Failure	4	4	16
Discontinued	6	9	8
Missing	0	< 1	0

*Primarily due to lack of MMR at 18 months.

0146

BOSUTINIB (SKI-606) AS SECOND-LINE THERAPY FOR CHRONIC PHASE CHRONIC MYELOID LEUKEMIA (CP CML) FOLLOWING IMATINIB FAILURE: ANALYSES OF CROSS-INTOLERANCE AND RESPONSE BY PRIOR RESPONSE TO IMATINIB

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Background. Bosutinib is an orally active, dual Src/Abl tyrosine kinase inhibitor (TKI) with minimal inhibitory activity against PDGFR or c-kit. *Aims.* This open-label, phase 1/2 study evaluated the efficacy and safety of bosutinib as second-line therapy in patients with Philadelphia chromosome-positive CP CML. *Methods.* After collection of informed consent, adults (aged ≥18 years) with resistance (n = 200) or intolerance (n = 88) to imatinib received daily oral bosutinib starting from 400 to 600 mg/day. *Results.* Of 288 CP patients enrolled, 53% were male, median age was 53 years (range, 18-91 years), and median time from CML diagnosis was 3.6 years (range, 0.1-15.1 years). Median follow-up was 2.5 years (range, 0.1-5.0 years), with 49% of imatinib-resistant and 47% of imatinib-intolerant patients still receiving treatment. Median bosutinib dose was 485 mg/day (range, 128-600 mg/day). Of 172 (86%) imatinib-resistant and 75 (85%) imatinib-intolerant patients achieving complete hematologic response (CHR), respectively, 69% and 81% retained their response as of the data cutoff. In the evaluable population, of 101 (54%) imatinib-resistant and 39 (49%) imatinib-intolerant patients achieving major cytogenetic response (MCyR), respectively, 68% and 90% retained their response as of the data cutoff. Of patients who had not previously achieved MCyR on imatinib, 45% and 28% achieved MCyR and CCyR, respectively, on bosutinib (Table). Complete cytogenetic response (CCyR) was achieved by 42% and 43% of imatinib-resistant and imatinib-intolerant patients, respectively. Among patients who achieved CCyR, major molecular and complete molecular (undetectable Bcr-Abl transcripts) responses, respectively, were observed in 69% and 52% of evaluable imatinib-resistant and 73% and 68% of evaluable imatinib-intolerant patients. MCyR and CHR were observed across 25 different Bcr-Abl kinase domain mutations, but not T315I. Grade ≥3 non-hematologic treatment-emergent adverse events (TEAEs) seen in ≥2% of patients were diarrhea (9%), rash (9%), and vomiting (3%). Diarrhea was predominantly grade 1/2, early in onset, and usually subsided within a month. Grade 3/4 lab abnormalities (≥10%) included thrombocytopenia (24%), neutropenia (17%), anemia (13%), hypermagnesemia (11%), and alanine transaminase elevation (10%). Overall, dose reductions and interruptions were required by 47% and 67% of patients, respectively; 12% of patients' dose escalated to bosutinib 600 mg/day. TEAEs led to treatment discontinuation in 17% of imatinib-resistant and 33% of imatinib-intolerant patients. Limited cross-intolerance between bosutinib and imatinib was observed: 8% of patients with imatinib intolerance re-experienced the same grade ≥3 AE on bosutinib and 11% discontinued bosutinib due to the same AE. Four of 32 patients with imatinib intolerance related to myelosuppression experienced grade ≥3 myelosuppression on bosutinib, and 3 of 10 patients with imatinib intolerance related to rash experienced a grade ≥3 rash on bosutinib; no patient with imatinib intolerance related to gastrointestinal events, edema, fatigue, hepatobiliary disorders, or muscle spasms experienced these grade ≥3 events on bosutinib. *Summary/Conclusions.* Bosutinib demonstrated promising activity as second-line therapy, with responses observed irrespective of prior response to imatinib. Bosutinib was also associated with an acceptable toxicity profile, with limited cross-intolerance. These results emphasize the therapeutic potential of bosutinib in patients with CP CML following imatinib failure.

Table 1.

n/n evaluable (%)	Imatinib-resistant (n = 200)	Imatinib-intolerant (n = 88)	Total (N = 288)
Hematologic response			
CHR	172/199 (86)	75/88 (85)	247/287 (86)
CHR in patients without baseline CHR	76/100 (76)	34/41 (83)	110/141 (78)
Cytogenetic response			
MCyR	101/156 (54)	39/80 (49)	140/266 (53)
CCyR	79/186 (42)	34/80 (43)	113/266 (42)
MCyR by best prior cytogenetic response to imatinib*			
MCyR on imatinib	64/80 (80)	28/39 (72)	92/119 (77)
No MCyR on imatinib	45/106 (46)	9/24 (38)	58/130 (45)
CCyR by best prior cytogenetic response to imatinib*			
MCyR on imatinib	59/80 (74)	27/39 (69)	86/119 (72)
No MCyR on imatinib	30/106 (28)	6/24 (25)	36/130 (28)

*Analysis is based on all treated patients with available data for best cytogenetic response to imatinib.

0147

IMPACT OF CYTOGENETICS AT DIAGNOSIS ON OUTCOME OF CML: RESULTS FROM THE RANDOMIZED GERMAN CML STUDY IV

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Background. The prognostic impact of cytogenetic findings in addition to the translocation t(9;22)(q34;q11) or the variant translocation

t(v;22) at diagnosis of chronic myeloid leukemia (CML) is conflicting. *Patients and Methods.* We used baseline and outcome data of 1030 patients (pts) (608 male, 422 female, median age 53, range 16-88) with chronic phase CML randomized to the German CML-Study IV (imatinib [IM] 800 mg [n=265] vs IM 400 mg [n=254] vs IM 400 mg + IFN [n=281] vs IM 400 mg after IFN failure [n=108] vs IM 400 mg + AraC [n=122]). We sought to investigate the impact of additional clonal cytogenetic findings at diagnosis on time to complete cytogenetic remission (CCR), as an accepted prognostic marker. Cytogenetic analysis was performed after 24- and/or 48 h culture on G-banded metaphases. If appropriate, fluorescent-in-situ-hybridization (FISH) was used in addition. *Results.* In total, at diagnosis 912/1030 pts (89%) had the translocation t(9;22)(q34;q11), 60/1030 pts (5.9%) showed a variant translocation t(v;22). 58/1030 (5.6%) had additional clonal cytogenetic findings [56 pts in addition to the translocation t(9;22) and 2 pts in addition to a variant translocation t(v;22)]. Out of these 30/1030 pts (2.9%) lacked the Y chromosome (-Y) and 28/1030 pts (2.7%) had additional numerical or structural aberrations except -Y. Median age, sex and treatment were similarly distributed except for -Y male pts who were older. For pts with the translocation t(9;22), with variant translocations t(v;22) and with the translocation t(9;22)(q34;q11)/t(v;22) with additional clonal chromosomal changes other than -Y, median time (years) to CCR was 0.98, 0.84 and 1.92. When comparing the groups "translocation t(9;22)"and "t(9;22)/t(v;22) with additional chromosomal findings other than -Y"and when comparing the groups "variant translocations t(v;22)"and "translocation t(9;22)/t(v;22) with additional chromosomal findings other than -Y"over the entire period of time, time to CCR was significantly shorter in the group "translocation t(9;22)"respectively "variant translocation t(v;22)"($p < 0.001$). No difference regarding time to CCR was seen when comparing the groups "translocation t(9;22)"and "t(v;22)"or the groups "translocation t(9;22)"and "-Y". *Conclusion.* We conclude that additional clonal cytogenetic findings at diagnosis have an impact on time to CCR and possibly on prognosis.

0148

FISH PATTERNS AND TREATMENT OUTCOME IN 12 PH-NEGATIVE BCR-ABL-POSITIVE CML CASES

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Background. Approximately 1% of the CML patients do not have t(9;22)(q34;q11) detectable by conventional cytogenetics, but carry *BCR-ABL* fusion gene revealed by fluorescence in situ hybridization (FISH) and/or reverse-transcriptase PCR (RT-PCR). Imatinib efficacy in this group of patients remains unclear. *Aim.* To define FISH patterns and imatinib treatment efficacy in Ph-negative BCR-ABL-positive CML patients. *Methods.* Initial diagnostics including chromosome banding analysis (CBA) and RT-PCR was done in 531 primary CML patients. CBA was performed after 24 hours culture. G-banding was performed by a trypsin-Giemsa method. Karyotypes were described according to ISCN (2005). At least 20 metaphases for each sample were analyzed. In Ph-negative patients who had *BCR-ABL* transcript FISH assay using Dual-Colour Dual-Fusion *BCR-ABL* Translocation Probe (Abbott, USA) was applied on at least 200 interphase nuclei (I-FISH) and on all available metaphases. CBA and FISH were performed at the time of diagnostics and every 6 months of imatinib treatment. Quantitative measurement of *BCR-ABL/ABL* transcripts ratio by real-time quantitative PCR (RQ-PCR) was done every 3-6 months. Detection of point mutations in the BCR-ABL tyrosine kinase domain was performed by direct sequencing of RT-PCR products. Compete cytogenetics response (CCgR) was assumed as ≤ 1% of FISH-positive nuclei (N. Testoni, Blood, 2009) Event-free survival (EFS) was calculated in respect of intention-to-treat and defined as the time from imatinib beginning until any of the following events occurred: any sign of treatment failure (according to the European LeukemiaNet criteria (M. Baccarani *et al.*, JCO, 2009)), progression to AP/BC or death of any reason. *Results.* Normal karyotype was detected in 12 newly diagnosed CML patients (2.3%). However, all of them harboured *BCR-ABL* fusion gene revealed by FISH and RT-PCR. Ph-negative group included 2 males and 10 females with median age of 51 years and median follow-up of 39-months. 4 patients had e13a2 transcript variant, 8 patients - e14a2. Three different FISH patterns were indentified (table).

Table 1.

D-FISH pattern	Chromosome localization of signals			Number of cases	Interpretation
	Fusion	Red	Green		
2R1G1F	1F(22)	1R (9) 1R (9)	1G (22)	9	Cryptic insertion of *ABL* into *BCR* gene on chromosome 22
1R2G1F	1F (9)	1R (9)	1G (22) 1G (22)	2	Cryptic insertion of *BCR* into *ABL* gene on chromosome 9
1R1G1F	1F(22)	1R (9)	1G (22)	1	Loss of *ASS*, 5'*ABL* and 3'*BCR* genes on chromosome 9

7 of 12 patients received imatinib treatment. 6 of 7 patients achieved complete hematological response at 3 months. Median number of *BCR-ABL*-positive nuclei at 12 months was 22% (range 1-50%), at 18 months - 40% (range 0-92%). Only 1 patient had CCgR (assessed by I-FISH) by 18 months. One patient progressed to AP and died subsequently. None of patients had BCR-ABL mutations. EFS in Ph-negative CML patients treated by imatinib was significantly lower than in 244 Ph-positive ones, for whom long-term follow-up data was available: 0.14±0.13 vs 0.62±0.03 (p=0.007), while overall survival was comparable in both groups: 0.83±0.15 vs 0.85±0.02% (p=0.88). *Conclusions.* Our data suggests that main mechanisms of fusion gene formation in Ph-negative CML are cryptic insertions of *ABL* into *BCR*, or vice versa. In our series treatment outcome in this group was significantly worse in comparison with Ph-positive CML patients. Resistance in observed group seems to have BCR-ABL-independent mechanisms, because of lack of BCR-ABL mutations, duplication or amplification. Absence of response criteria for data obtained by I-FISH assay hampered to refer such patients to treatment failure earlier than 18 months.

0149

CHRONIC PHASE CML PATIENTS ON TYROSINE KINASE INHIBITORS HAVE A POLYFUNCTIONAL CELLULAR IMMUNE RESPONSE AGAINST INFLUENZA BUT AN IMPAIRED IGM HUMORAL RESPONSE AGAINST PNEUMOCOCCUS AFTER VACCINATION

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The tyrosine kinase inhibitors (TKI) imatinib, nilotinib and dasatinib are remarkably effective as single-agent treatments for chronic myeloid leukaemia (CML) in chronic phase (CP). However little is known of their potential impact on the immune system; this is of particular interest in terms of the long-term effects of TKI on tumour immune surveillance and susceptibility to infections. To date there are no human *in vivo* studies to assess how these molecular-targeted drugs affect immune function in patients and data from *in vitro* and animal studies with imatinib have been contradictory, ranging from impaired antigen-specific T-cell response to enhanced stimulation of tolerant T cells. Few data are available to assess potential immunomodulatory effects of the second-generation TKIs nilotinib and dasatinib. The aim of this study was to prospectively analyze humoral and cellular immune responses to vaccination against influenza virus (Flu) and Pneumococcus in CP-CML patients treated with imatinib, dasatinib or nilotinib compared to healthy controls. Fifty CP-CML patients on standard dose TKIs (IM, n=22; dasatinib, n=15; nilotinib, n=13) and 15 healthy controls were vaccinated against Flu (Influenza vaccine Ph. Eur. 2008/2009, CSL biotherapies) and pneumococcus (Pneumovax II, Sanofi Pasteur MSD). Samples were collected before and 1 and 3 months post-vaccination. We analyzed the immunological T-cell response to influenza virus both quantitatively and qualitatively using flow cytometry for intracellular TNF-α, IFN-γ, IL-2 and the cytotoxicity marker CD107a. Titers of IgM and IgG anti-pneumococcal were determined using ELISA, and the proportion of B-

cell subsets (IgM memory and switched memory B-cells) were measured using flow cytometry. CD8 and CD4 T-cell responses to Flu vaccination were not significantly different between patients and controls: after vaccination, T cell responses against Flu were detected in 18/35 patients on TKI and 9/11 healthy control. Polyfunctional T cells were detected in 6/10 evaluable patients and 4/8 healthy controls. In contrast a significantly lower proportion of patients mounted an anti-pneumococcal IgM response at 4 weeks post vaccination (defined as IgM serum titer >80 U/ml and 4-fold increase in titer from baseline) compared to healthy controls (20/45 versus 11/12 respectively, p=0.004). The median frequency of IgM memory B cells in controls was 13.9% (range 7.06 to 18.45); the frequency of IgM memory B cells in CML patients on TKI was below this range in 17 of 36 (Fisher exact test, p=0.004). Overall, T and B cell responses to vaccination were not significantly different when comparing patients on imatinib, dasatinib and nilotinib although the relatively small number of patients in each group precludes any definitive conclusions. These data have significant implications for the use of TKI together with immunotherapy, and in patients receiving vaccination against infectious agents. Contrary to some *in vitro* studies, TKI do not appear to impair the cellular immune response to viruses *in vivo*. However we found that compared to controls, CML patients on TKI have an impaired IgM antibody response to pneumococcus, associated with a selective reduction in the IgM memory B cell subset. We are currently investigating the mechanism underlying this observation.

0150

RECOMMENDATIONS TO MEET THE NEW STATISTICAL CHALLENGES ARISING FROM EFFICACY ENDPOINTS BEYOND OVERALL SURVIVAL IN CLINICAL TRIALS ON CHRONIC MYELOID LEUKEMIA

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Background. Overall survival (OS) used to be the primary endpoint for efficacy evaluation of treatments in chronic myeloid leukemia (CML). However, due to the success of tyrosine kinase inhibitors, the mortality is low and possible significant differences between treatments will not be observed for a long time. There is the need to arrive at insights on therapeutic improvement earlier on. Thus, remission parameters regarded as surrogate markers for OS are considered, either as new primary endpoints by themselves or as part of a composite endpoint. *Aims.* To master the new statistical challenges, we suggest recommendations for sound statistical analyses of efficacy parameters in CML. *Methods.* For "time-to-first-remission"endpoints (first complete cytogenetic remission, first major (or complete) molecular remission), Kaplan-Meier analysis may not to be applied. Methodological issues also with regard to composite endpoints are discussed. *Results.* A prerequisite for Kaplan-Meier analysis is "non-informative censoring", i.e. the event of interest might be observed in the future. With the competing event "death before a possible observation of a first remission"this prerequisite is no longer met, instead the probability of a later first remission is reduced to zero. Erroneous censoring of competing risks leads to systematically overestimated probabilities of observing the event of interest. The appropriate approach to estimate these probabilities is the calculation of the cumulative incidence function (CIF). However, the date of first remission is hardly known exactly and its detection depends on protocol compliance. Differences in evaluation frequencies may lead to a seriously biased effect size between two randomized treatments. A composite endpoint "failure-free survival (FFS)"might be defined as survival until one of the following events is observed: Failure of achievement or loss of a certain remission status, severe toxicity, disease progression, or death. The last two events are usually summarized as progression-free (PFS), the last three events as event-free survival (EFS). With FFS, missing-value problems regarding the events linked to insufficient remission are likely and the wide range of failure causes implies difficulties with respect to interpretation. In contrast to severe toxicity, progression, or death, remission failures alone are much less critical events. *Conclusions.* For above reasons, we do not recommend to use time to (a certain level of) remission (TTR) or complex composite endpoints as the primary endpoint of a clinical trial. Despite delayed discovery times, TTR analysis remains a useful tool to support judgment on the velocity of drug response and on the time until a certain remission should be waited for. If established as a surrogate marker for OS, the remission status at such a time could be chosen as primary endpoint. In accordance with accuracy and ease of interpretation, the order of preference for time-to-event endpoints is "overall survival", progression-free"and "event-free survival". Still, the chance to identify essential treatment differences earlier due to a higher number of failures could speak for EFS where the severity of possible events is reasonably comparable. If regarded as (secondary) endpoints, TTR parameters and composite endpoints demand analysis of all (competing) events and caution with outcome interpretation.

0151

THE EFFICACY AND SAFETY OF HIGHER DOSES OF IMATINIB FOR THE TREATMENT OF NEWLY DIAGNOSED, PREVIOUSLY UNTREATED CHRONIC MYELOID LEUKEMIA PATIENTS IN CHRONIC PHASE - SYSTEMATIC REVIEW AND META-ANALYSIS

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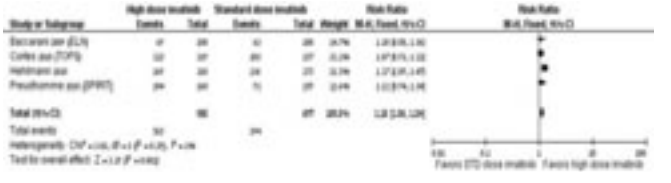
Background. Imatinib at a dose of 400mg daily is considered frontline treatment in chronic phase (CP) chronic myeloid leukemia (CML). Doses of 600mg or 800mg daily have proven efficacy in the accelerated phase or in the event of unsatisfactory response to standard dose imatinib. Recent randomized controlled trials of standard dose versus higher doses of imatinib as first-line treatment for CP-CML have demonstrated conflicting results regarding treatment outcomes. *Aims.* We aimed to evaluate the efficacy and safety of higher doses of imatinib (≥ 600mg daily) compared with standard doses (400mg daily) for newly diagnosed, previously untreated CP-CML patients. *Methods.* Systematic review and meta-analysis of randomized controlled trials comparing frontline treatment with single agent imatinib 400mg daily vs. higher doses (≥ 600mg daily) in patients with CP-CML. The Cochrane Library, MEDLINE, conference proceedings and references were searched until February 2011. Two reviewers appraised the quality of trials and extracted data. Outcomes assessed were: Complete cytogenetic response (CCyR) and major molecular response (MMolR) at 12 months; progression to accelerated phase (AP) / blastic crisis (BC); all-cause mortality at the end of follow-up; adverse events at 12 months. Relative risks (RR) were estimated and pooled. *Results.* Our search yielded four trials, published between the years 2009-2010 and randomizing 1645 patients. Three trials compared the standard dose of 400 mg daily with 800mg daily and one with 600mg daily. Three trials included patients of all Sokal risk scores, while one trial included only high risk Sokal score patients. The median actual dose of imatinib in the high dose arm trials ranged from 590mg to 720mg. *Efficacy analysis*: CCyR at 12 months was improved in the high dose imatinib arm (RR 1.15, 95% CI 1.06-1.24, 4 trials, figure). High dose imatinib also improved MMolR at 12 months (RR 1.36, 95% CI 1.08-1.70, 4 trials). However, there was no difference in all-cause mortality (RR 0.79, 95% CI 0.52-1.22, 3 trials) or progression to AP/BC (RR 0.83, 95% CI 0.45-1.50, 3 trials) at the end of follow-up. *Safety analysis*: Adverse events requiring discontinuation were more common in the high dose imatinib arm (RR 1.98, 95% CI 1.20-3.26, 3 trials), as were grade III/IV neutropenia, thrombocytopenia and edema: RR 1.57, 95% CI 1.15-2.14 (3 trials), RR 1.91, 95% CI 1.28-2.93 (3 trials) and RR 2.44, 95% CI 1.21-4.92 (2 trials), respectively. There was no difference in grade III/IV anemia or myalgias. *Conclusions.* Higher doses of imatinib significantly improved CCyR and MMolR, as compared to standard dose imatinib. There was no difference in all-cause mortality or progression to AC/BC, although this should be taken with reservation due to the short time of follow-up. Moreover, higher doses were associated with greater toxicity. Thus, there is currently insufficient evidence to support the routine use of higher imatinib doses as frontline treatment for CP-CML. Extended follow-up is needed to evaluate if the superior CCyR and MMolR with higher doses of imatinib will translate to long-term clinical benefit.

Table 1. Complete cytogenetic response at 12 months.



0152

THE PREVIOUS RESPONSE TO IMATINIB IS THE MAIN PREDICTOR OF CCYR ACHIEVEMENT ON SECOND LINE TKI TREATMENT

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Background. New tyrosine-kynase inhibitors (TKI) is the best possibility to overcome the imatinib resistance in patients with chronic myeloid leukemia (CML). Still we need more data to predict TKI's efficacy for appropriate choose treatment strategy. *Aims.* The aim of the study was to evaluate several prognostic markers of new TKI's efficacy in imatinib resistant patients. *Patients and Methods.* 47 imatinib-resistant pts (male:female ratio 21:26) with chronic phase of CML were analyzed retrospectively. Patients were treated with nilotinib (34 pts) or dasatinib (13 pts) since 2005 to 2010 with median follow-up on new TKIs 16,4 mos (3-66 mos). The median imatinib pretreatment time was 27 mos (2-83 mos). The median follow-up duration from the diagnosis was 45 mos (8-190 mos). The median age at the beginning of new TKIs - 50,4 years (26-84). There were 11, 17, 14 pts with low, intermediate and high Sokal risk, respectively (among 42 evaluable patients). *Results* Probability of CCyR achievement was 65 % (the rate 51,1% (23/45)). It was significantly higher among secondary than in primary resistant patients: 81% (rate 66,7% (10/15)) vs 50% (rate 39,3% (11/28)), p=0,045. It also depends on the best cytogenetic response (0-65% vs >65% Ph-positive cells) during imatinib treatment: 73% (rate 67,9% (19/28)) and 41% (rate 25% (4/16)), p=0,02. The probability of CCyR was higher in pts with complete hematologic response (CHR) at the start of 2nd TKI treatment: 78% (rate 66.7% (20/30)) with CHR vs 18% (rate 2/14)) without CHR, p=0,01. It also was relatively, but not significantly higher in low+intermediate than in high Sokal risk groups: 80% (rate 63% (17/27)) vs 50% (rate 38,5% (5/13)), respectively, p=0,101. In multivariant analysis (Cox regression) the significance of CHR and best CyR were confirmed, p<0,05. During 2nd line TKI treatment time to partial CyR predicted CCyR achievement - the probability of CCyR was 93% (rate 17/19 - 89.5%)) vs 83% (5/6 - 83.3%) in patients with PCyR achieved before or later 6 mos, respectively, p=0,022. Probability of CCyR loss was 10% (rate 9,1% (2/22)), we failed to reveal any factors, influencing it. Probability of progression-free survival (PFS) by 5,5 years was - 88% (progression rate - 4,3% (2/47)). It was better in patients with low and intermediate than in high Sokal risk group: 100% (progression rate 0/29) vs 68% (rate 15,8% (2/13)), p=0,048. Probability of survival from the start of TKI2 by 5,5 years was 92% (death rate 4,3% (2/47)) (only CML-related deaths were considered). The probability of survival among patients with low+intermediate Sokal risk score was 100% (death rate 0/29) and 67% (death rate 15,4% (2/13)) among high risk patients, p=0,046. Other factors didn't influence the patient's overall or progression free survival. The therapy was stopped in 21 patients: 16 due to resistance, 5 due to intolerance. *Conclusion.* Previous efficacy of imatinib and stable CHR at the start of therapy, but not Sokal risks, appear to be the main predictors of CCyR on 2nd TKI treatment. Only Sokal risk groups influenced overall and progression free survival in our cohort of patients.

0153

DYNAMICS OF MOLECULAR RESPONSE TO STANDARD-DOSE IMATINIB IN NEW CP CHRONIC MYELOID LEUKEMIA PATIENTS AFTER ACHIEVING CMR4.0

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Background. Imatinib has been the standard therapy in CML patients, and sustained undetectable BCR-ABL using real time quantitative polymerase chain reaction (RQ-PCR) is often referred to as complete molecular response (CMR). With prolonged imatinib therapy, the numbers of patients with CMR are increasing, and an estimated 40% of chronic phase (CP) CML patients will achieve CMR after 5 years on imatinib therapy. However, the degree of CMR might be different in each laboratory depending on the sensitivity of RQ-PCR assays, and it is important to confirm what level of sensitivity is necessary to determine the stability of patients on therapy. Although the significance of achievement of major molecular response (MMR) has been extensively studies in the previous studies, according to our knowledge, significance of achievement of CMR has not been determined yet. *Aims.* In this study, we investigated the dynamics of molecular response to imatinib after achieving $CMR^{4.0}$ in CP CML patients, and investigated their current clinical status to determine whether the achievement of $CMR^{4.0}$ represents the level of stability in patients on imatinib therapy. *Methods.* Fifty three patients who achieved $CMR^{4.0}$ with imatinib therapy after diagnosed as new CP CML in Seoul St. Mary's Hospital from 15Aug 2001 to 18 Dec 2009 were monitored by RQ-PCR assay at regular intervals to evaluate the molecular response. Patients' median age was 43 (range 17 - 68) and median follow-up duration were 71.9 months (range 9.7 - 108.8 months) after commencing imatinib and 19.6 months (range 2.8 - 88.8 months) after achieving $CMR^{4.0}$. Molecular response was expressed as a ratio of BCR-ABL to ABL% according to the international scale (IS), and $CMR^{4.0}$, $CMR^{4.5}$ and $CMR^{5.0}$ are defined as 4-log, 4.5-log and 5-log reductions from the baseline, respectively. *Results.* With the estimated detection limit of 5.0 logs below the standardized baseline in our RQ-PCR assay, we could discriminate $CMR^{4.0}$, $CMR^{4.5}$ and $CMR^{5.0}$, and different dynamics of molecular responses were demonstrated in each group as shown in Table 1.

Table 1. Molecular dynamics after achieving CMR4.0.

Dynamics of molecular response	Patient grouping according to the degree of CMR		
	Group 1 $CMR^{4.0}$, but not $CMR^{4.5}$ (N = 9)	Group 2 $CMR^{4.5}$, but not $CMR^{5.0}$ (N = 13)	Group 3 $CMR^{5.0}$ (N = 31)
Undetectable BCR-ABL levels N (%)	0 (0)	0 (0)	21 (67.7)
Decreasing BCR-ABL levels N (%)	2 (22.2)	6 (46.2)	0 (0)
Fluctuation under CMR level N (%)	6 (66.7)	4 (30.8)	0 (0)
Fluctuation across CMR level N (%)	1 (11.1)	3 (23.1)	7 (22.6)
MMR, but loss of CMR* N (%)	0 (0)	0 (0)	3 (9.7)

* CMR is defined differently in each group: 4-log, 4.5-log and 5-log reduction from the baseline in group 1, 2 and 3, respectively

Among 53 patients with $CMR^{4.0}$, 9 patients achieved $CMR^{4.0}$, but not $CMR^{4.5}$ (Group 1), 13 patients achieved $CMR^{4.5}$, but not $CMR^{5.0}$ (Group 2), and 31 patients obtained $CMR^{5.0}$ (Group 3). All patients have maintained complete hematologic response (CHR), complete cytogenetic response (CCyR) and MMR without disease progression, and survival rate was 98.1% (52/53) with 1 patient died from complications after SCT. Although 7 patients showed fluctuating BCR-ABL levels under/across $CMR^{4.0}$ levels, all of them maintained $CMR^{4.0}$ without further progression to advanced disease. *Conclusions.* Although different molecular dynamics were observed after achieving $CMR^{4.0}$, this study demonstrates that the achievement of $CMR^{4.0}$ represents a stable level where the risk of progression including loss of MMR is extremely low. Analysis of molecular dynamics after achieving $CMR^{4.0}$ examined here can be extended to expand our understanding of molecular profiles in CML patients through clinical application in a larger cohort with longer follow-up. With further follow-up, other possible significances of achieving CMR could be determined.

0154

THE EFFECT OF COMPLETE MOLECULAR RESPONSE ON PROGRESSION-FREE SURVIVAL IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA RECEIVING IMATINIB MESYLATE

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Background. Achievement of major molecular response (MMR) at 18th month and stable or improving MMR anytime during the treatment of chronic myeloid leukemia (CML) were defined as optimal re-

sponse by the European LeukemiaNet (ELN) based on previous studies. Importance of MMR is obvious. However, results of studies regarding the prognostic significance of complete molecular response (CMR) are conflicting. *Aims.* To investigate the effect of CMR achieved by imatinib mesylate (IM) on progression-free survival (PFS) in patients with CML. Secondary aim of the study was to compare disease outcomes in BCR-ABL positive and negative patients with MMR. *Methods.* Data from 106 CML patients receiving imatinib mesylate therapy were evaluated retrospectively. Of those, 35 patients were excluded because MMR could not be achieved. Demographic and clinical data of the remaining 71 patients are shown in Table 1. Remission definition and cytogenetic and molecular monitoring during the treatment was performed in accordance with the criteria of the ELN. CMR was defined as no detection of BCR-ABL by two consecutive polymerase chain reaction analyses at least three months apart. Any treatment failure was defined as progression. PFS was estimated as the time elapsed from the beginning of the treatment until the progression or last contact. Overall survival (OS) was calculated as the time between the beginning of the treatment until death or last contact. Demographic data were compared by descriptive statistics, $\chi 2$, Student's t-test, and Mann Whitney U tests. Survival analyses and curves were performed by log-rank test and Kaplan-Meier method, respectively. *Results.* CMR was obtained in 59 out of 71 (83.1%) patients with MMR. Median PFS was not reached in both BCR-ABL positive and negative groups. 3-year PFS was 74.1% and 89.8% for BCR-ABL PCR positive and negative patients, respectively (p=0.3). Only one patient has died among 71 patients. 3-year OS was 100% vs. 97.1% for BCR-ABL PCR negative and positive patients, respectively (p=0.7). There were no differences between groups regarding disease outcomes including treatment failure, disease transformation, and death (Table 2). *Conclusions.* Although this is a retrospective study including only a limited number of patients with CMR, our results show that achieving CMR in patients with CML has no effect on either PFS or disease outcomes. CMR should not be an essential goal in the treatment of CML patients.

Table 1.

Demographic and clinical data			
Variables	BCR-ABL PCR (-) (n=59)	BCR-ABL PCR (+) (n=12)	P
Age (Mean ± SEM)-years	47.24 ± 1.89	47.58 ± 5.11	0.9
Female-n (%)	33 (55.9)	7 (58.3)	1.0
Sokal risk score-n (%)			0.5
• Low	31 (58.5)	7 (63.6)	
• Intermediate	17 (32.1)	2 (18.2)	
• High	5 (9.4)	2 (18.2)	
Time since diagnosis-mo.			0.6
• Median	44.0	40.5	
• Range	10.0-144.0	12.0-98.0	
Time to imatinib mesylate (IM)-mo.			0.6
• Median	1.0	1.0	
• Range	0.0-55.0	0.0-48.0	
Time since the beginning of IM-mo.			0.4
• Median	41.0	40.0	
• Range	9.0-92.0	12.0-98.0	
CCgR at 12 mo.-n (%)	46 (80.7)	9 (81.8)	0.9
MMR at 18 mo.-n (%)	46 (80.7)	7 (72.7)	0.7
Time to first MMR-mo.			0.4
• Median	12.0	16.0	
• Range	3.0-81.0	3.0-58.0	

Table 2.

Disease outcomes			
Outcomes	BCR-ABL PCR (-) (n=59)	BCR-ABL PCR (+) (n=12)	P
Treatment failure-n (%)	10 (16.9)	3 (25.0)	0.1
• Loss of CHR	1 (1.7)	2 (16.7)	
• Loss of CCgR	2 (3.4)	0	
• Loss of MMR	7 (11.9)	1 (8.3)	
Accelerated phase-n (%)	1 (1.7)	0	1.0
Blastic transformation-n (%)	1 (1.7)	2 (16.7)	0.07
Death-n (%)	1 (1.7)	0	1.0

Clinical hemoglobinopathies 1

0155

SERUM SCLEROSTIN CORRELATES WITH BONE MINERAL DENSITY IN PATIENTS WITH THALASSEMIA MAJOR AND OSTEOPOROSIS; IMPLICATIONS INTO THE MANAGEMENT OF THALASSEMIA-RELATED OSTEOPOROSIS

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Background. Osteoporosis represents an important cause of morbidity in adult patients with thalassemia major (TM). Its pathogenesis includes increased osteoclast activity and osteoblast deregulation. Osteocyte is a key cell for the control of bone remodeling. Sclerostin is a secreted inhibitor of the wingless-type and integrase 1 (Wnt) canonical pathway which is produced by osteocytes and has inhibitory effects on osteoblasts. *Aims.* The aim of this study was to evaluate the circulating levels of sclerostin in patients with TM-related osteoporosis and explore possible correlations with clinical and laboratory data. *Methods.* Sixty-six patients with TM-related osteoporosis were studied. These patients were blindly randomized to receive zoledronic acid (ZOL) at a dose of 4 mg, iv, every 6 months (n=23) or every 3 months (n=21), or to receive placebo every 3 months (n=22) for a 12-month period. Bone mineral density (BMD) of the lumbar spine (L1-L4), femoral neck (FN) and distal radius (R) was determined using DXA before and 12 months post-ZOL treatment. Circulating sclerostin was measured in the serum of all patients at baseline and after 12 months of therapy, using an ELISA methodology (Biomedica Medizinprodukte, Vienna, Austria), along with a series of serum bone indices: i) bone resorption markers [C-terminal telopeptide of collagen type-1 (CTX) and tartrate resistant-acid phosphatase (TRACP-5b)]; ii) bone formation markers [bone-alkaline phosphatase (bALP), osteocalcin and C-terminal propeptide of collagen type-I (CICP)]; iii) osteoclast regulators [RANKL, osteoprotegerin (OPG) and osteopontin] and iv) dickkopf-1 (Dkk-1), another inhibitor of Wnt signaling. The above bone markers were also evaluated in 30, age- and gender-matched, healthy controls, while sclerostin was also measured in 62 women with post-menopausal osteoporosis. *Results.* At baseline, patients with TM had elevated circulating levels of sclerostin (median: 605 pg/mL, range: 22-1227 pg/mL) compared to healthy controls (250 pg/mL, 0-720 pg/mL, p<0.001) and reduced levels of sclerostin compared with postmenopausal women with osteoporosis (840 pg/mL, 181-1704 pg/mL, p<0.001). TM patients had also increased values of CTX (p<0.001), Dkk-1 (p<0.001), bALP (p<0.001), CICP (p=0.003), TRACP-5b (p<0.01), and sRANKL/OPG ratio (p=0.001). Sclerostin levels correlated with BMD in all studied sites L1-L4 (r=0.454, p<0.001), R (r=0.324, p=0.01) and FN (r=0.268, p=0.035). Similar results were obtained for post-menopausal women with osteoporosis for L1-L4 BMD (r=0.501, p<0.001). The other Wnt inhibitor, Dkk-1 also correlated with BMD of L1-L4 (r=-0.290, p=0.022) and R (r=-0.415, p=0.001). Patients who received ZOL did not alter their sclerostin levels after 12 months of therapy, but reduced their circulating Dkk-1 (from 39.6±16.6 to 28.9±16.3 pmol/L, p=0.004). In contrast, placebo group patients showed an increase of sclerostin levels (p=0.01) and a borderline increase of Dkk-1 (p=0.08). *Summary/Conclusions.* These results suggest that circulating sclerostin is elevated in TM patients with osteoporosis and correlated with their BMD. Furthermore, the high Dkk-1 serum levels and their association with BMD support the notion of a disrupted Wnt signaling in patients with TM-related osteoporosis which leads to osteoblast deregulation. These findings give the rationale for the use of novel drugs targeting sclerostin and Dkk-1 in TM patients with osteoporosis.

0156

LIVER IRON CONCENTRATION AND MORBIDITY IN PATIENTS WITH THALASSEMIA INTERMEDIA

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Background. Patients with thalassemia intermedia (TI) can have significant iron overload, irrespective of transfusion status, secondary to increased intestinal iron absorption. Correlation between serum ferritin levels ≥ 1000 ng/ml and the rate of clinical complications in TI has been studied; however, data on the effect of elevated liver iron concentration (LIC) on morbidity is lacking. *Aims.* To evaluate the association between LIC and several morbidities in patients with TI. *Methods.* A retrospective study of 168 TI patients treated at two centers in Beirut, Lebanon and Milan, Italy; for whom an LIC measurement was available. None of the patients were receiving iron chelation therapy. Data on demographics (age and gender), splenectomy status, transfusion status, and presence of clinical complications were retrieved. Mean values of SF, fetal (HbF) and total hemoglobin (Hb) levels, as well as platelet and nucleated red blood cell (NRBC) counts were retrieved. For LIC, direct determination of iron burden was performed using R2 MRI in Lebanon and T2* MRI in Italy using established methodology. *Results.* The mean age was 35.2 ± 12.6 years with 42.9% being males. A total of 121 (72%) patients were splenectomized and 44 (26.2%) were transfusion independent. The mean LIC was 8.4 ± 6.7 mg Fe/g dry weight (dw). On bivariate analysis, mean LIC values were significantly higher in patients with leg ulcers (P<0.05), thrombosis (P<0.01), pulmonary hypertension (P<0.001), abnormal liver function (P<0.001), hypothyroidism (P<0.05), osteoporosis (P<0.001), and hypogonadism (P<0.001) than those without. On multivariate logistic regression analysis, after adjusting for age, gender, splenectomy status, transfusion status, and laboratory indices, a 1-mg Fe/g dw increase in LIC was independently associated with higher odds of thrombosis (AOR: 1.12, 95% CI: 1.05-1.20), pulmonary hypertension (AOR: 1.08, 95% CI: 1.02-1.14), hypothyroidism (AOR: 1.05, 95% CI: 1.01-1.11), osteoporosis (AOR: 1.08, 95% CI: 1.02-1.14), and hypogonadism (AOR: 1.10, 95% CI: 1.03-1.16). *Summary/Conclusions.* Elevated LIC is associated with vascular and endocrine complications in patients with TI.

0157

EVALUATING PLATELET FUNCTION IN PATIENTS WITH THALASSEMIA USING THE PFA-100 SYSTEM; THE EFFECT OF CHELATION TREATMENT

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Background. Patients with thalassemia are at increased risk for developing thromboembolic events. Prophylactic use of aspirin, though widely adopted in these patients, is mainly based on theoretical *Background.* Furthermore, the effect of iron chelation therapy in platelet aggregation is not known. *Aim.* To evaluate the use of a screening method for platelet function in patients with thalassemia and to assess how the results of the method are affected by iron chelation therapy. *Methods.* Samples from the patients, after informed consent was obtained, were evaluated using the PFA-100 platelet function analyzer (Dade Behring Inc. Deerfield, IL, USA). This instrument uses small membranes coated with either collagen and epinephrine (Col/Epi) or collagen and ADP (Col/ADP). Blood samples pass through the membranes at a high shear rate, simulating the *in vivo* hemodynamics in the small capillaries. The PFA test is considered a rapid, accurate screening test to measure both platelet adhesion and aggregation (primary hemostasis). Sixteen patients (10 females, mean age: 33 ± 6.4 years) followed in our unit with either thalassemia major (12 patients) or thalassemia intermedia (4 patients) were included in the study. Patients were on chelation treatment with either desferioxamine (DFO) (2 patients), deferiprone (5 patients) or combination therapy of DFO and deferiprone (5 patients). Fourteen patients underwent a 2nd evaluation, 2-4 weeks after starting or switching chelation therapy to deferasirox. *Results.* Mean PFA-100 levels at the first evaluation were 114.9 ± 13.3sec, which were within the reported reference values. There were no differences in regards to gender or type of thalassemia. After initiation of therapy with deferasirox there was a significant increase in the PFA-100 levels from a mean value of 113 ±12.9sec to 132.6 ± 23.1 (p<0.05). In 6 of these patients the Col/Epi closure time was longer than the reference value with 4 of these 6 patients showing also prolongation of the Col/ADP closure time, indicating a more profound effect on platelet function. The prolongation of Col/Epi closure time parallels the changes in renal function, a well described phenomenon observed during treatment with deferasirox (p<0.05) *Conclusions.* A significant change was noted in the results of the PFA-100 evaluation after initiation of treatment with deferasirox. A possible mechanism for this phenomenon may be the inhibition of the heme-containing enzyme cyclooxygenase of the platelets, which produces thromboxane A2 from arachidonic acid, a potent regulator of platelets aggregation. This observation may have significant clinical applications for in patients receiving deferasirox. Toxicity from the chelator may be prevented by avoiding other drugs which have similar effect to platelets aggregation. Moreover, the need for concomitant antithrombotic prophylaxis with aspirin needs to be reconsidered. Larger long-term studies are required to validate these observations.

0158

STUDY OF RENAL IRON OVERLOAD BY T2* MRI IN A LARGE COHORT OF THALASSEMIA MAJOR PATIENTS

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Background. Renal dysfunction has been reported in adult subjects with thalassemia major (TM) since 1975. One of the main cause is the iron overload consequent to regular transfusions. Multiecho T2* MRI is a well-established technique for cardiac and hepatic iron overload assessment, but there very few report concerning the kidneys. *Aims.* The aims of this study were to describe the T2* values of the kidneys in patients with TM, to investigate the correlation between renal and myocardial or hepatic siderosis and biventricular cardiac function. *Methods.* 119 TM patients (58 men, 30.7 ± 8.2 years) enrolled in the Myocardial Iron Overload (MIOT) networks underwent MRI. For the measurement of iron overload, multiecho T2* sequences were used. The left ventricle was segmented into a 16-segments standardized model and the T2* value on each segment was calculated as well as the global value. In the liver, the T2* value was assessed in a single region of interest (ROI) in a homogeneous area of the parenchyma. For each kidney, T2* values were calculated in three different ROIs and were averaged to obtain a representative value for the kidney. The mean T2* value over the kidneys was also calculated. Cine images were obtained to quantify biventricular morphological and functional parameters in a standard way. *Results.* T2* values in the right kidney were significant lower than in the left kidney (40.3±11.9 ms vs 44.1±12.7 ms, P<0.0001). The mean T2* value over the kidneys was 42.2±11.9 ms and 40 patients (33.6%) had a pathological value (T2*<36 ms, lower limit of normal evaluated on 20 healthy subjects). The mean T2* value did not show a significant difference amongst men ad women (43.2±11.7 ms versus 41.3±12.1 ms, P=0.378). The mean T2* values increased with age in a significant manner (r=0.321, P<0.0001). There was a significant negative correlation between serum ferritin levels and mean renal T2* values (r=-0.446, P<0.0001, figure 1 left).

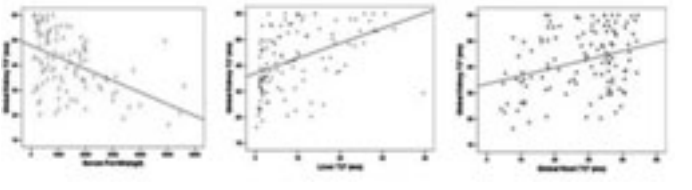

Figure 1.

Significant positive correlations of the mean T2* values were demonstrated for liver (r=0.511, P<0.0001, figure 1 center) and global heart (r=0.262, P=0.004, figure 1 right) T2* values. No correlation was found between renal iron overload and bi-ventricular function parameters. *Conclusions.* Systemic T2* differences between left and right kidneys were found, with significant lower values in the right one. Mean T2* value increased with age. We confirmed that kidney iron deposition was not very common in TM, but it was correlated with iron deposition in liver and heart.

0159

SAFE AND EFFICIENT CHELATION BY DEFERASIROX IN THALASSEMIA MAJOR PATIENTS WITH LOW LIVER IRON CONCENTRATIONS

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Background. Chelation treatment of iron overload due to chronic blood (RBC) transfusions has dramatically improved life experience, morbidity and mortality in Thalassemia major (TM) patients. Different treatment regimen are used, especially for chronically transfused patients with low or even normal liver iron concentration. *Aims.* We wanted to study the efficiency and safety of iron chelation therapy with Deferasirox (DSX) in TM patients with normal or low liver iron concentration (LIC) and chronic blood transfusion. *Methods.* This study included regularly transfused TM patients who were on iron chelation treatment with low liver iron overload. Inclusion criteria were LIC < 1200 µg/g-liver wet weight (conversion factor 6 for mg/g-dry wgt). Liver iron measurements by SQUID biomagnetic susceptometry (BLS) and/or MRI-R2 were performed in intervals of 6 to 12 months. For each measurement interval, the ratio of daily iron influx and DSX dose rate was calculated. This represents the equilibrium molar efficacy for iron balance. *Results.* Nineteen TM patients (9 females, mean age 14 y) were treated with DSX (median dose 19 mg/kg/d, range: 2 - 38 mg/kg/d) for 6 to 71 months. In all TM patients no severe side effects were observed and creatinine was in the normal range of < 0.9 mg/dl throughout the treatment with DSX. Median RBC transfusion rate was 8500 ml/y, equivalent to about 2 erythrocyte concentrates per 3 weeks and a daily iron influx of 16.2 mg/d. The median LIC was 778 µg/g-liver wet weight (range: 460 - 1122 µg/g). From baseline treatment interval to the endpoint of treatment observation, liver iron decreased by 124 - 4689 µg/g-liver, and serum ferritin decreased by -596 to 8283 µg/l. For all measurement intervals, molar chelation efficacies between 18 % and 56 % were calculated at equilibrium with a median efficacy of 31 % (interquartile range = 16 %). This agrees with molar efficacies of DSX reported earlier but for relatively higher LIC and chelation doses (Blood 2005; 106(11):#2690 and Blood 2007; 110(11):#2776). *Conclusion.* Iron chelation therapy with DSX enables TM patients to stay on a relatively low iron burden, however, it is necessary to find a balance between the continuous iron accumulation from RBC transfusions and the toxicity of iron chelation therapy. Under these circumstances iron chelation therapy with DSX in TM patients with normal to low liver iron overload is safe, and does not result in increased creatinine levels or severe side effects and is as efficient as in patients with high liver iron overload.

0160

ONSET OF CARDIAC IRON LOADING IN A LARGE AND HOMOGENOUS COHORT OF THALASSEMIA MAJOR PEDIATRIC PATIENTS

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Background. Heart failure remains the main cause of mortality in thalassemia major (TM). Magnetic Resonance Imaging (MRI) by the T2* approach is the unique non invasive technique for highly reproducible quantifications of myocardial iron burden and is the gold standard for quantifying biventricular function parameters. It is important to determine the appropriate age to start MRI screening, because its high cost and no large availability. Few data are available in the literature. *Aims.* The aim of this study was to address this issue in a cohort of paediatric patients selected from a large TM population homogenous for geographical origin and treatment approach. *Methods.* We studied retrospectively 72 TM patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network with an age < 18 years (47 males, 4.2-17.9 years old, mean age 13.0±3.7 years). Myocardial iron overload (MIO) was measured by T2* multislice multiecho technique. Biventricular function parameters were quantitatively evaluated by cine images. *Results.* The global heart T2* value was 29±11 ms. No MIO was detected in the 33% of the patients; 44% of the patients showed a heterogeneous MIO with a global T2*value ≥ 20 ms; 10% showed a heterogeneous MIO with a T2* global value < 20 ms and 13% had a homogeneous MIO. No significant correlation was found between global heart T2* value and age. OR for a global heart T2* < 20 ms was 1.13 (P = 0.18) per year. The global heart T2* value did not show significant differences according to the sex (male 30.2±11.0 ms versus female 28.7±11.8 ms, P=0.568). None of patients with an abnormal global heart T2* value (<20 ms) was under 8 years of age. Global heart T2* value was negatively correlated with mean serum ferritin levels. OR for high serum ferritin levels (≥ 1500 ng/ml) was 8.4 (1.01-69.37 OR 95%CI) for abnormal global heart T2* values (<20 ms). The global heart T2* value did not show a significant difference with respect to the chelation therapy (P=0.322). No significant correlations were found between the global heart T2* values and the bi-atrial areas or the left ventricular (LV) and right ventricular (RV) morphological and functional parameters. Five patients showed a LV ejection fraction (EF) < 57% and one patient showed a RV EF < 52%. None of them was under 8 years of age. *Conclusion.* The MRI screening for both iron overload and function assessment can be started for TM patients at the age of 8 years. At this age not sedation is generally needed. If the availability of cardiac MRI is low, the serum ferritin levels can be used as a discriminating factor.

0161

QUANTITATIVE IRON OVERLOAD ASSESSMENT BY T2* MAGNETIC RESONANCE IMAGING: INFLUENCE OF MAGNETIC FIELD

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Background. The gradient echo multiecho T2* MRI technique is the most robust method for the sensitive and reproducible quantification of iron overload in transfusion dependent patients. The 1.5T MRI scanner is generally used in the clinical arena. However, given that 3T scanners are becoming largely widespread, there is a growing need to assess the practicality of evaluating MIO at 3T. *Aims.* This study aimed to establish the relationship between T2* values at 3T and 1.5T over the range of clinical interest of tissue iron concentrations. *Methods.* 38 transfusion-dependent patients (22 males, 36±7 years) were scanned at 1.5T and 3T. In the liver the T2* value was determined over a circular region of interest. For the heart, the T2* value on each of the 16 segments was calculated as well as the global value. The relationship between R2* (1000/T2*) values at 3T and 1.5T is expected to be linear and can be approximated as: (R2*3T=aR2*1.5T + I) Eq.1, so the relationship was found out in terms of R2* values. T2* values were transformed into their reciprocal R2*. The R2* values at 3T were plotted versus the correspondent values at 1.5T. The data were fitted to a straight line, of which the slope and the y-intercept were extracted. In the clinical practice is most common to use T2* values as measure of iron overload. So, the T2* values at 3T were expressed as a function of the values at 1.5T by inverting both the members of the equation 1. *Results.* Figure 1A shows the liver R2* values at 3T plotted against the corresponding values at 1.5T. At very high iron tissue concentrations the T2* assessment is lacking of precision because technical constraints, so the patients with a R2* value at 1.5 T greater than 1000 Hz were excluded from the fitting (cross-shaped marker). The line of best

fit had a slope of 1.917±0.061 and an intercept of -9.145±13.083 Hz. The R-squared value for the fit was 0.973. Figure 1B shows the global heart R2* values at 3T plotted against the corresponding values at 1.5T for the patients. The line of best fit for the patients had a slope of 1.961±0.053 and an intercept of -17.570±3.160 Hz. The R-squared value for the fit was 0.975. The fit was performed even for all the segmental T2* values. The line of the best fit had a slope of 2.008 ± 0.043 and an intercept of -19.205 ± 2.787 Hz. The R-squared value for the fit was 0.784. Specific conversion formulas for liver and global heart T2* values at 3T vs T2* at 1.5T are indicated in Figure 1C. *Conclusion.* For both liver and heart, the R2* values at 3T were about twice that at 1.5 T with an intercept depending on the non-iron component of the tissue. A conversion formula between T2* values at 3T and 1.5T was introduced and it may be used in the clinical arena by the centers that use 3T scanners.

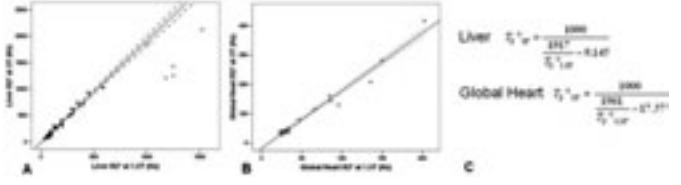

Figure 1.

0162

DIFFERENT PATTERNS OF MYOCARDIAL IRON OVERLOAD BY MULTISLICE T2* CARDIOVASCULAR MR AS MARKERS OF RISK FOR CARDIAC DYSFUNCTION IN THALASSEMIA MAJOR

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Background. The multislice multiecho T2* Cardiovascular Magnetic Resonance (CMR) technique allows to detect different patterns of myocardial iron overload (MIO). *Aims.* The aim of this study was to verify the risk of biventricular dysfunction related to different patterns of MIO in a large cohort of thalassemia major (TM) patients. *Methods.* 1135 TM patients (538 M, 30±19 years) enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) network underwent CMR . For the assessment of MIO, three short-axis views of the left ventricle (LV) were acquired and the myocardium was segmented into 16-segments standardized LV model. The T2* value on each segment was calculated as well as the global T2* value. Biventricular function parameters were quantitatively evaluated by cine images. *Results.* Four groups of patients were identified: homogeneous MIO (all segments with T2*<20 ms) (N=173), heterogeneous MIO (some segments with T2*≥20 ms and other segments with T2*<20 ms) and global heart T2*<20 ms (N=160), heterogeneous MIO and global heart T2*≥20 ms (N=33) and no MIO (all segments with T2*≥20 ms) (N=465). The LV ejection fraction (EF) was significant different among the groups (P<0.0001) (Figure 1, top). Odds Ratio for LV dysfunction (LV EF<57%) was 4.8 (3.1-7.3 OR 95%CI; P<0.0001) for patients with homogeneous MIO vs patients with no MIO and 1.9 (1.2-3.2 OR 95%CI; P=0.007) for patients with heterogeneous MIO and global heart T2*<20 vs patients with no MIO. The right ventricular (RV) EF was significant different among the groups (P<0.0001) (Figure 1, bottom). Odds Ratio for RV dysfunction (RV EF<55%) was 2.1 (1.4-3.2 OR 95%CI; P=0.001) for patients with homogeneous MIO vs patients with no MIO. *Conclusions.* Biventricular dysfunction is correlated with MIO distribution decreasing from the patients with homogeneous MIO to the patients with no MIO. Homogeneous MIO and heterogeneous MIO with a global heart T2*<20 predicts a significantly higher risk to develop cardiac dysfunction suggesting an intensive chelation therapy in this group of patients.

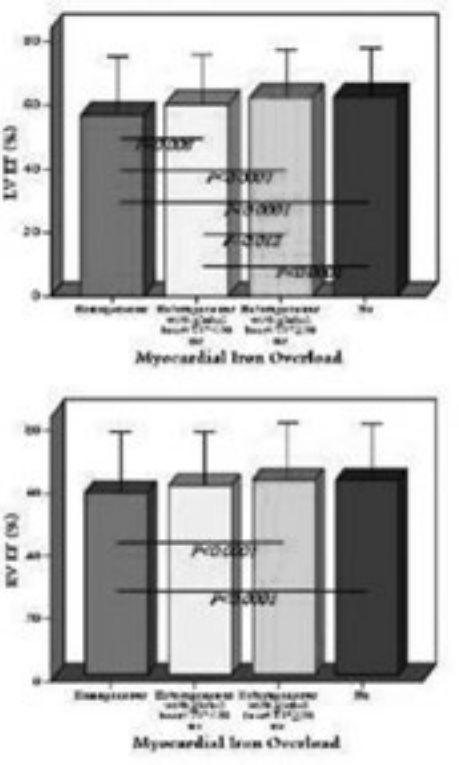

Figure 1.

0163

THYROID DYSFUNCTION IN PEDIATRIC EGYPTIAN THALASSEMICS: WHAT ABOUT IODINE DEFICIENCY?

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Introduction. Primary hypothyroidism is one of the most frequent endocrinal complications observed in patients suffering from thalassemia (10-50%). Long standing anemia, hypoxia and hemosiderosis were proposed as a cause. Iodine deficiency is an important contributing factor for hypothyroidism. Iodine deficiency is not uncommon among Egyptian children, its contribution to hypothyroidism in thalassemia major patients was not studied. The urinary iodine concentration is the most useful test for assessing iodine nutrition in populations. The aim of the present study was to assess the thyroid hormones and the urinary iodine level in Egyptian thalassemic pediatric patients. *Study design.* Sixty thalassemia patients (31 males, 29 females), mean age 14.4±3.83 years) followed up at the hematology clinic were randomly selected to participate in this study together with thirty six age and sex matched controls after the parents gave their consent. All patients were subjected to thorough clinical examination. Serum evaluation of T3, T4, TSH and urinary iodine. *Results.* A highly significant difference was observed in urinary iodine, T3, T4 and TSH between the thalassemic and the control groups (P<0.001). T3 was low in 81.7% (n=49), T4 in 68.3% (n=41) while TSH was elevated in 81.7% (n=49) of the patients. Overt hypothyroidism (low T4 and elevated TSH >10 uIU/ml) was present in 45% (n=27)of the patients, while all controls were euthyroid with normal urinary iodine. Severe iodine deficiency was present in 15% (n=9), moderate deficiency in 45%(n=27) and mild deficiency in 40% (n=24) of the patients . Thyroid function tests results varied among these groups of iodine deficiency. A negative correlation was found between urinary iodine and both serum ferritin and TSH (r=-0.413, P<0.001, and r=0.881, P< 0.001 respectively) and both serum ferritin and age (r=0.355, P<0.001, r=0.491, P<0.001). *Conclusion.* Correction or supplementation of iodine to thalassemia patients may have a role in prevention or correction of hypothyroidism in thalassemia patients especially in areas where iodine deficiency is common like Egypt.

0164

A MRI PROSPECTIVE SURVEY ON HEART AND LIVER IRON AND CARDIAC FUNCTION IN THALASSEMIA MAJOR PATIENTS TREATED WITH DEFERASIROX VERSUS DEFERIPRONE AND DESFERRIOXAMINE IN MONOTHERAPY

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Background. No prospective data are available about the efficacy of deferasirox versus deferiprone and desferrioxamine in monotherapy. *Aims.* Our study aimed to prospectively assess the efficacy of deferasirox versus deferiprone and desferrioxamine in monotherapy in a large cohort of thalassemia major (TM) patients by quantitative Magnetic Resonance (MR). *Methods.* Among the 1135 TM patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network we selected those with an MR follow up study at 18±3 months who had been received one chelator alone between the 2 MR scans, We identified three groups of patients: 80 treated with DFX, 39 with DFP and 74 with DFO. Iron overload was measured by T2* multiecho technique. Biventricular function parameters were quantitatively evaluated by cine images. *Results.* Excellent/good levels of compliance were similar in the DFX (98.8%) vs DFP (94.9%) and DFO (95.9%) groups. The percentage of patients who maintained a normal global heart T2* value (≥20 ms) was comparable for DFX (98.1%) vs DFP (100%) and DFO (98.1%) groups. The percentage of patients that maintained a normal left ventricular ejection fraction (LVEF>57%) was significantly lower in DFX (75.8%) versus DFP group (100%) (P=0.002), but no versus DFO group (83.1%). Among the patients with myocardial iron overload at baseline, in all three groups there was a significant improvement in the global heart T2* value (DFX: +3.5±4.7 ms P=0.001, DFP: +8.8±8.6 ms P=0.015 and DFO: 3.7±5.5 ms P=0.001) and a reduction in the number of pathological segments (DFX: -2.4±3.8 P=0.003, DFP: -6.0±5.6 ms P=0.012 and DFO: -2.9±3.7 ms P=0.001).

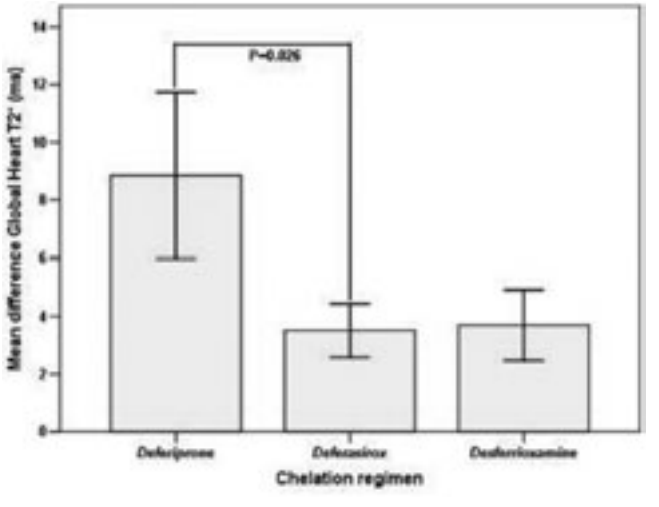

Figure 1.

Only in the DFP group there was a significant improvement in left and right ventricular ejection functions (+5.0±6.4% P=0.045 and +6.8±3.7% P=0.001, respectively). The improvement in the global heart T2* was significantly lower in the DFX versus the DFP group (P=0.026, see figure), but it was not significantly different in the DFX versus the DFO group. The improvement in the LVEF and in the RVEF was higher in the DFP group than in the DFX group at the limit of the significance (P=0.066 and P=0.062, respectively); it was comparable between DFX and DFO groups. Among the patients with hepatic iron at baseline (T2*<9.2 ms) the changes were not significantly different in DFX versus the other groups. *Conclusions.* Prospectively in a large clinical setting of TM patients, DFX monotherapy was significantly less effective than DFP in improving myocardial siderosis and in maintaining a normal left ventricular ejection fraction.

0165

THE PALATABILITY AND TOLERABILITY OF DEFERASIROX TAKEN WITH DIFFERENT LIQUIDS OR FOOD

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Background. Deferasirox is an oral iron chelator indicated for the treatment of transfusional hemosiderosis in patients aged ≥2 years. The recommended mode of administration is to be taken on an empty stomach in water, apple juice or orange juice ≥30 minutes before food. Post-marketing reports have noted discontinuation or reduced compliance of deferasirox secondary to palatability and gastrointestinal adverse events. Registration trials with deferasirox did not evaluate food combinations to maintain predictable plasma levels since single dose pharmacokinetic studies suggested that bioavailability was increased by food. *Aims.* Assess palatability and tolerability of deferasirox when taken with meals, various liquids, and crushed and added to food. *Methods.* A single-arm, open-label, multi-center study (NCT00845871) enrolling patients with transfusional hemosiderosis (minimum entry serum ferritin ≥500 µg/L) aged ≥2 years with thalassemia major, sickle cell disease, low or intermediate-1 risk MDS or other anemias, who were on, starting, or resuming treatment with deferasirox. The study began with a 1-month run-in phase with deferasirox dosed according to prescribing information, followed by a 3-month assessment phase where subjects chose each week from 5 options: with/without meals; morning/evening; crushed and added to soft food/liquid of choice. Palatability was assessed with a Five-Point Facial Hedonic Scale with additional questions capturing GI effects. *Results.* Sixty-five patients were enrolled (Table 1).

Table 1.

Summary of underlying hematological disorder, serum ferritin, and patients with GI events by age

Category	Age ≥2 to <10 years (n=10)	Age 10 to <60 years (n=44)	Age ≥60 years (n=11)	Total (N=65)
Median age, years (range)	6.0 (3–9)	17.5 (10–48)	74.0 (60–83)	18.0 (3–83)
Hematological condition				
Sickle cell disease, n (%)	3 (30)	22 (50)	0	25 (38.5)
Thalassemia major, n (%)	5 (50)	15 (34.1)	0	20 (30.8)
Myelodysplastic syndrome, n (%)	0	0	9 (81.8)	9 (13.8)
Other anemia, n (%)	2 (20)	7 (15.9)	2 (18.2)	11 (16.9)
Serum ferritin				
Median baseline[a], µg/L (range)	2680 (800–6880)	2200.5 (560–8660)	4[illegible] ([illegible])	
Median end-of-study[b], µg/L (range)	2442.5 (1234–5340)	1968 (440–8040)	3670 (1677–6040)	
Patients with GI events during run-in phase (n=62; by age group: n=9/43/10, respectively)				
Diarrhea, n	1	9	5	15
Abdominal pain, n	1	6	1	8
Nausea, n	0	5	4	9
Vomiting, n	0	2	2	4
Patients with GI events during assessment phase (N=65)				
Diarrhea, n	1	3	3	7
Abdominal pain, n	3	9	2	14
Nausea, n	1	4	2	7
Vomiting, n	0	4	2	6

[a] 68% of patients received deferasirox prior to study; [b] 4 months of treatment

Palatability ratings improved with the introduction of food. The mean percentage of weeks that each patient rated a mode as "dislike extremely"or "somewhat dislike"was lower during the assessment phase as compared to the run-in phase. No particular mode was clearly more palatable than others. The most popular soft foods chosen were apple sauce and yogurt; the most popular liquids chosen were orange and apple juice. An age effect was observed for GI disturbances during the (fasting) run-in phase with older subjects (≥60) reporting higher rates of nausea (40% versus 9.6%, P=0.04) and diarrhea (50% versus 19%, P=0.09) compared to younger subjects. Gastrointestinal adverse events decreased with the addition of food. There were 46 occurrences of diarrhea in 15 patients (n=62) during run-in versus 29 occurrences in 7 patients (n=65) during assessment (P=0.08 by patients); there were 2 patients with diarrhea in both the run-in and assessment phases. Most diarrheal events occurred ≤2 hours after dose. The rates of other GI events in the run-in versus assessment phases included: 54 versus 47 abdominal pain events; 31 versus 23 nausea events; and 10 versus 15 vomiting events. There were no marked differences in renal or liver function tests between phases. *Conclusions.* Administration of deferasirox with food was rated by patients as more palatable and appeared to lessen GI adverse events. This study suggests that the addition of food may improve the palatability and tolerability of deferasirox among those experiencing difficulties with the approved dosing schedule. Further evaluations of safety and the effect of food on pharmacokinetics at steady state are required.

0166

INCIDENCE OF ARTERIAL AND VENOUS THROMBOEMBOLIC EVENTS AMONG PATIENTS WITH THALASSEMIA. ANALYSIS OF PREDISPOSING FACTORS

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Bakground/Aims. Homozygous beta-thalassemia has been considered a hypercoagulable state, particularly following splenectomy. However, few large series of patients have been reported, describing the frequency of thrombotic complications in this group of diseases. We herein present our experience on venous and arterial thrombotic events, encountered among a large group of 538 thalassemic patients and investigate the relevant risk factors. *Patients and Methods.* Medical records of all thalassemic patients of three Thalasemia Units were studied, and all venous and arterial thromboembolic events were collected and analyzed. Three hundred and sixty-one patients (67.1%) had thalassemia major (TM), 168 (31.2%) had thalassemia intermedia (TI), and 9 had Hemoglobin (Hb) H disease (1.7%). In this population the prevalence of thromboembolic events was analyzed, in relation to disease genotype, patient's age, frequency of transfusions, serum ferritin levels, type of iron chelation, the presence of HCV infection, adequacy of hepatic function, history of previous splenectomy, Hb levels, MCV, platelet count, mean platelet volume (MPV), presence of nucleated red blood cells (RBC), total serum globulin levels, and the presence of FV Leiden-, FIIG20210A-, beta-Fibrinogen-455G=>A-, FXIII-, MTHFRC667T and MTHRF1298CT mutations, which have been associated with a hereditary thrombophilic state. *Results.* Totally, 17 (3.2%) venous and 7 (1.3%) arterial thromboembolic events were recorded. In particular, there were 7 episodes of portal vein thrombosis, 4 of pulmonary embolism, 4 of pulmonary hypertension, 3 of central venous catheter thrombosis, extended to left atrium, 3 of deep vein thrombosis (DVT) and 3 ischemic cerebral attacks. The frequencies of thrombophilic mutations in the studied population were: 2.5% for FV Leiden, 1.7% for FII G20210A and 7.1% for MTHFR C667T polymorphism homozygocity. These mutations were not related to any increase in the incidence of thrombosis in patients with TM and TI. Statistical analysis of the remaining parameters studied, revealed that genotype IVSI-6, and previous splenectomy were independent risk factors for the appearance of both, venous and arterial thrombosis. The prevalence of venous thrombosis in TM and TI were 2.49% and 7.7% respectively, whereas that of arterial thrombosis was estimated at 0% for TM and 4.2% for TI. By removing central venous catheter events the prevalence of venous thrombosis in TM was calculated 1.66%. Fourteen of the 17 venous thrombotic episodes and all arterial thrombotic events occurred in splenectomized patients. These events occurred 8 days to 45 years post-splenectomy. Platelet count at the onset of thrombotic event ranged between 250 and 1400x103/μl, but was not a significant factor for the event. TI patients with a thrombotic episode were sporadically transfused, while they were splenectomised due to severe hypersplenism. *Conclusions.* TI patients exhibit substantially higher risk for both, venous and arterial thrombosis as compared to TM patients. Splenectomy and disease genotype, associated with an intermediate phenotype were shown to be the major contributing factors for thrombosis. For TI patients, not regularly transfused, the presence of abnormal and of nucleated RBCs in circulation and endothelial damage are considered major factors contributing to thrombosis.

0167

NEW INSIGHTS ON β-THALASSEMIA IN GAZA: HIGH FREQUENCY AND MILDER PHENOTYPE AMONG IVS1-1 HOMOZYGOUS PATIENTS WITH HIGH LEVELS OF FETAL HEMOGLOBIN

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Background. Beta thalassemia is a very common disease in the Gaza strip. The rate of carriership is estimated to be 4.3% and there are around 300 homozygous patients treated by transfusions. Premarital screening is being implemented since 2000 and this is the only mean of prevention as prenatal diagnosis is not performed routinely. The aim of the present study is to understand better the correlation between genotype and phenotype in the thalassemic population of Gaza. *Patients and Methods.* Peripheral blood samples were obtained from 82 patients with β thalassemia major from 54 families, after signing an informed consent. Analysis of β-globin mutations and XmnI polymorphism were carried out using PCR followed by either Allele Specific Oligonucleotide hybridization, sequencing and endonuclease restriction. *Results.* Thirteen different mutations have been identified, which have been previously describes in Mediterranean populations. However, the distribution of the mutated alleles among the patients in Gaza is quite unique. The most common mutation was IVS1-1 G-A, which was prevalent in 31.5% of the thalassemic alleles. The IVS1-110 mutation, which is the most frequent mutation in the Eastern Mediterranean as well as in other Arab countries, was found in 25% of alleles. In addition, the mutations of IVS1-6, Frameshift 5, Nonsense 39, IVS1-(-1) were found among 9%, 8%, 7% and 6% of the chromosomes respectively. Two rare mutations were also found. The novel Poly A mutation AATAAA- A——-, which was reported previously only in Gaza, was found in an additional family. In addition, the 25 bp deletion which is prevalent in the Arab Golf countries, mostly in Bahrain, was first diagnosed in a Palestinian family. Fifteen patients were homozygotes for IVS1-1 and 20 were homozygotes for IVS1-110 mutations. The homozygotes for IVS1-1 mutation required less blood, since they consumed only 13 packed red cell units a year while those with IVS1-110 consumed 24 blood units a year ($p<0.00001$). Their mean hemoglobin level was 8.3gr/dl and 7.68gr/dl (Figure A,B) respectively, and there was no significant difference in age or gender distribution between these two groups. The reason for the difference in blood requirements between the 2 groups are over expression of fetal hemoglobin (HbF): The mean HbF level was 46% in the homozygotes for IVS 1-1 and 6% for those with IVS 1-110 ($p<0.0001$) (Figure C). The XmnI polymorphism at the gamma globin gene, which is known to be associated with elevated HbF levels, was found in 7 out of 15 IVS1-1 patients. The XmnI positive patients had HbF level of 76.86% compared to 18.76% ($p<0.0001$). *Conclusion.* The distribution of β-thalassemia among patients in Gaza is unique with a highest frequency of IVS1-1 mutation which is a βo mutation resulting in severe β thalassemia major. Counter to expectation, these patients required less blood transfusions, due to persistent production of HbF. The association between IVS 1-1 mutation with high expression of HbF has not been previously described.

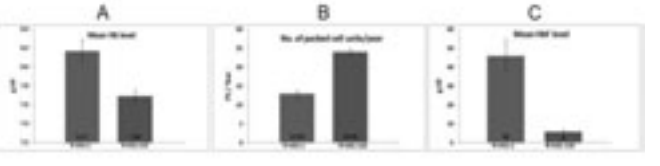

Figure 1.

0168

MATERNAL AND FETAL COMPLICATIONS IN THALASSEMIA MAJOR: OUTCOMES FROM A 10 YEAR STUDY AT MOUNT SINAI HOSPITAL IN TORONTO, CANADA

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Background. Thalassemia major is an inherited disorder of ineffective erythropoiesis, resulting from severely defective or absent production of beta globin chains. Lifelong chronic transfusion support leads to iron overload, affecting the pituitary-gonadal axis as well as cardiac and hepatic siderosis. With advances in the detection of iron overload and improved chelation therapy, it has been increasingly possible for women to successfully undergo pregnancy. This is the first centre in North America to date that has studied pregnancy outcomes in this population. *Aims.* To identify maternal and fetal complications in pregnant women with Beta Thalassemia Major, with reference to the Ontario population. *Methods.* A retrospective study of consecutive deliveries of thalassemia major patients at Mount Sinai Hospital Special Pregnancy Program, from September 2000 through December 2010 was conducted. Maternal demographics, pregnancy complications including transfusions, mode of delivery, apgar scores and fetal complications were recorded. Patients were identified from the Special Pregnancy Database and the Delivery Databases of Mt. Sinai Hospital. Electronic patient records from the Toronto General Hospital and Mt.Sinai Hospital paper charts were reviewed. *Results.* A total of 44 women were identified. There were 41 singleton and 2 sets of twin live birth deliveries and 1 neonatal death. Median maternal age was 32(range 21 to 41) years with a median gestational age of 38.5 (range 27 to 41) weeks. Only 15% of women delivered before 37 weeks. For 56% of women it was their first live birth. 39% of deliveries were by C-section. One mother developed mitral regurgitation during the pregnancy. Gestational diabetes developed in 9% and pregnancy induced hypertension (PIH) in 2/44 (4.5%) There were no cases of intra-uterine growth retardation. Median birth weight was 3158 (930-4860). 13% of neonates were born less than 2.5 kg. Mean apgar scores at 1 and 5 minutes were 8.3 ± 1.5 and 9.0 ± 0.2 respectively. There were no fetal anomalies. *Summary/Conclusions.* Although the C-section rate was higher than the 2005/2006 Ontario rate of 28% it was at the lower end of the reported range in Thalassemia, of 26-93%. This relatively low C section rate probably reflects optimal transfusion support and suppression of marrow expansion, preserving normal pelvic anatomy. Preterm labour and neonates with a low birth weight were higher than the 2005/ 2006 Ontario rates of 8.6% and 7% respectively. Gestational diabetes was higher than the 4% found in the Ontario data, indicating pituitary and pancreatic iron deposition. Other maternal and fetal outcomes in our study did not differ from averages for healthy women. Pregnancy outcomes in women with thalassemia major are excellent if care is provided by an experienced multidisciplinary team including hematologists and high risk pregnancy obstetricians.

0169

RELATIONSHIP BETWEEN SERUM FERRITIN AND LIVER IRON CONCENTRATION IN HEMOGLOBIN E THALASSEMIA

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Background. Hemoglobin E thalassemia, the most common form of severe thalassemia worldwide, is associated with body iron loading even in the absence of frequent transfusions. *Aims.* The aim of the study was to determine the degree of relationship between serum ferritin and liver iron concentration in hemoglobin E thalassemia patients. *Methods.* Approvals were obtained from the human research ethics committees at The University of Kelaniya, The University of Western Australia, and the University Health Network, Toronto. Informed consent was obtained from all subjects. Hemoglobin E thalassemia subjects were recruited from the National Thalassaemia Centre, Kurunegala, Sri Lanka. Patients were then categorized into to two groups (1) those who had received less than or equal to 20 transfusions lifelong (N=25) and (2) those who had received more than 20 transfusions lifelong (N=61). LIC was measured for all subjects using spin density projection assisted R2-MRI (FerriScan®). *Results.* The mean age of subjects was 27.3 ± 16.1 [range 7.8 to 57.4] years for group 1 and 23.5 ± 10.1 [range 8.3 to 60.2] years for group 2. The figure shows the relationships between serum ferritin and LIC for groups 1 and 2. Solid lines are linear regression fits to the data; gradient 29.9 (± standard error 10.1) ng ferritin/L per mg Fe/g dw for group 1 and gradient 71.0 (± standard error 15.1) ng ferritin/L per mg Fe/g dw for group 2. Dashed lines represent the 95% confidence bands of the line of best fit. For group 1, there was a weak (r^2=0.28) but significant (p=0.007) correlation between serum ferritin and LIC. For group 2, there was also a weak (r^2=0.27) but significant (p< 0.0001) correlation with LIC. However, for both groups the 95% prediction intervals for LIC for a given serum ferritin were so broad as to make serum ferritin of little clinical value in determining liver iron loading. For example, a serum ferritin of 1500 ng/L was associated with 95% prediction intervals of LIC of 21.2 to 83.1 mg Fe/g dw (group 1) and 0.3 to 13.2 mg Fe/g dw (group 2). *Conclusions.* Serum ferritin measurements have limited clinical value for assessing the degree of iron loading of patients with hemoglobin E thalassemia.

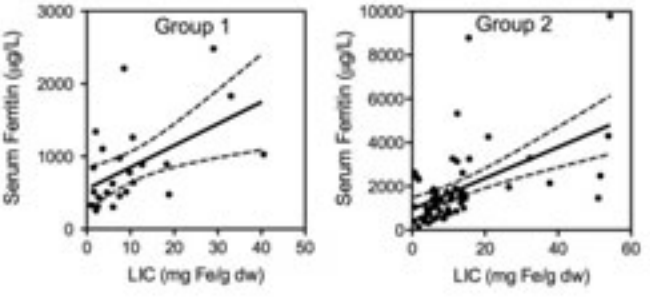

Figure 1.

0170

PUBERTAL EVALUATION OF FEMALES AND MALES WITH B-THALASSEMIC MAJOR IN RELATION TO CHELATION REGIMEN

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While transfusion and iron chelation therapy have increased longevity of patients with beta-thalassemia major (BTM), yet puberty disorders and infertility have become more apparent. *Aim of the study.* To evaluate the prevalence of delayed puberty and the pituitary gonadal axis in transfusion-dependent BTM patients in relation to chelation type and degree of iron overload. Also, to assess the spermatogenic function in pubertal males with BTM males. *Patients and Methods.* A two year prospective study of 93 regularly transfused thalassemics; 42 males and 51 females; 14 years and above. Sixty-Two (66.7%) were on deferiprone (DFP), 21 (24%) on desferrioxamine (DFO), and 10(9.3%) on combination DFO & DFP. At study entry, after baseline compliance analysis, endocrinal evaluation were performed. Those with delayed and arrested puberty were tested for pituitary -testicular axis and pituitary-ovarian function by gonadotrophin-releasing hormone (GnRH) and human chorionic gonadotropin (HCG) tests. Spermiograms were done for pubertal males. Patients with delayed puberty with good pituitary and gonadal responses were shifted to combined chelation therapy, and yearly pubertal assessment and spermiogram for pubertal males. *Results.* Median age was 20 years, Thirteen male patients (30.9%) had normal puberty; they were on either DFP 7(53.8%), DFO 2(15.4%) or combination 4(30.8%), while twenty- nine (69%) had puberty disorder (21 on DFP, 8 on DFO); Out of non pubertal males; 13(44.8%) had good pituitary and testicular response to LHRH stimulation. Forty seven % of studied females had pubertal disorders; delayed puberty (13.7%), primary amenorrhea (17.6%) or secondary amenorrhea (15.7%) and 75% of the delayed puberty were on DFO alone. During two years follow-up using intensive combination chelation therapy(DFP + DFO), 7 of good pituitary responders males progressed to puberty and six females had reversal of their hypogonadism (24.9%).Total sperm count and sperm motility were decreased in 4 (25%) and 6 (35.3%) patients respectively. In conclusion, The risk of hypogonadism in B-TM patients remained high. Intensive combination chelation for 2 years lead to better sexual development in both sexes.

0171

HEART T2* FOR PREDICTION OF CARDIAC COMPLICATIONS IN WELL-TREATED TM PATIENTS

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[10]*Serv. Talassemia, Osp. "V. Emanuele III", Gela, Italy*
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Background. T2* Magnetic Resonance Imaging (MRI) technique allows noninvasive quantification of organ-specific iron burden, playing a key role in the management of thalassemia major (TM) patients. There are few data on the incidence of heart failure and arrhythmias in TM patients according to baseline T2* values. *Aims.* The aim of this study was to establish prospectively the risk of cardiac complications in a large cohort of well-treated TM patients. *Methods.* We considered 527 TM patients (252 males, mean age 30±9 years) for who clinical data relative to a period of 5 years after the first MRI were collected in a central data base. At time of the first scan mean ferritin levels were1653±1559 ng/l, global heart was 27±13 ms, and excellent/good level of compliance were present in the 96% of the study population. *Results.* At 5 years of follow-up, we recorded 24 cardiac events: 4 episodes of cardiac failure, 15 of arrhythmia, 1 of pulmonary hypertension and 4 of other cardiac complications. The majority of these events (21/24) happened within the first 24 months subsequent to the MRI, so we considered this follow-up period. At the first MRI scan, in patients with cardiac complications the global heart T2* was 22.5 ±12.4 ms. In comparison with global heart T2* values ≥20 ms, there was not a significantly increased risk of cardiac complications associated with global heart T2* values <20 ms (HR= 2.028 P=0.09). In the heart failure patients the global heart T2* was 19±12 ms. In comparison with global heart T2* values ≥20 ms, there was not a significantly increased risk of heart failure associated with global heart T2* values <20 ms (HR=1.9 P=0.524) or <10 ms (HR=2.6 P=0.443). In the arrhythmic patients the global heart T2* was 25±13 ms. In comparison with global heart T2* values ≥20 ms, there was not a significantly increased risk of arrhythmia associated with global heart T2* values <20 ms (HR=2.1 P=0.179) or <10 ms (HR=0.8 P=0.824). During the follow up changes in the chelation therapy (type and/or dose-frequencies) were found in > 25% of the study population. *Conclusion.* We detected very few cardiac events, almost all concentrated in the first 24 months. In a large cohort of well-treated TM patients heart T2* lost its power in predicting cardiac events probably due to a patient-specific adjustment of the chelation therapy MRI-guided.

0172

A MRI PROSPECTIVE SURVEY ON CARDIAC AND HEPATIC IRON AND CARDIAC FUNCTION IN THALASSEMIA MAJOR PATIENTS TREATED WITH SEQUENTIAL DEFERIPRON-DESFERRIOXAMINE VERSUS DEFERASIROX.

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Background. No data are available in literature about possible different changes in cardiac and hepatic iron and in cardiac function in thalassemia major (TM) patients treated with sequential deferipron-desferrioxamine (DFP-DFO) versus deferasirox (DFX). Magnetic Resonance (MR) is the unique non invasive suitable technique to evaluated quantitatively this issue. *Aims.* Our aim was to prospectively assess the efficacy of the DFP-DFO vs DFX in a large cohort of TM patients by quantitative MR. *Methods.* Among the first 1135 TM patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network, 392 patients performed a MR follow up study at 18±3 months according to the protocol. We evaluated prospectively 35 patients treated with DFP-DFO versus 80 patients treated with DFX between the 2 MR scans. Cardiac iron was evaluated by T2* multiecho multislice technique. Biventricular function parameters were quantitatively evaluated by cine images. Liver iron was measured by T2* multiecho technique. *Results.* Excellent/good levels of compliance were similar in the two groups (DFP-DFO 97.1% vs DFX 98.8%; P=0.544). Among the patients with no significant myocardial iron overload (MIO) at baseline (global heart T2*≥20 ms), there were no significant differences between groups to maintain the patients without myocardial iron overload (DFP-DFO 96% vs DFX 98%; P=0.536). Among the patients with MIO at baseline, in both groups there was a significant improvement in the global heart T2* value (DFP-DFO: 4.8±3.9 ms P=0.004 and DFX: 3.5±4.7 P=0.001) and a significant reduction in the number of pathological segments (DFP-DFO: -3.2±3.8 P=0.026 and DFX: -2.4±3.8 P=0.003). Only in sequential group there was a significant increment in the left and right ventricular ejection fractions (4.3±5.1% P=0.035 and 6.7±6.6% P=0.017, respectively). The improvement in the global heart T2* was not significantly different between groups. The improvement in the left as well in the right ventricular ejection fractions was significantly different between groups (P=0.009 and P=0.015, respectively) (Figure 1). Among the patients with hepatic iron at baseline (T2*<9.2 ms), only in the DFX group there was a significant improvement in the liver T2* value (2.6±5.3 ms P=0.001). The changes in liver T2* were significantly higher in DFX group than in DFP-DFO (0.5±2.0 ms) group (P=0.030). *Conclusions.* Prospectively no significant differences on cardiac iron were found between TM patients treated with sequential DFP-DFO and TM patients treated with DFX, although the DFP-DFO was significantly more effective in improving biventricular function. DFX was significantly more effective in reducing hepatic siderosis.

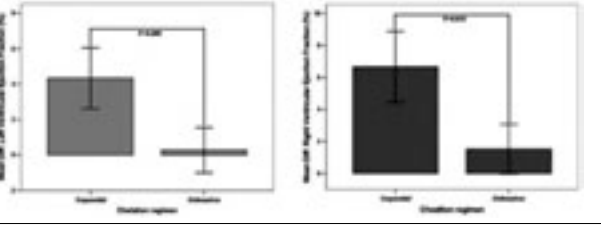

Figure 1.

Clinical thrombosis

0173

SELECTION CRITERIA OF PATIENTS WITH VENOUS THROMBOEMBOLISM FOR LABORATORY INVESTIGATION OF INHERITED THROMBOPHILIA

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Background. Laboratory investigation for inherited thrombophilia is warranted in young patients, especially those with severe venous thromboembolism (VTE) occurred spontaneously or recurrently. Investigation of older patients is discouraged, especially when events are mild or provoked. Such policy could miss a number of carriers, leaving undiagnosed their kindreds. *Aims.* To investigate whether clinical parameters are predictive of the presence of inherited thrombophilia in VTE patients. *Patients and Methods.* We analyzed the files of 1,835 patients referred to our Thrombosis Center between 1996 and 2009. The median age at the first VTE was 37 years (range 0-89); 736 were males (40.1%). Patients were stratified according to family history of VTE, age of first VTE (<45 years), type of first VTE (defined severe for proximal DVT and/or pulmonary embolism and mild for distal DVT or superficial vein thrombosis), circumstances of first VTE (unprovoked or provoked), history of recurrent VTE. Multiple regression was carried out labelling as dependent variable diagnosis of overall thrombophilia or severe thrombophilia (antithrombin or protein C or protein S deficiency, homozygous or multiple defects, n=211) or mild thrombophilia (heterozygous factor V Leiden or prothrombin G20210A, n=415). *Results.* Diagnosis of overall thrombophilia was associated with family history (p=0.005), severity of VTE (p=0.008) and recurrent events (p<0.0001); the aforementioned criteria were all absent only in 8% of patients with thrombophilia. Among patients with thrombophilia 30% had clinical onset >45 years, 62% had a first provoked VTE, and 11% had both. Severe thrombophilia was associated with family history (p=0.02), first unprovoked VTE (p=0.015) and recurrent events (p=0.04). Mild thrombophilia was associated with family history (p=0.05), severity of VTE (p=0.03) and recurrent events (p<0.0001). *Conclusions.* Family history, clinical severity and recurrence of VTE are strong predictors of inherited thrombophilia, and at least one of these parameters is present in more than 90% of cases. Selection of the patients to be investigated according only to age and/or circumstances of the first VTE could miss diagnosis of thrombophilia in a relevant number of cases.

0174

THE IMPACT OF VENOUS THROMBOEMBOLISM IN CRITICALLY ILL PATIENTS: A META-ANALYSIS OF MAJOR CLINICAL OUTCOMES

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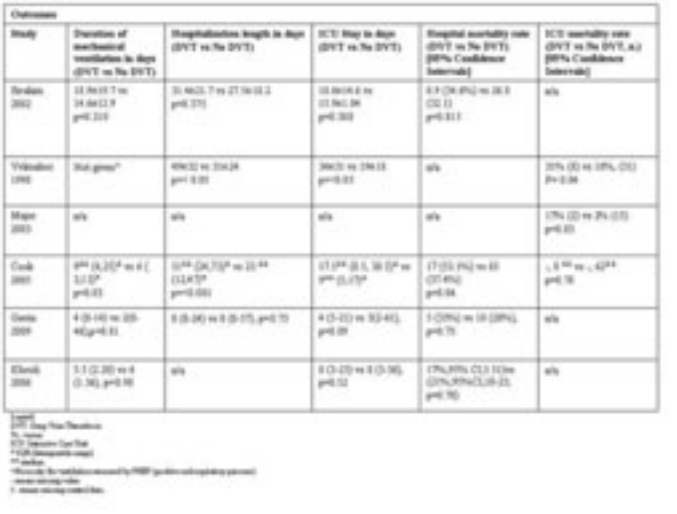
Background. Critically ill patients are at high risk of developing venous thromboembolism (VTE) during their stay in the intensive care unit (ICU) because of premorbid medical and surgical conditions. The clinical consequences of Deep Vein Thrombosis (DVT) have the potential to be serious yet are frequently unrecognized in the Intensive Care Unit (ICU). In contrast to the extensive documentation on the short and long-term outcomes of patients with DVT evaluated in other clinical settings, little is known about the clinical course of this disease in the ICU setting. We hypothesized that both undetected and clinically evident VTE would affect the prognosis of critically ill patients. *Purpose.* To systematically review whether a diagnosis of DVT in critically ill patients affects clinically important outcomes including length of stay, duration of mechanical ventilation and mortality. *Material and Methods.* MEDLINE and EMBASE databases were searched up to June 2010. Two reviewers performed study selection independently. Studies were selected if evaluate one or more of the following outcomes: hospital and ICU mortality, duration of patient stay in hospital and in ICU, and duration of mechanical ventilation. Two investigators independently extracted and reviewed data from each study; including study and patient characteristics and outcomes. Association between DVT and hospital and ICU mortality, and the mean difference of duration of patient stay in hospital and in ICU, and duration of mechanical ventilation in patients with and without DVT were calculated using a random-effects model (DerSimonan and Laird method). Pooled results are reported as relative risk (RR) and mean difference and are presented with 95% confidence interval (CI) and with 2-sided P values. A P value of .05 or less was considered statistically significant. Statistical heterogeneity was evaluated using the I2 statistic, which assesses the appropriateness of pooling the individual study results [22]. The I2 value provides an estimate of the amount of variance across studies due to heterogeneity rather than chance. Cohen's Kappa for inter-rater agreement was used to assess inter-rater reliability. *Results.* Six studies for a total of 1518 patients were included in the systematic review. Patients diagnosed with DVT compared to those without DVT had increased ICU and hospital stay (7.3 days (95% CI 1.4 to 13.2; P= 0.02) and 16.5 days (95% CI 1.51 to 30.59; P= 0.03), respectively. Duration of mechanical ventilation appeared to be increased in patients with DVT although this difference was not statistically significant (weighted mean difference: 3.41 days 95 % CI -1.12 to 7.94; P=0.14). Patients diagnosed with DVT also had a marginally significant increase in the RR of hospital mortality (RR 1.31 95%CI,0.99 to 1.74,P=0.06), and a non statistically significant increase in the RR of ICU mortality (RR 1.96; 95% CI 0.74 to 5.19; P = 0.17). *Conclusions.* A diagnosis of DVT upon ICU admission appears to affect clinically important outcomes including length of ICU and hospital stay and hospital mortality. Further research involving larger prospective study designs are warranted.

Table 1.



0175

VENOUS THROMBOEMBOLISM IN ADOLESCENTS WITH CANCER - A SINGLE CENTRE EXPERIENCE

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Background. The association between cancer and venous thromboembolism (VTE) is well documented with a reported incidence of 4.1% from a large adult series. Risk factors for thrombosis include the underlying neoplasm and its therapy, patient age, other predisposing medical conditions and immobilization. Younger age is considered protective. The presence of central venous catheters (CVC) is perhaps the single most important predisposing factor in the development of VTE in children. The British Committee for Standards in Haematology (BCSH) has recently released a guideline on the prevention, investigation and management of VTE in children below 16 years of age. In March 2010 the NICE VTE thromboprophylaxis guidelines for adults aged 18 and above were introduced. Our adolescent unit treats approximately 72 teenagers (aged 13-19) with cancer each year; most of whom have CVCs inserted to facilitate treatment. There is a paucity of evidence for the risk of developing a VTE across this specific age range and lack of clarity in recommendations for patients between 16-18 years of age. This has prompted an evaluation of the VTE episodes in our unit and to audit our procedure against both current national guidelines. *Aims.* 1) To identify incidence of VTE in an adolescent cancer population and any specific patient characteristics and risk factors. 2) Audit our population and current practice

against both sets of guidance. 3) Confirm our diagnostic methods and treatment follow national guidelines. *Methods.* Retrospective casenote review of all adolescents with cancer diagnosed between 2005 and 2010 who developed a clinically significant VTE during treatment. Patient records were cross referenced with pharmacy records of patients treated with dalteparin (treatment of choice in our unit). Data were collated on a proforma and entered onto an electronic database. *Results.* 483 patients were treated in our unit between 2005 and 2010. 50/483 developed VTE during the course of their treatment (11.6%). 29/50 episodes were in girls, and 21/50 in boys. At least 21 VTE were line-associated. 7 patients had sagittal sinus thromboses of which 5 were contemporaneous to L-asparaginase. L-asparaginase administration was also associated with 11 other VTE (line-related, lower limb DVT and PE). Other notable risk factors include immobility due to surgery in 4 patients. Of note, one patient who was appropriately risk assessed pre-surgery (managed with graduated compression stockings and dalteparin) subsequently developed a line-associated VTE. There was a high degree of compliance with diagnostic techniques and therapy as recommended by both the BCSH and NICE guidelines. *Summary/Conclusions.* Adolescents with cancer are at significant risk of developing VTE; in particular female patients with indwelling CVCs. Our incidence of VTE is higher than reported in adult patients with a cancer diagnosis. At the very least, all adolescent patients should be screened using the NICE guidelines. However, further analysis of risk factors and morbidity will help develop tailored recommendations for this specific patient population. <br type='_moz' />

0176

SUCCESSFUL IMLPLEMENTATION OF AN ELECTRONIC (COMPUTER BASED) MODEL OF MANDATORY RISK ASSESSMENT FOR VENOUS THROMBOEMBOLISM IN PATIENTS ADMITTED TO A MEDIUM SIZED GENERAL HOSPITAL

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Background. Venous thrombo-embolism (VTE) amongst the hospitalised patients is a serious and prevalent problem jeopardizing patient safety. Most consensus based guidelines on this subject highlight the need for recognising and identifying the individuals at risk. The process of risk assessment allows the health care providers to take appropriate preventive measures e.g. administration of prophylactic doses of Enoxaparin. The success of the risk assessment process itself depends on awareness amongst the health providers about this issue. This is best achieved by putting in place a system of alerts, reminders and mandatory intervention. *Aim.* Over the last 3 years, we developed and implemented a computer based electronic model of mandatory assessment for the risk of VTE in all adult patients admitted to our hospital. *Methods.* Using the fully integrated Electronic Patient Record system, a computer program was developed to introduce VTE risk assessment as a step in the admission process. This program also provided electronic reminders to the health care staff to perform the VTE risk assessment and prescribe thrombo-prophylaxis. As an electronic tool it makes auditing easy and accurate and enables the clinical leadership to address areas with lesser compliance. We have now analysed our VTE risk assessment and thrombo-prophylaxis data for the last 33 months and our results show evidence that electronic models of VTE risk assessment is practically possible and effective. *Results.* Before the introduction of our VTE risk assessment tool, there was no formal process of risk assessment. The tool raised the profile of VTE risk assessment throughout the hospital and there was a rapid and significant increase in the number of patients having formal VTE risk assessment. In the last 3 months we have achieved a hospital wide average of 95.6% of adult in-patients being assessed for the risk of VTE. Prior to the implementation of risk assessment, the only measure of VTE prevention was prescribing rates for Enoxaparin prophylaxis. Our data shows that before the implementation of the tool, an average of 26% of all adult in-patients received thrombo-prophylaxis. The use of this tool has improved the thrombo-prophylaxis by about 10%. Nevertheless, the data shows that there still is a gap between the number of patients assessed and those prescribed thrombo-prophylaxis. In the last three months we achieved VTE risk assessment in over 95% of patients admitted, demonstrating 51.6% at risk of VTE, but only 35% received Enoxaparin. *Summary/Conclusions.* Risk assessment for VTE is an essential step for reducing hospital acquired thrombosis and enhancing patient safety. Our data shows that an electronic model of VTE risk assessment is easy to implement and successful in achieving high rates of assessment. However, the prescription of thrombo-prophylaxis lags behind and this needs to be studied further. In the recent years, the focus has been on the need for accurate risk assessment. Now that computer based systems make that achievable, is it time to shift our focus to practical implementation of thrombo-prophylaxis?

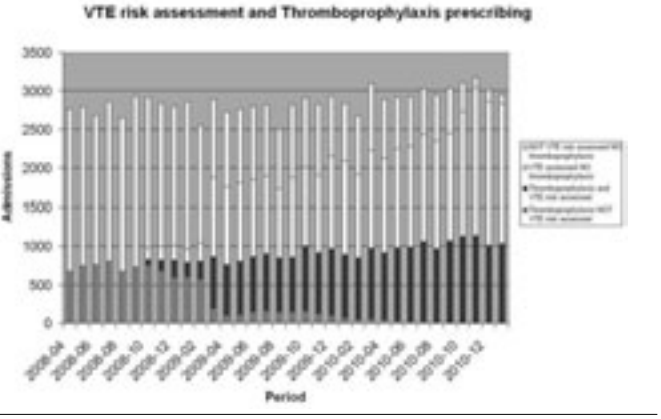

Figure 1. Summary snap shot of our data.

0177

THE RISK OF VENOTHROMBOEMBOLISM (VTE) AMONG ASIAN PATIENTS (PTS) WITH LYMPHOPROLIFERATIVE DISORDERS (LPD) RECEIVING IMMUNOMODULATORY AGENTS (IMIDS) IS LOWER, BUT CORRELATES WITH CUMULATIVE EXPOSURE

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Background. In contrast to western populations, the risk of VTE in Asian pts with LPD receiving IMIDS including thalidomide and lenalidomide is unknown. With limited evidence postulating a negligible risk, VTE prophylaxis is commonly omitted. *Aims.* We sought to evaluate the incidence and risk factors of VTE in our LPD pts exposed to IMIDS, and explore the role of anti-platelet agents (APA) in the prevention of VTE. *Methods.* From the comprehensive multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL) registry maintained in a tertiary institution, newly diagnosed pts from 2000 to 2009 with an objectively confirmed diagnosis of VTE were identified. Their characteristics and exposure to IMIDS and APA were compared against other pts in the registry. Thalidomide and lenalidomide were available for treatment of LPD from 2003 and 2008 respectively. Cumulative thalidomide exposure is measured in milligrams- week (mg-wk) [dose x duration]. Thromboprophylaxis with warfarin or heparin was not mandated in this patient population receiving MM treatment. *Results.* Among 731 consecutive LPD pts (320 MM and 411 NHL) entered into the registry, and prospectively followed, 239 pts (33% overall, 72% in MM and 2% in NHL) have been exposed to IMIDS, and 17 developed VTE event (7.1%). All VTE events were reported in MM pts only. Risk of VTE was not associated with pt (gender, ethnicity, age) or disease (stage, tumor burden, immunoglobulin subtype) characteristics at presentation. The median time to VTE was 16 months (range, 2 -134) from the time of MM diagnosis. Five patients developed VTE during induction treatment, while 12 developed VTE post-induction (maintenance or relapse)($P<0.001$). Among pts exposed to thalidomide (N=235), the median cumulative dose was 3750 mg-week (range, 100 -37,100). VTE risk was 3.4% for cumulative dose less than 3750 mg-wk, compared with 10.5% for patents receiving more than 3750 mg-wk ($P=0.03$). Among 239 pts with complete medication records available, 72 received APA (aspirin, ticlopidine, clopidogrel) indicated for other co-morbidities while receiving IMIDS. Interestingly, there was a near significant trend of a higher VTE risk for patients receiving concomitant APA (11.1%) compared with those who did not (4.8%) ($P=0.07$). *Conclusions.* Although VTE risk in Asian pts receiving IMIDS is lower compared with Western literature, it still confers a significant morbidity risk. Events appear to occur later in the course of the disease and were significantly influenced by the cumulative exposure to IMIDS. Concomitant use of an APA did not preclude the risk of developing VTE. Perhaps, the co-morbidities which indicated the use of APA could be risk factors for VTE. As IMIDS have become an integral part of MM treatment, and the survival of MM patients have improved, an optimal strategy for prophylaxis needs to be defined and validated, since duration of IMID use tends to be longer in MM pts.

0178

THE CLINICAL SIGNIFICANCE OF JAK2V617F MUTATION FOR PHILADELPHIA-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASES IN PATIENTS WITH SPLANCHNIC VEIN THROMBOSIS

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Background. Polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF), collectively known as Philadelphia-negative (Ph-negative) chronic myeloproliferative diseases (MPDs) represent commonest causes of splanchnic vein thrombosis (SVT), including Budd-Chiari syndrome (BCS) and portal vein thrombosis (PVT). JAK2V617F mutation has been demonstrated in most Ph-negative chronic MPDs. High prevalence of JAK2V617F mutation in SVT reported in recent studies raised suspicion of an underlying MPD. *Aims.* We assessed the diagnostic value of JAK2V617F mutation in 68 patients with SVT (42 PVT, 19 BCS, 7 combined PVT and BCS) under follow up at outpatients clinics of Hematology and Gastroenterohepatology Departments of Istanbul Faculty of Medicine between years 2007 and 2010. *Methods.* Patients with SVT presenting with portal hypertension with/without cirrhosis were enrolled. Screening was performed for prothrombotic states, including genotyping for factor V Leiden, factor II, protein C, protein S, and antithrombin deficiencies, homocysteinemia and antiphospholipid antibodies. JAK2V617F mutation was detected by fluorescent resonance energy transfer probes and LightCycler techniques. Genotype assessment was based on melting curve analysis. *Results.* Patient features with respect to JAK2V617F mutation status are demonstrated in Table 1.

Table 1. Patient features regarding JAK2V617F status in SVT.

All patients	Without JAK2V617F, n=39 (mean [SD])	With JAK2V617F, n=29 (mean [SD])	P
Age, y	42 [12.5]	45 [12.7]	0.398
Women, %	22 (56.4%)	19 (65.5%)	0.611
Hemoglobin, g/dl	12.2 [2.3]	12.8 [2.8]	0.312
Hematocrit, %	36.6 [7.18]	38.8 [8.46]	0.263
WBC count, /mm³	5803 [3114]	10525 [6547]	0.001
Platelet count, /mm³	179333 [153921]	369103 [300724]	0.004
LDH, U/L	431 [184.5]	523 [229.1]	0.08
Cirrhosis, %	21 (53.8%)	14 (48.3%)	0.834
Pro-thrombotic states ,%	15 (38.5%)	5 (17.2%)	0.103
Combined thrombosis ,%	2 (5.1%)	5 (17.2%)	0.127
Essential thrombocythemia ,%	1(2.6%)	5 (17.2%)	0.016
Polycythemia vera ,%	1(2.6%)	8 (27.6%)	0.002

JAK2V617F mutation was present in 42.1% of patients with BCS, 38.1 % of PVT and 71.4% of combined PVT and BCS. Thirteen of 15 (86.6%) with overt MPD and 16 of 53 (30.1%) without overt MPD (patients with either normal blood counts or cytopenias), including 6 of 16 with BCS (37.5%), 7 of 33 with PVT (21.2%) and 3 of 4 with combined BCS and PVT (75%) possessed JAK2V617F mutation. JAK2V617F was associated with significantly higher platelet and leukocyte counts (Table 1). Most patients with JAK2V617F mutation had peripheral blood cell counts within normal range except for higher mean values for leukocytes (10525/mm3 (SD 6547). There was a trend for higher LDH levels in JAK2V617F mutation carriers than in noncarriers (mean 523 U/L (SD 229.1), mean 431 U/L (SD 184.5), respectively; p=0.08). There was a significant positive correlation between JAK2V617F mutation status and leukocyte and platelet counts (r=0.445 and r=0.384, respectively). Receiver Operating Characteristic (ROC) curve analysis determined a platelet count of 190000/mm3 (area under curve; AUC=0.724, p=0.002) and a leukocyte count of 8150/mm3 (AUC=0.76, p=0.001) as best cutt-off values for highest sensitivity and specificity ratios of JAK2V617F mutation in SVT. No relationship was observed between prothrombotic risk factors, and JAK2V617F status, sites of thrombosis (PVT or BCS), presence of combined thrombosis and Hb, Htc, platelet and leukocyte counts. *Conclusions.* Signs of myeloproliferation may not be evident on peripheral blood if SVT is accompained by portal hypertension and a hypersplenic state. In our study, despite absence of overt signs of MPH, a substantial proportion of patients with SVT were shown to carry the JAK2V617F mutation. We found no relationship between prothrombotic risk factors and JAK2V617F status, site of thrombosis and presence of combined thrombosis. Our experience confirms that simple and rapid JAK2V617F testing on peripheral blood represents a key element of diagnosis of latent MPD in SVT.

0179

CLINICAL PHENOTYPE IN CONGENITAL ANTITHROMBIN DEFICIENCY

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Backround. Antithrombin (AT) is a potent inactivator of thrombin and factor Xa and the major inhibitor of blood coagulation. Inherited AT deficiency is considered as the strongest genetic factor increasing the risk of thrombosis 20-50 fold. The prevalence of heterozygous AT deficiency in general population ranges between 0.02% and 0.3%, its proportion among the patients with venous thrombosis (VT) and pulmonary embolism (PE) is about 5%. AT level may be detected by functional and immunologic assays, however, the reduction of functional activity is more predictive for the risk of thrombosis. At least 70% of a normal functional level is essential to ensure effective hemostasis control. Most patients with congenital AT deficiency have AT levels between 40% and 60%. Wehether the risk for thrombosis correlates with the severity of AT decrease is still not clear. *Methods.* We present the clinical phenotype in 37 patients with inherited AT deficiency (22F/15M) with a mean age of 41 yrs (range 9-65 yrs) and mean AT activity of 51±9%. The diagnosis was based on repeated AT testing (chromogenic assay Anrithrombin Berichrom, Siemens) and exclusion of acquired causes of AT decrease. The prevalence, type and recurrence of thrombosis, age at first thrombosis, concomitant risk factors and the need for sustained anticoagulation therapy were evaluated with regard to gender and antithrombin levels of 30-40% and >40%. *Results.* The prevalence of VTE and PE in entire patient group was 28/37 (76%) and 10/37(27%), respectively. The first episode occured at mean age of 29 yrs. Four patients had symptoms of ischemic stroke and two sinus venous thrombosis. Nine patients with AT of 36-66% were asymptomatic at the diagnosis in age 22-57 yrs. In symptomatic women (n=16) and men (n=12) the age at the first episode was 24 yrs (2 weeks-46 yrs) and 33 yrs (18-52 yrs), respectively. PE was more frequent in women (43% v.s. 25%; RR:1.72), while recurrent VT was present in 50% men and only 25% of women; RR:2.0. In men 75% episodes of VTE were idiopatic, while in 81% women concomitant risk factor was present, represented mostly by oral contraceptives (31%) and pregnancy (31%). The frequency of VTE family history was equal in both groups (75%), however, genetic markers were negative in all but one patient heterozygous for FVLeiden. Interestingly, there was no difference between the two patient's groups with AT≤40% (n=10) and AT>40% (n=27) in the prevalence of the VTE (80% v.s. 74%) or recurrent VT (20% v.s. 30%). However, albeit non-significantly, the incidence of PE and continuous warfarin use were higher in patients with AT≤40% than in the other ones: 40% v.s. 22% and 50% v.s 37%, respectively. *Conclusion.* Our results demostrate inherited AT deficiency to be a strong risk factor of VTE. In our cohort oral contraceptives and pregnancy were cause of an earlier VT manifestation in young women than men. Despite the rarity of the disorder appropriate diagnostic and preventive measures should be taken in all persons with a strong family history of VTE, in pregnancy or before contraceptives prescription in particular.

0180

UPPER EXTREMITY DEEP VENOUS THROMBOSIS AND THROMBOPHILIA

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Background. Deep venous thrombosis (DVT) is much less common in the upper than in lower extremity. Furthermore, there is limited information on risk factors for and the prognosis of upper extremity (UE) DVT in general population. Our aim was to study the contribution of genetic and acquired thrombophilic defects to the appearance of acute episode of UEDVT, either isolated or associated with pulmonary embolism (PE). *Materials and Methods.* By December 2008, 4503 patients objectively diagnosed with venous thromboembolism (VTE) on whom a thrombophilia study was carried out had included in RIETE (Registro Informatizado de la Enfermedad Tromboembólica), is an ongoing, international multicenter, observational registry of consecutive patients with symptomatic, objectively confirmed, acute VTE. Thrombophilia screening included: tests for antithrombin (AT), protein C (PC) and protein S (PS) deficiencies, factor V Leiden (FVL) and prothrombin G20210A (PTG20210A) mutations, resistance to activated protein C (APC-R), hyperhomocysteinemia, and antiphospholipid sindrome (APS). Odds ratios (OR) and 95% confidence intervals (CI) were calculated using unconditional logistic regression. *Results.* The mean age of our population was 43 ±18 years. 1852 patients (41%) presented positive results. 3.9% suffered UEDVT, and 0.3% UEDVT+PE. The risk of suffering UE-DVT or UEDVT+PE was calculated according to thrombophilia factors which, following adjustment for chronic lung diseases, chronic heart failure, abnormal creatinine levels, post-operative, immobility, cancer, prior VTE, journeys longer than 6 hours over three preceding weeks, oestrogen therapy during two previous months, legs varicosities, body mass index, and idiopathic VTE. There were not association between any of the trombophilic defects studied and UEDVT or UEDVT+PE (OR: 0.5; CI 95% 0.2 -1.2) and (OR: 1; CI 95 % 0.1-8.2), respectively. *Conclusions.* AT, PC and PS deficiencies, FVL and PTG20210A mutations, APC-R, hyperhomocysteinemia, and APS carriers there are not risk developing UEDVT or UEDVT+PE. From our results thrombophilia study in patients with UEDVT would not be recommended.

0181

FACTOR VIII LEVELS AND RISK OF RECURRENCE OF VENOUS THROMBOEMBOLISM

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Background. Patients presenting with a first episode of venous thromboembolism (VTE) have a well known risk of thrombotic recurrence potentially resulting in significant both morbidity and mortality rates. The role played by the different thrombophilic markers, and in particular elevation of factor VIII plasma levels, remains controversial. *Aims.* The main goal of the current study was to evaluate the possible existence of a correlation between elevated factor VIII levels and recurrence of a first episode of VTE. *Methods.* We carried out a retrospective observational study including 220 patients from our healthcare area with a diagnosis of a first episode of VTE from 1998 to 2010. Patients with coexistent active malignancy, chronic liver disease or chronic renal failure were excluded from the study. Overall 151 patients were eligible for evaluation. Both demographic and clinical features were recorded; these included age, sex, number of thrombotic episodes, type of VTE episode and number of patients who suffered subsequent thrombotic recurrence. Factor VIII measurement was performed in citrated plasma by coagulometric method using factor VIII deficient plasma from Stago (STA Factor VIII, Diagnostica Stago, Asniéres sur Seine, France) and an STA-R coagulometer (Diagnostica Stago). FVIII levels above 150 IU/dL were considered as elevated (normal range 55-150 IU/dL) *Results.* Median age of patients enrolled at diagnosis of the first thrombotic event was 45 years (range 9-80). Eighty three of them (55%) were men and 68 (45%) women. Median number of thrombotic episodes was 1 (range 1-4). The first episode of VTE was a distal deep venous thrombosis (DVT) in 83 cases (55%), a proximal DVT in 34 (22.5%), a pulmonary embolism in 23 (15.2%), a cerebral venous thrombosis in 7 (4.6%) and a mesenteric venous thrombosis in 4 (2.6%). Median factor VIII levels of the whole series of patients was 147.5 IU/dL (range 11-300 IU/dL). Out of 151 patients eligible for FVIII measurement 45 (29.8%) suffered thrombotic recurrence. Twenty seven out of 69 (39.1%) subjects who showed initially elevated FVIII levels (>150 IU/dL) presented with recurrent VTE versus 18 out of 82 (21.95%) of those whose FVIII plasma concentrations were within the normal range (p=0.021). *Summary/Conclusions.* Our findings confirm the statistically significant association existing between elevated FVIII plasma levels and thrombotic recurrence in patients with a previous episode of VTE. Inclusion of FVIII measurement in the routine thrombophilia screening is thus mandatory in order to identify this subset of patients. Whether such individuals are candidates to long term anticoagulant treatment on the basis of this higher risk of VTE recurrence remains unclear and future trials addressing this issue are warranted. email-address: anagodoy1006@hotmail.com

0182

ANTIPHOSPHOLIPID ANTIBODIES IN PEDIATRIC PATIENTS WITH SYSTEMIC LUPUS ERYTHREMATOSUS-LONG TERM CLINICAL AND LABORATORY FOLLOWUP STATUS STUDY

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Background. Anti-phospholipid antibodies (APLA) have been reported in 38-87% patients with pediatric systemic lupus erythrematosus (p-SLE). Although studies have analyzed the frequency of APLA in p-SLE patients, there are limited studies on long term follow up of p-SLE patients and their APLA status. In a previous study (Rheumatol Int 2005: 25:530-535), we analyzed 27 p-SLE patients for the prevalence of APLA; in the present study we further reviewed the APLA status and its relation with clinical outcome of this cohort of patients for a further 7 years followup period. *Aim.* To evaluate long term prevalence of APLA in p-SLE and its correlation with clinical outcome. *Methods.* Twenty seven p-SLE patients were tested for APLA and their clinical status was followed up. APLA testing included: i) lupus anticoagulant (LA) [by kaolin clotting time, diluted Russell viper venom time (LA Screen and LA Confirm, Dade Behring) and STACLOT-LA kit] ii) anticardiolipin antibodies (ACA)-IgG, IgM and iii) anti-β2 glycoprotein-1 (β2 GP1) antibodies-IgG, IgM (Orgentec GmBH by ELISA). Criteria for defining antiphospholipid syndrome (APS) was as outlined previously (Miyakis S, *et al*. J Thromb Haemost 2006; 4: 295-306). Patients with active disease were managed with high-doses corticosteroids along with cyclophosphamide in addition to hydroxychloroquine. Children in remission were receiving maintenance doses of prednisolone and/or hydroxychloroquine. *Results.* Out of the initial cohort of 27 patients, followup APLA testing was available in 21 patients. Six patients in whom no followup APLA testing was available included -1 patient who had died at the time of previous study, 3 who were lost to followup and 2 who died shortly after study. Therefore the present study analyzed 21 p-SLE patients for their follow up APLA and clinical status. These patients were tested for APLA on 49 occasions in further 7 year followup period. Seven (33.3%) of these 21 patients were never positive for any of the APLA, 5 (23.8%) were positive for APLA only once, 9 (42.9%) were positive for APLA intermittently. Most common APLA positivity was for LA seen in 7 (33.3%) patients. IgG and IgM ACA was positive in 3 (14.3%) patients each. Anti-β2 GP1 antibodies were tested in 11 patients on followup, of which 3 (27.3%) showed positivity. In all, 4 (19%) patients showed positivity for more than 1 APLA. Out of these 21 patients, 3 (14.3%) patients had thrombosis and all 3 patients were positive for APLA, 1 for IgG and IgM ACA and 2 for LA. Two of these fulfilled the criteria for APS. There were 2 fatalities in this cohort while on followup, both of these had thrombosis and were positive for APLA. *Conclusion.* Pediatric patients with SLE on treatment frequently test positive for APLA. Thrombosis ending fatally was infrequent in this cohort, however, when present was associated with APLA positivity. On the other hand presence or persistence of these antibodies was not always associated with thrombosis. Our

study suggests that p-SLE children should be tested routinely for APLA, as this would identify patients with an increased risk for thrombotic manifestations. email: shanonaseem@yahoo.co.in

0183

IMPROVING ADHERENCE TO VTE (VENOUS THROMBOEMBOLISM) PROPHYLAXIS

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VTE (venous thromboembolism) is estimated to cause 60,000 deaths per annum in the UK. Hospital in-patients have many risk factors for VTE, such as acute illness, reduced mobility or surgery. As a result, NICE guidelines and government CQUIN targets aim to nationally reduce the morbidity and mortality associated with in-patient VTE through mechanical and pharmaceutical prophylaxis following focused risk assessment. This formed the initiative behind our study into improving education and awareness of VTE prophylaxis. Our aim was to assess and improve local trust adherence to VTE prophylaxis. Based in a major hospital in Central London, we set out to identify the barriers that needed to be addressed in order to improve compliance, and to implement changes that would raise awareness and reinforce the importance of VTE prophylaxis. By auditing inpatient populations on two separate occasions we were able to assess changes in compliance over a period of 6 months. Initially we ran a baseline audit to assess compliance prior to any awareness programme. Between audits, we ran a trust wide education programme: the introduction of a VTE risk assessment proforma and specialised drug charts, and creating new hospital guidelines. Flowcharts for prophylaxis were displayed around the trust to aid staff. We then reaudited to assess whether compliance had improved. Our audit standard was the NICE venous thromboprophylaxis guideline CG92 (2010) and excluded patients in ITU, obstetrics and gynaecology, and paediatrics.

Figure 1. Flowchart.

Our audit tool was an amended version of the Department of Health VTE audit tool designed locally. The audits ran as a 'snap-shot' of the hospital with all eligible wards being audited within a few days, in January and July 2010. Audit 1 (January 2010) n = 250; Male to female ratio = 1.05:1; 64% aged greater than 60 years, 26% aged between 40-60 years; 11% with no risk factors, 35% with one risk factor and 54% with two or more risk factors. 13% of patients contraindicated for pharmaceutical prophylaxis. Audit 2 (July 2010) n=242; Male to female ratio = 0.9:1; 61% aged greater than 60 years, 23% aged between 40-60 years; 13% with no risk factors, 26% with one risk factor and 60% with two or more risk factors. 4% of patients contraindicated for pharmaceutical prophylaxis. Initially no standardised risk assessment on admission, 28% completed forms after education programme. Initial VTE prophylaxis prescribed for 49% of eligible patients, climbing to 74% after education programme. A concentrated education programme clearly improves compliance of VTE prophylaxis. However, it is a danger that without continued efforts, VTE prophylaxis and risk assessment can be overlooked. CQUIN targets of 90% are imposed on all NHS trusts for VTE prophylaxis and risk assessment, and it is likely some trusts will struggle without an awareness programme. Electronic patient records would increase the ability to not only audit VTE prophylaxis but also act as a reminder to risk assess accordingly for patients. Ideas for future development would be formal teaching at compulsory induction sessions for new employees and assigning a specialist nurse role in the trust.

0184

ANTITHROMBOTIC THERAPY IN NON-NEOPLASTIC CHRONIC PORTAL VENOUS THROMBOSIS IN CIRRHOSIS: RECANALIZATION AND LIVER FUNCTION EVALUATION

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Introduction. Non-neoplastic chronic portal vein thrombosis (PVT) is a frecuent diagnosis in the course of liver cirrhosis, with reported prevalences of 0.6% to 15,8%. PVT can motivate life-threatening complications due to worsening portal hypertension, so anticoagulation therapy is challenging in these patients. OBJECTIVE: To analyze the response to antithrombotic therapy and changes in liver function tests in 28 patients with chronic PVT associated with cirrhosis. *Patients and Methods.* 28 consecutive patients with liver cirrhosis and chronic PVT were treated with antithrombotic therapy from 2004 to 2009. Hepatocellular carcinoma and known thrombophilic risks were ruled out. Therapy consisted in 15 days of therapeutic doses of low molecular weight heparin (LMWH) (enoxaparin) adjusted according to baseline coagulability (Table 1), followed by either prophylactic doses (40mg/day) of LMWH or acenocoumarol (target INR 2-3), during 6 months. Response was evaluated after 6 months.

Table 1.

Range of baseline coagulation study and adjusted therapeutic doses of LMWH. N: Normal range; LH: Low hypocoagulability; MH: Moderate hypocoagulability; MSH: Moderate-Severe hypocoagulability.

Test	N	LH	MH	MSH
Platelet count /mm³	> 120000	80000-120000	50000-80000	30000-50000
INR	< 1.4	1.4-1.8	1.8-2.2	1.8-2.2
APTT sec.	< 37	37-40	40-45	40-45
Fibrinogen mg/dl	> 200	150-200	100-150	100-150
LMWH doses	1mg/kg/12h	0.75mg/kg/12h	0.5mg/kg/12h	1mg/kg/24h

If recanalization was complete, therapy was suspended. If recanalization was partial or no recanalization was observed, therapy was continued until response. RESULTS: From the 28 patients studied, 19 (68%) were males with a median age of 53 years (range 35-77). Cirrhosis was due to alcoholism (25%), virus (54%), mixed in 1 patient and other causes in 3 patients. PVT involved the portal trunk and/or branches in 19/28 (68%) patients, mesenteric vein in 2 patients and portal trunk and/or branches, mesenteric and/or splenic vein thrombosis coexisted in 7 patients. 19/28 (68%) of the patients had moderate or moderate-severe hypocoagulability range. Complete and partial thrombosis was seen in 18 and 10 patients at diagnosis, respectively.

From the 28 patients, 18 (64%) responded to antithrombotic therapy after 6 months, with a complete recanalization in 13 patients 13/18 (72%) and partial in 5/18 patients (28%). None of the 28 patients presented hemorrhagic complications and none showed platelets counts below baseline values. 17 from the 18 patients who responded, showed altered liver function tests before therapy. After 6 months, 8/17 (47%) improved liver function (only one patient had received antiviral therapy). After a median follow up of 42 months (range 7-67), 15/18 (83%) patients continued showing complete or partial response while 3 patients progressed. Of note, 3 patients of this group could proceed to further liver transplantation. *Conclusions*: Antithrombotic therapy in chronic PVT in cirrhotic patients resulted in a high response rate (64%) in our study, with a complete recanalization in 72% of the cases. Adjusted dose scheme according to level of hypocoagulability seems to be effective and safe, since 63% of the subgroups of moderate and moderate-severe hypocoagulability responded with no haemorrhagic complications.

0185

THROMBOPHILIC FACTORS IN IDIOPATHIC GANGRENE OF LIMBS

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Background. Gangrene of limbs result from thrombotic occlusion of peripheral arteries. Most commonly gangrene occurs because of alteration in the hemostatic balance, caused by hypercoagulability which is out of proportion to the response of fibrinolytic system of the body. Such a hypercoagulable or thrombophilic state resulting in gangrene is usually secondary to septicaemia, autoimmune conditions, vasculitides, measles, chickenpox, malignancy, ergotism, protein C, protein S or antithrombin deficiency. However, gangrene of limbs has been reported in patients without any underlying obvious primary etiology. We describe here the laboratory profile of thrombophilic factors in patients with idiopathic gangrene of limbs. *Aim.* To study the role of inherited and acquired thromobophilic factors in patients with idiopathic gangrene of limbs and to determine differences between adults and pediatric cases, if any. *Methodology.* We analyzed and compared thrombophilic factors in 22 patients with idiopathic gangrene of limbs, who presented at our centre over a 9 year period (January 2002- January 2011). The parameters which were studied include- factor V leiden mutation (FVL), protein C levels (PC), protein S levels (PS), antithrombin levels (AT), anticardiolipin antibodies (ACA) and lupus anticoagulant (LA). Patients were tested for these factors after 6 weeks of the acute event/or after 4 weeks of cessation oral anticoagulant therapy. PC, PS, AT were tested on automated coagulation analyser STA Compact (Stago Diagnostica), ACA with a commercial ELISA kit (Organtec GmBH), LA by screen and confirm kits (Dade Behring) and FVL by method described by Bertina *et al. Results.* During the study period 2700 patients with thrombosis were referred to laboratory for workup of thrombophilic factors, of which 62 patients (2.3%) had idiopathic gangrene of limbs. Complete data for all the above parameters was available in 22 cases, which were included in the present study for analysis of thrombophilic factors. In the 22 cases analyzed, male: female ratio was 1:1 with mean age of 20 years (range= 27 day- 49 years). Among these 22 patients, 14 (63.6%) were children (1 was a neonate) and 8 (36.7%) were adults. In this cohort, none of the patients had FVL mutation, PC or AT deficiency. 4 children had 1 or more underlying thrombophilic factors .ACA positivity, transient LA positivity and Protein S deficiency were present in a single case each. One patient tested positive for both LA and ACA. Among the adult patients, however only patient had Protein S deficiency. This patient also had a history of recurrent stroke. *Conclusion.* Our results show that idiopathic gangrene of limbs is an uncommon condition. Overall, testing for the above parameters would contribute to underlying etiology in only 23% patients. Acquired risk factors were slightly more common in this study population. Significant differences could not be established between adults and pediatric patients due to limited sample size. Alternative factors including fibrinolytic parameters and platelet activation markers need to be evaluated in this setting of idiopathic gangrene of limbs, as these might be indicative of underlying etiology.

0186

RANDOMIZED COMPARISON OF THE DAWN AC COMPUTER PROGRAM AND A SIMPLE MANUAL NOMOGRAM FOR QUALITY OF WARFARIN DOSING

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Background. In patients receiving warfarin, the quality of anticoagulant control as measured by the time-in-therapeutic range (TTR) for the International Normalized Ratio (INR) is a key determinant of risk for thromboembolic and bleeding events. Computer programs can assist physicians in optimizing TTR, but are expensive. *Aim.* The purpose of this study was to determine whether a computer system (DAWN AC) was non-inferior to a two-step manual nomogram used in a hospital anticoagulation clinic, for quality of warfarin dosing. *Methods.* Patients receiving warfarin maintenance therapy with target INR range 2-3 in the anticoagulation clinic were randomized to management with the newly acquired DAWN AC computer system or the clinic's standard of care, a simple manual dosing nomogram. After an initial run-in phase, study data collection started on February 1st 2010 and was completed on August 8th 2010. Primary outcome was the mean TTR calculated by the Rosendaal linear interpolation method. The non-inferiority margin was set at 4.5% lower TTR for DAWN AC compared with the nomogram. Approval from the Research Ethics Board of Hamilton Health Sciences was obtained and no patient informed consent was required. *Results.* Of the 1,298 patients initially randomized, 1,127 were still managed by the clinic after the run-in phase and entered the study on February 1st; 564 were managed with DAWN AC, and 563 with the manual nomogram. The mean age of study patients was 69 ± 14 years and 62% were male. Main indications for anticoagulation were atrial fibrillation (48%) and prosthetic heart valves (25%). Mean follow-up was 172 days, encompassing 8,344 INR values and 155,041 patient days. Adherence to recommended warfarin doses was higher in the DAWN AC than in the nomogram group (99 vs. 90%; p<0.0001), and the average interval between consecutive INR measurements was similar in the two groups (21 ± 12 vs. 21 ± 13 days; p=0.1987). In the primary analysis, mean TTR in the DAWN AC group was non-inferior to mean TTR in the nomogram group (71.0% ± 23.3 vs. 71.9% ± 22.9; non-inferiority p=0.0052). The effect of DAWN AC vs. nomogram on TTR was compared among subgroups for age, gender, warfarin pill size and primary indication and no significant interactions were found. *Conclusions.* Among patients receiving warfarin maintenance therapy with a target INR of 2-3 in an anticoagulation clinic, quality of anticoagulant control with the DAWN AC computer program was non-inferior to a simple two-step manual dosing nomogram. The nomogram could be a useful dosing tool for physicians without access to a computerized warfarin dosing system.

0187

MOTOR DISABILITY IN PATIENTS ON ORAL ANTICOAGULANT THERAPY (OAT): PRELIMINARY RESULTS FROM AN ITALIAN EPIDEMIOLOGICAL STUDY

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Background. OAT is chronically administered to prevent thrombotic events in a broad group of diseases having a narrow therapeutic range to balance the risk of hemorrhage and thrombosis, thus requiring a close monitoring of prothrombin time (PT) and the international normalized ratio (INR). Although PT/INR can be tested by a healthcare professional or by the patients (pts) themselves, the majority of pts are usually followed by an hematological specialized center for both analysis and OAT management, thus resulting in the need of hospital visits and in complex burden of problem related to frailty and several degree of disability afflicting OAT pts. Although these concerns are commonly observed in the daily clinical practice, the incidence of disability in OAT pts is unknown. *Aims and Methods.* In order to address this issue, we performed a cross-sectional evaluation of the motor disability (MD) in a group of consecutive outpatients on OAT, by handling in a MD assessment questionnaire at the moment of therapy schedule delivering. MD was assessed using Barthel Index (BI), which was used as basic ADL ability scale, and was classified as mild

(BI>66%), moderate (BI: 33-66%) and severe (BI<33%). *Results.* There were 122 patients (73 male); median age was of 71 (27-91) years. Disability was present in 37/122 (30%) patients; mild in 27%, moderate in 2% and severe in 1% of patients, respectively. The frequency of BI items reduction revealed that bladder function and complex activities are the most frequently impaired basic ADL in OAT pts (mean item reduction: bladder -14%, ascending and descending stairs -11%, bathing -8%, dressing -6%). *Conclusions.* Although preliminary and related to a limited series of patients, our data provide new insights on a neglected issue in OAT pts, such as disability. Indeed, MD is a frequent feature, but extended data analysis is required to achieve a better understanding about the disablement process, onset, causes, related risk factors and progression. A targeted rehabilitative approach to prevent and treat disability may lead to positive effects on both patient's quality of life and caregiver work-load. Home care management for MD affected OAT pts may be a suitable option, but requires a trained healthcare professionals team and adequate resources.

0188

PRELIMINARY RESULTS OF OUR STUDY DOES NOT SUPPORT THE ASSUMPTION THAT FREQUENCIES OF THROMBOPHILIA CAUSES ARE DIFFERENT BETWEEN EARLY AND LATE LOSSES IN CASES WITH RECURRENT PREGNANCY LOSS

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Background. Recurrent pregnancy loss (RPL) is defined by two or more failed pregnancies according to American Society for Reproductive Medicine. There is a large and contradictory literature on the association between maternal inherited thrombophilia and RPL occurring in the first trimester. Evaluation for an inherited thrombophilia should be considered in cases of RPL after nine weeks associated with evidence of placental ischemia and infarction and maternal vessel thrombosis. *Aims.* The purpose of our study is to compare the frequencies of hereditary thrombophilia factors among cases who had pregnancy loss before 10th week of gestation and after in Turkish RPL cases. *Methods.* Sixty three cases included in study whose ages range between 28,43 ± 3,88 years (mean ± standart deviation) (21-37 years). Cases with genital anatomical or chromosomal anomalies were excluded. All cases were investigated for activated protein C resistance, factor V Leiden and prothrombin gene 20210GA polymorphisms, antithrombin, protein C and S deficiencies. The number of losses were between 2 to 5 (median 2). Thirteen cases explained that thay had at least one healthy childbirth (secondary RPL). *Results.* According to our findings, 33,3% of RPL cases had at least one thrombophilia factor. The frequencies of thrombophilia causes in RPL cases and their comparisons according to whether they were early or late losses were shown in table. *Summary/Conclusions*. Preliminary results of our study support that the frequencies of thrombophilia causes are not different between cases who had early or late RPL. Studies larger and evaluating abortus materials histopathologically are needed to support our findings opposite to suggestion that early RPL cases are not subject to investigate for thrombophilia factors.

Table 1. Thrombophilia factors in RPL cases.

Cause	All Frequency (%)	Early RPL (n= 39)	Late RPL (n= 24)	p value
Activated protein C resistance (<120 seconds)	17,3% (9/52) (5,8% F V Leiden negative)	20,0% (7/35)	11,8% (2/17)	0,376
Factor V Leiden gene	9,7% (6/62) (1,6% homozygous)	10,2% (4/39)	8,7% (2/23)	0,607
Prothrombin gene 20210GA	3,4% (2/59)	2,6% (1/38)	4,8% (1/21)	0,589
Antithrombin deficiency (<80%)	1,6% (1/63)	0,0% (0/39)	4,2% (1/24)	0,381
Protein C deficiency (<70%)	1,7% (1/58)	2,7% (1/37)	0,0% (0/21)	0,638
Protein S deficiency (<60%)	14,3% (9/63)	10,2% (4/39)	20,8% (5/24)	0,212
At least one cause	33,3% (21/63)	33,3% (13/39)	33,3% (8/24)	0,611

0189

ARE JAK2 MUTATION AND PAI -1 POLYMORPHISM RISK FACTORS FOR VASCULAR EVENTS IN ET PATIENTS?

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Background. The treatment of ET patients focuses mainly on lowering the risk and incidence of thrombotic and haemorrhagic complications. The tyrosine kinase activating JAK2 V617F point mutation has been found in 50% of Essential Thrombocythemia (ET)patients and the relationship between the occurance of this mutation and thrombotic events has been widely suggested. Additionally, it has recently been found that a 4G/5G polymorphism located in the promotor region of plasminogen activator inhibitor -1(PAI-1) can also be a risk factor for deep vein thrombosis, a complication often observed in ET patients. *Aim.* In this study we tried to identify the most important risk factors for vascular events based on evaluation of pathogenetic and prothrombotic mutations as well as hemostatic risk factors . *Methods.* We have examined 106 patients with ET (80 females and 26 males, mean age 54(23-82)).The control group (CG) consisted of 20 healthy persons: 6 males and 14 females (mean age 41(31-54)). We searched for JAK2V617F, Factor V Leiden, G20210A prothrombin gene, MTHFR C667T gene mutations and PAI-1 polimorphism. We also evaluated plasma levels of: factors I, VIII, XII, AT, protein C and S and serum levels of: homocysteine, folic acid, witamin B12 . *Results.* The JAK2 V617F point mutation was detected in 48 (45,28%) ET patients. In ET patients with JAK2 point mutation compared to ET patients without JAK2 mutation, the higher levels of:red blood cells(RBC), white blood cells(WBC), hemoglobin (HGB)and hematocrit (HCT) were found. The results were as follows: RBC: median 4.77 T/l; P25-75%, 4.46-5.26 and 4.22T/l,3.8-4.52,p<0.001, WBC: median 8.5G/l,P25-75%,6.8-9.9 and 7.2G/l,5.7-8.7,p<0.05, HGB: median 14.2g%,P25-75%,13.5-15.0 and 13.0g%,11.8-3.9,p<0.001,HCT:median 41.5%,P25-75%,38.7-45.6 and 37.5%,34.0-40.2,p<0.001 respectively. JAK2 positive patients had lower CRP and free protein S levels compare to JAK2 negative ones (CRP: median 1.5 P25-75%,0.7-2.1 and 2.0; 1.75-2.45mg/l, p<0.05 and protein S: median 78.25%,P25-75%,72.4-94.65 and 90.96%,79.7-103.0, p <0.05). In 21(19.8%) patients from ET group 37 thrombotic complications occurred and in 22(20.75%)ET patients bleeding episodes were noticed. 14(32.56%)patients with thrombotic complications were JAK2 positive, while only 5(11.63%) patients with bleeding episodes were JAK2 positive. The difference beetween these group was statistically significant (p<0.05). In JAK2 positive patients thrombotic episodes were found more often, while JAK2 negative patients were more prone to bleeding complications. The hiperhomocysteinemia was detected in 41(38.7%) of ET patients. The median homocysteine level was higher in ET patients compared to CG (11.10; P25-75%,8.74-13.20and 9.21,7.94-10.60umol/l, <0.05).What is more, the folic acid level was lower in ET patients compared to patients from CG (median 7.96,P25-75%,5.16-10.8 and 12.6,9.03.18.8 ng/ml, p<0.001).In ET group PAI-1 4G/4G polimorphism was found in 34(32.08%)persons while 4G/5G polimorphism was detected in 42 persons (39.62%).These mutations were present in the same proportions in patients with thrombotic and bleeding complications. *Conclusions.* The pathogenetic JAK2 point mutation seems to be also a thrombotic risk factor in ET patients. Higher RBC, HGB, WBC, HCT levels observed in JAK2 positive patients may aggravate the thrombotic risk. Hiperhomocysteinemia observed in ET patients may be due to lower folic acid level. PAI-1 polimorphism seems not to be a risk factor for thrombotic and bleeding complications.

0190

INHERITED THROMBOPHILIA MUTATION ASSOCIATIONS: A THOUSAND VARIABLES, ONE CLINICAL DECISION - CAN CURRENT GUIDELINES ADEQUATELY REPRESENT THE REAL POPULATION?

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Background. Inherited thrombophilias are mutations with a Mendelian transmission that predispose carriers to a variably increased risk of venous or arterial thromboembolism. Commercially available testing kits include several such mutations, the clinical applicability of some being unproven. While the higher-risk mutations are associated with published risk-benefit analyses that clearly favour primary or secondary prophylaxis, other mutations confer a small increase in thrombotic risk that does not balance the increased bleeding risk of prophylaxis. Still other mutations carry a theoretically increased risk which has yet to be demonstrated in robust clinical series. The coexpression of more than one high-risk mutation facilitates the clinical decision to introduce prophylaxis, as the risk-benefit balance further shifts towards anticoagulation or antiaggregation; however, decisions become increasingly subjective as the number of low-risk or unproved-risk mutations increases, increasing with it the perceived theoretical thromboembolic risk. *Aims.* To determine the frequency of hereditary thrombophilia mutation associations in a sample of patients, to characterize the clinical difficulties of deciding whether or not to institute prophylaxis. *Methods.* We reviewed all requests for molecular biology analysis of inherited thrombophilias, from patients with thromboembolic events, obstetric complications or familial thrombosis, received in our center between June 2005 (introduction of the strip-assay currently used) and January 2011, containing complete results for Factor V Leiden (FVL), Factor V HR2 Haplotype (FVHR2), Prothrombin G20210A, Beta-Fibrinogen 455 G>A, Plasminogen Activator Inhibitor 1 4G/5G and 5,10-Methylenetetrahydrofolate Reductase (MTFHR) C677T and A1298C, as well as levels of Antithrombin (AT) and Proteins C (PC) and S (PS). *Results.* We identified 2048 individual patients fulfilling the inclusion criteria; 27 (1.3%) had no alterations in the parameters analyzed; 537 (26.2%) were high-risk patients with homozygosity for FVL or deficiencies of AT, PC or PS (irrespective of other mutations), and 7 (0.3%) were heterozygous for both FVL and FVHR2 (irrespective of other mutations). Single heterozygous mutations were found in 142 (6.9%), while 72 (3.5%) carried single homozygous mutations (excluding FVL), of whom 49 (2.4%) were homozygous for a MTHFR mutation. Considering coexpression of low-risk mutations, 587 (28.7%) expressed two mutations, 469 (22.9%) expressed three, 182 (8.9%) expressed four, 23 (1.1%) expressed five and 2 (0.1%) expressed six. *Conclusions.* In this series we identified only 1.3% of patients with neither mutations in the strip-assay kit used, nor deficiencies of AT, PC and PS. A total of 26.5% presented with high-risk mutations which are well characterized in the literature, while 10.4% had low-risk mutations which are also well described. However, the majority of patients (51.6%) presented with two or three simultaneous low-risk mutations and a significant number (10.1%) with four or more mutations. This study highlights that fact that, though published series focus on individual mutations, the majority of patients have complex associations of low-risk mutations, the interaction of which is not well understood. Due to the number of mathematical combinations possible (over 1200), relevant cohorts analyzing the thrombotic risk of each are impossible to obtain. Current recommendations need to take into account this grey area, and further define guidelines for anticoagulation and antiaggregation.

Cytogenetics and molecular diagnostics

0191

SECONDARY CHROMOSOMAL AND MOLECULAR ABNORMALITIES IN ACUTE PROMYELOCYTIC LEUKEMIA: DISTRIBUTION AND IMPACT ON OUTCOME

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Background. Acute promyelocytic leukemia (APL) characterized by the translocation t(15;17)(q22;q12), resulting in the fusion gene *PML-RARA*, is a distinct subset of acute myeloid leukemia. Since the introduction of all-trans retinoic acid (ATRA) this AML subgroup has a favourable prognosis. *Aims.* To evaluate the incidence of additional chromosomal and molecular abnormalities in APL patients. *Methods.* The study included 106 adult APL patients (median age, 45.5 years; range 18-72 years) entered on 2 protocols (APL95 and AML HD98B) of the German-Austrian AML Study Group (AMLSG) between 1995 and 2004. All patients were treated with ATRA in combination with intensive chemotherapy. Beside cytogenetic analysis, we evaluated the mutational statues of *ASXL1*, *CEBPA*, *DNMT3A*, *FLT3* internal tandem duplication (ITD), *FLT3* tyrosine kinase domain (TKD), *IDH1*, *IDH2*, *MLL*, *NPM1*, *NRAS*, *RUNX1* and *TET2*. *Results.* Median follow up for survival was 6.5 years. In 36 of the 106 patients (34%), at least one additional chromosomal abnormality was detected. The most common secondary chromosomal abnormalities were trisomy 8 in 33% (n=12) and chromosome 7 abnormalities in 19% (n=7), including deletions of the long arm (del(7q), n=6) as well as one balanced translocation t(2;7)(p21;q22). Additional abnormalities affected chromosome 17 in 14% (n=5) of the patients, including isochromosomes (n=3), derivative chromosomes (n=2) and one deletion of the short arm of chromosome 17 (del(17p)). Activating *FLT3* mutations were the most frequent secondary molecular abnormalities with 38% for *FLT3*-ITD and 14% for *FLT3*-TKD. At least one activating *FLT3* mutation was identified in 48% of the patients. In trend, activating *FLT3* mutations were inversely correlated with secondary chromosomal abnormalities (p=0.08). All other gene mutations occurred in a very low frequency, *ASXL1* mutation in 1.5%, monoallelic *CEBPA* mutation in 1.5%, *DNMT3A* in 1.8%, *IDH1* in 1%, *NRAS* in 5% and *TET2* in 4%. None of the patients harboured *IDH2*, *MLL*, *NPM1* or *RUNX1* mutations. Patients with activating *FLT3* mutations had a higher frequency of additional gene mutations (17%) versus those without (5%). Overall survival (OS) after 6 years was 71% (95%-CI 63-81%) for the whole cohort. A significant inferior survival was observed in patients with at least one gene mutation (n=46) versus in those without (n=45) (p=0.05). If presence or absence of secondary cytogenetic abnormalities were combined with presence or absence of at least one gene mutation, three risk groups with significantly different survival could be defined: A, absence of secondary cytogenetic abnormalities and gene mutations; B, presence of secondary cytogenetic abnormalities or gene mutations; C, presence of secondary cytogenetic abnormalities as well as gene mutations. OS rates at 6 years were 84%, 64% and 46% for group A, B and C, respectively. A Cox regression model on OS revealed higher age (HR for a difference of 10 years, 1.38, p=0.04), WBC above 10/nl (HR, 2.6, p=0.03) and cytogenetic/molecular risk group (HR for change from A to B or B to C, 2.1, p=0.03) as unfavourable prognostic variables. *Conclusions.* In our study on 106 APL patients secondary chromosomal abnormalities and concurrent gene mutations had an adverse impact on outcome.

0192

PROSPECTIVE EVALUATION OF AN AUTOMATED, CARTRIDGE-BASED SYSTEM FOR QUANTITATION OF BCR-ABL1 TRANSCRIPTS BY COMPARISON WITH RQ-PCR ALIGNED TO THE INTERNATIONAL SCALE: RESULTS FROM THE ICORG 0802 TRIAL

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Background. Molecular monitoring of BCR-ABL1 transcripts by real-time quantitative (RQ)-PCR is an integral part of the management of patients with chronic myeloid leukaemia. Sequential BCR-ABL1 transcript results demonstrate the kinetics of response to tyrosine kinase inhibitor (TKI) therapy, correlate with progression free survival and can indicate impending loss of response. Inter-laboratory standardisation is necessary to provide consistent interpretation of patient results allowing therapeutic decisions and accurate comparison of clinical trial data. To date, standardisation to the International Scale (IS) has required sample exchanges with a national or international reference laboratory in order to establish and validate a methodology specific conversion factor (CF). Current methods for BCR-ABL1 RQ-PCR require time consuming preparatory, analysis and reporting steps. Automated and standardised BCR-ABL1 quantitation platforms would negate the requirement for a relatively labour-intensive, manual methodology and significantly improve turn around times enabling prompt clinical decisions. *Aims.* To evaluate the performance, in the diagnostic and residual disease settings, of the automated, cartridge-based Xpert BCR-ABL Monitor/GeneXpert (Cepheid), a rapid, closed-system, nested real-time PCR assay requiring minimal pre- and post-analytical manipulations. This evaluation was implemented as part of the ongoing All Ireland Co-operative Oncology Research Group (ICORG) 0802 trial: a phase 2, multi-centre study of nilotinib 300mg twice daily as first line treatment of newly diagnosed, Ph-positive, chronic phase CML (ClinicalTrials.gov NCT00809211). *Methods.* Paired, peripheral blood analyses of 36 evaluable CML patients were performed at diagnosis and at 128 subsequent three-monthly timepoints. BCR-ABL1/ABL1 measurements were performed on all samples by RQ-PCR methodologies aligned to IS and by the Xpert BCR-ABL Monitor system. *Results.* At diagnosis both techniques had comparable results in 35/36 (97.2%) samples: IS, median BCR-ABL1/ABL1 41.0% (range 15.0->100.0%, non-linear >10%); Xpert BCR-ABL Monitor, median BCR-ABL1/ABL1 44.0% (range 16.0-100.0%) in patients expressing e13a2 or e14a2 BCR-ABL1 transcripts. The automated system was unable to identify and quantify e19a2 BCR-ABL1 transcripts in one patient. For follow up samples, the failure rate of the manual methodology was 4.7% (n=6). In the 122 paired analyses, correlation between methodologies, without automated system IS conversion, over a five log range (IS BCR-ABL1/ABL1 100-0.001%) was favourable (r2=0.845) with a progressive decline in correlation noted with each decreasing log IS BCR-ABL1/ABL1 level. Analysis at clinically relevant levels revealed no significant difference between the Xpert BCR-ABL Monitor and IS RQ-PCR in identifying major molecular responses (44.3% vs 41.8%) although a trend was apparent for the automated system to overestimate complete molecular responses (≤0.001 IS RQ-PCR) when compared with IS RQ-PCR (10.7% vs 4.9%). *Summary.* The Xpert BCR-ABL Monitor is capable of providing rapid (<2 hours) quantitative BCR-ABL1/ABL1 results with standardised kit reagents that require minimal pre-analytical steps thus significantly reducing potential contamination while maintaining maximal amplification efficiency. The system performs well in the diagnostic and residual disease settings but would not obviate the requirement for RNA isolation for identification of variant BCR-ABL1 species or acquired resistance-associated mutations. A reagent lot-specific IS CF would enhance applicability in monitoring TKI therapy with confirmation required by IS RQ-PCR for clinically relevant molecular responses.

0193

HIGH THROUGHPUT TRANSCRIPTOME SEQUENCING AND SNP ANALYSIS UNVEIL A CRYPTIC ETV6/ABL FUSION IN A PH NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASM WITH NORMAL KARYOTYPE

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Background. In recent years, the discovery of new genetic lesions such as mutations of JAK2 and TET2 genes has provided crucial insight to understand the molecular pathogenesis of chronic myeloproliferative neoplasms (MPNs). Nonetheless, the underlying genetic lesion remains unknown in many cases and this represents a major obstacle to define the most appropriate treatment in many patients. High throughput transcriptome sequencing and Single Nucleotide Polymorphism (SNP) analysis may represent a rapid and widely applicable tool to identify genetic lesions in these patients. By this approach, cryptic genetic lesions can be identified and at least in some cases, this may offer the possibility to apply new molecular targeted therapies. *Patients and Methods.* In 1997 a diagnosis of atypical Ph-, chronic MPN was posed to a 63 years old woman who was followed-up and treated with hydroxyurea only when indicated to control excessive leukocytosis and splenomegaly. The cytogenetic analysis performed at diagnosis and repeated in year 2009, showed a normal karyotype and the molecular analysis (FISH and RT-PCR) repeatedly proved negative for BCR-ABL p210, p190 and p230. Whole transcriptome sequencing (RNAseq) and SNP analysis were performed on peripheral blood granulocytes derived RNA and DNA using an Illumina or Affymetrix Platform, respectively. SNP analysis was also performed on DNA derived from purified CD3 positive T-lymphocytes to discriminate individual variations from neoplasm-associated genotype alterations. The identified chimeric fusion gene was validated with FISH, RT-PCR and conventional sequencing. Imatinib was administered at 400mg/die and the fusion transcript monitored with RQ-PCR during treatment. *Results.* The parallel SNP analysis performed on peripheral blood CD3+ T lymphocytes as well as on granulocytes allowed to demonstrate on these latter cells only, the presence of a deletion on the long arm of chromosome 9 and a duplicated region on the short arm of chromosome 12. The simultaneous RNAseq unveiled the presence of an ETV6/ABL chimeric fusion transcript as the only fusion gene emerging among a huge amount of potentially interesting results. RT-PCR and direct sequencing of the PCR product confirmed the presence of a fusion between ETV6 exon 5 and ABL exon 2. FISH analysis with fluorescent molecular probes encompassing ETV6 and ABL genes showed the co localization of the two probes on chromosome 9 thus confirming the presence of a cryptic translocation, as previously described in some cases of ETV6-ABL fusions. Due to the reported sensitivity of ETV6-ABL positive diseases to TKI, Imatinib was given to the patient at 400 mg/die. A rapid and complete normalization of blood counts and splenomegaly was documented in less than 2 weeks; the molecular monitoring of response is currently ongoing by sequential RQ-PCR analysis of the fusion transcript. *Conclusion.* Our results underline the clinical utility of high throughput whole transcriptome sequencing to identify cryptic genetic lesions in patients with a normal karyotype. This approach can lead to tailored, effective treatments.

0194

SCREENING FOR FUSION GENES INVOLVING PDGFRA OR PDGFRB IN PATIENTS WITH EOSINOPHILIA-ASSOCIATED MYELOPROLIFERATIVE NEOPLASMS USING THE FLUIDIGM BIOMARK REAL-TIME PCR SYSTEM AND 48.48 DYNAMIC ARRAY

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Background. Identification of fusion genes with involvement of the platelet derived growth factor receptor alpha or beta (PDGFRA or

PDGFRB) in eosinophilia-associated myeloproliferative neoplasms (Eos-MPN) is challenging due to the presence of multiple partner genes with heterogeneous breakpoints. However, accurate detection of these fusions is important for clinical management as they are associated with excellent response to imatinib mesylate. *Aims.* To develop quantitative real time PCR (RQ-PCR) assays to detect clinically significant overexpression of the 3'-region of PDGFRA and PDGFRB that may be indicative of an underlying gene fusion. Six RQ-PCR assays were designed for each gene; three assays 3' to known breakpoints and three assays 5' to known breakpoints. Where no fusion transcript is present, normal PDGFRA and PDGFRB expression (relative to the control gene assays) should be observed for all assays. However, when a fusion transcript is present the three assays 3' to the breakpoint region may detect overexpression compared to the three assays 5' to the breakpoint region. *Methods.* Using the BioMark real-time PCR system and 48.48 Dynamic Array (Fluidigm) we tested pre-amplified cDNA from haematologically normal controls (n=16), samples from patients with unexplained eosinophilia that had previously tested negative for PDGFRA (n=12) or PDGFRB (n=3) fusions using conventional assays and samples with known PDGFRA (n=11) or PDGFRB (n=4) fusions. PDGFRA and PDGFRB expression was analysed using 6 RQ-PCR assays per gene and control gene assays for ABL1, BCR and GUSB. The 48.48 dynamic array allowed 2304 real time PCRs to be performed in one run enabling 48 samples to be analysed simultaneously in triplicate using 16 RQ-PCR assays. *Results.* Median Ct values were used to calculate the delta-delta Ct value for each assay and each sample using ABL1, BCR or GUSB as a reference. These data were then normalised to the expression level observed in the calibrator sample (normal human leukocytes). The difference between the median expression calculated for the assays 5' and 3' to known breakpoints was used to determine the relative overexpression of the 3' end of PDGFRA or PDGFRB. The median relative overexpression was 44.3 fold (PDGFRA, $p<0.0001$) for samples with PDGFRA fusions and 59.3 fold (PDGFRB, $p=0.003$) for samples with PDGFRB fusions compared with 0.66 fold (PDGFRA) and -0.06 fold (PDGFRB) in normal controls relative to ABL1. No significant overexpression of the 3' end of the genes was found in the patients with unexplained eosinophilia when compared to normal controls (PDGFRA, $p=0.186$; PDGFRB, $p=0.401$). Statistically significant overexpression was observed in cases with known PDGFRA and PDGFRB fusions compared to normal when the data were analysed using BCR and GUSB as reference genes. *Conclusions.* This simple, rapid and high throughput screen can be used to detect clinically significant overexpression of PDGFRA and PDGFRB in Eos-MPN. The flexible nature of the dynamic array means that further assays could be designed to facilitate high throughput screening for other gene fusions in haematological malignancies.

0195

GENOMIC PROFILING USING SNP-BASED MICROARRAYS AS A DIAGNOSTIC TOOL IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. In acute lymphoblastic leukemia (ALL) specific chromosomal abnormalities provide important diagnostic and prognostic information. In recent years, it has become clear that ALL cells also frequently harbor relevant disease related submicroscopic chromosomal abnormalities (Kuiper *et al.* Leukemia 2007, 21, 1258-1266; Mullighan *et al.*, Nature 2007, 446, 758-764). Although conventional karyotyping is generally considered as the gold standard in the genetic diagnosis of ALL, this method is limited by its low success rate due to an inadequate metaphase yield and/or a poor banding quality, and its inherent capacity to detect only those copy number changes that are large enough to be microscopically visible (5-10 Mb in size). Implementation of genome-wide high-resolution copy number profiling using genomic microarrays in routine diagnostics would significantly improve the detection of clinically relevant abnormalities and, at the same time, would overcome the above mentioned limitation of karyotyping, since no culturing of the clinical samples is required. *Aims.* We explored whether microarray-based genomic profiling would be feasible as an alternative method to detect diagnostic and prognostic relevant genomic copy number aberrations (CNAs) in a routine clinical diagnostic setting. In addition, we aimed to develop a practical workflow for fast, objective and routine interpretation of CNAs obtained by microarray-based genomic profiling, thereby facilitating its application in a routine clinical diagnostic setting. *Methods.* We performed both conventional cytogenetic analysis and microarray-based genomic profiling, using the 250K Affymetrix® GeneChips arrays, of 60 ALL cases and subsequently compared conventional karyotypes with microarray-deduced copy number profiles. *Results.* Microarray-based genomic profiling resulted in a CNA detection rate of 93%, whereas for conventional karyotyping this was 61%. In addition, many small (< 5 Mb) genetic lesions were encountered using the microarray platform. Many of these, sometimes focal, lesions harbor clinically relevant ALL-related genes such as CDKN2A/B, ETV6, PAX5 and IKZF1. *Summary/Conclusions.* We conclude that microarray-based genomic profiling serves as a robust tool in the genetic diagnosis of ALL. In addition, it outreaches conventional karyotyping in CNA detection, both in terms of sensitivity and specificity. Since balanced chromosomal abnormalities were not identified, it is recommended to apply FISH and/or other targeted methods for the detection of translocations with a known diagnostic and/or prognostic impact (e.g. t(9;22)/BCR-ABL1).

0196

PAX5 DELETION IS COMMON AND CONCURRENTLY OCCURS WITH CDKN2A DELETION IN B LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background and Aims. The *PAX5* is essential in normal B-cell lymphopoiesis and deregulation of *PAX5* function is believed to contribute to leukemogenesis in B-ALL. Although the common cytogenetic changes in ALL may serve as diagnostic and prognostic markers, they are detectable at only low rates in ALL. In this study, we investigated the clinical utility of *PAX5* deletion in childhood and adult B-ALL. *Methods.* We performed a comprehensive study using FISH, G-banding and IHC, to identify *PAX5* deletion and immunoexpression in 102 *CD19+* clinical B-ALL cases (79 children and 33 adults) and investigated its relationship with common cytogenetic changes including *BCR-ABL1, ETV6-RUNX1* and *MLL* rearrangements, and *CDKN2A* deletion. *Results.* The incidences of translocations and deletions were 2.5% and 10.0% in 33 children, and 0.0% and 18.2% in adults, respectively. The incidence of *PAX5* deletion was higher than those of *BCR-ABL1* (8.9%) or *MLL* rearrangements (5.1%) in children and than that of *MLL* rearrangement (3.1%) in adults. Most of patients with *PAX5* deletion (83.3% of children and 100.0% of adults with *PAX5* deletion) had concurrent *CDKN2A* deletion. *PAX5* deletions were detected both in patients with positive and negative *PAX5* immunoexpression. *Conclusions.* In this study, we found that *PAX5* is a common target in leukemogenesis of B-ALL along with *CDKN2A*. Owing to its frequent deletion in B-ALL, *PAX5* could be used as one of the cytogenetic markers in diagnosis and monitoring of the disease. No correlation between immunoexpression of *PAX5* and deletion of *PAX5* suggests allele-specific regulation and haploinsufficiency of *PAX5*. As a marker for minimal residual disease, early relapse or response to therapy, *PAX5* appears to be more widely applicable than BCR-ABL1 or MLL rearrangment in B-ALL.

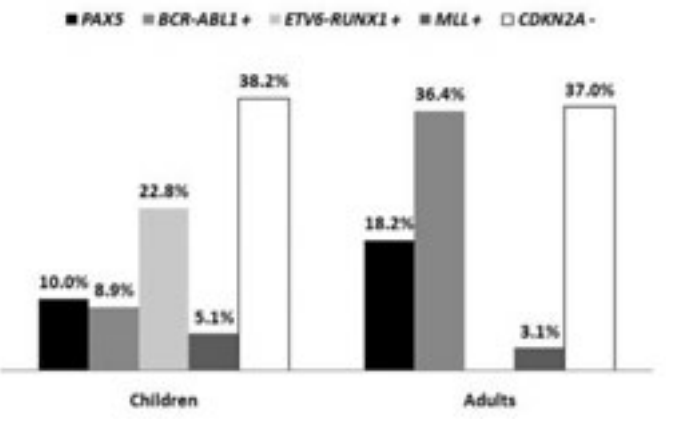

Figure 1. Incidence of PAX5 deletion, BCR-ABL1 rearrangement.

0197

SENSITIVE AND QUANTITATIVE DETECTION OF LOW LEVEL KIT D816V MUTATION IN PATIENTS WITH SYSTEMIC MASTOCYTOSIS USING A NANOFLUIDIC DIGITAL PCR ARRAY

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Background. Systemic mastocytosis (SM) is characterised by the abnormal proliferation and accumulation of clonally derived mast cells and is classified as a myeloproliferative neoplasm (MPN). The acquired activating point mutation 2447 A>T (D816V) in exon 17 of KIT can be detected in 80 - 95% of patients with adult onset SM. Because of its transforming activity, D816V is considered to play a primary role in SM and is included in the consensus WHO SM classification criteria. Development of a sensitive assay for detection of KIT D816V is important diagnostically and also has predictive significance since the mutation confers resistance to the kinase inhibitor imatinib mesylate. *Aims.* Analysis of D816V in SM is challenging as clonally derived cells are often patchily distributed in the bone marrow (BM) and absent or present only at very low level in peripheral blood (PB. Techniques such as direct sequencing are only able to detect mutational loads >20% and therefore we aimed to develop an assay to facilitate low level, quantitative detection of the mutated allele. *Methods.* A mutation specific digital PCR assay was developed for sensitive detection and quantification of D816V using the Fluidigm Biomark real time PCR system and BioMark digital array. DNA samples from putative SM cases (n=24) and normal controls (n=12) were analysed in a blinded fashion for the presence of the D816V mutation and results were compared to those obtained using an allele-specific competitive blocker (ACB) PCR assay. Analysis of serial dilutions of the HMC-1 cell line (50% - 0.01% D816V) were tested to determine the limit of detection of the assay. *Results.* Testing of patient DNA by digital PCR showed 97% concordance with the ACB PCR results with the levels of quantification ranging from 0.01% - 37.9%. One case was found to be negative by ACB PCR but showed amplification in 3 reaction chambers in the digital PCR assay (mutational load of 0.01%). Amplification of D816V was not observed in normal control samples (n=12) or negative control panels demonstrating that the assay is highly specific for the mutated allele and that no amplification occurs from the wild type allele. Testing of HMC-1 cell line DNA serially diluted in DNA extracted from normal peripheral blood determined that the lowest limit of detection of the digital PCR assay was 0.01% compared to 0.1% for the ACB PCR. *Conclusions*. The nanofluidic digital PCR array used in conjunction with a novel mutation specific real time PCR assay allowed sensitive detection and quantification of KIT D816V to 0.01% in genomic DNA samples. The use of the digital array enables enhanced detection of rare mutated alleles in a high background of wild type DNA as the sample is partitioned prior to amplification. The detection sensitivity of 0.01% is greater than that achieved using any other methodology published to date. This enhanced sensitivity may increase the detection of D816V in samples with low mast cell content helping to improve the diagnosis and clinical management of patients with SM.

0198

THE FREQUENCY AND IMPLICATION OF GENE MUTATIONS INVOLVING RECEPTOR TYROSINE KINASES AND RAS PATHWAYS IN CHILDHOOD ACUTE MYELOID LEUKEMIA WITH FOUR MAJOR RECURRENT GENETIC ABNORMALITIES

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Background. Two-hit model of leukemogenesis has been proposed for acute myeloid leukemia (AML). Recurrent chromosomal translocations of t(8;21), inv(16), t(15;17), and *MLL* rearrangements are considered class II mutations. The implication of class I mutations in childhood AML with the 4 major recurrent genetic abnormalities is not clear. *Aims.* We sought to determine the frequencies of class I mutations involving receptor tyrosine kinases (RTK)/JAK2/RAS signaling pathways in childhood AML with the 4 major recurrent genetic abnormalities and to assess their prognostic impact. *Methods.* Two hundred and one consecutive patients with childhood AML were diagnosed between 1996 and 2010. Ninety-nine patients had the 4 major recurrent genetic abnormalities which were detected by cytogenetic analysis and/or RT-PCR. *MLL* gene rearrangement was first screened by cytogenetic, Southern blot or FISH analysis, followed by RT-PCR to detect common *MLL* fusion transcripts and cDNA panhandle PCR to identify the rare *MLL* partner genes. Class I gene mutations, including *FLT3*/ITD, *FLT3*/TKD, *C-KIT*, and *C-FMS* (RTKs); *N-RAS*, *K-RAS*, and *PTPN11* (RAS pathway); and *JAK2*V617F, were examined. Mutational analyses were performed by DNA/cDNA PCR followed by direct sequencing for genes of RTKs and RAS pathways, and allele-specific PCR for *JAK2*V617F. Fifty-seven patients treated with Taiwan Pediatric Oncology Group APL and AML 97A (for non-APL) protocols were analyzed for the prognostic impact of gene mutations. *Results.* The frequencies of gene mutations in each genetic subtype of AML are shown in Table.1.

Table 1.

The frequencies of gene mutations in each genetic subgroup of childhood AML

Genetic subgroup	Pt no.	FLT3/ITD	FLT3/TKD	C-KIT	C-FMS	JAK2V617F	N-RAS	K-RAS	PTPN11
t(15;17)	18	4	4	0	0	0	0	1	1
t(8;21)	39	0	0	15	0	3	0	3	0
inv(16)	17	0	1	7	0	1	4	1	0
11q23-abn	27	0	4	0	2	0	3	3	1

Mutations involving RTKs/ JAK2/ RAS pathways occurred in 51.3 % of t(8;21)AML, 70.6% of inv(16), 62.5% of t(15;17) and 40.7% of *MLL* translocations. Taken together, 53.5 % of patients had class I gene mutations; 38% for RTKs, 17% for RAS pathway and 3% for JAK2V617F, in the whole cohort of AML patients carrying 4 major recurrent genetic abnormalities; 5 of them had multiple gene mutations. The 5-year event free survival (EFS) of 57 patients was 72±6% (mean±S.E.), 92% for patients with t(15;17), 70% for inv(16), 68% for t(8;21), and 62% for *MLL* rearrangement (P= 0.2). The 5-year overall survival (OS) of the whole group was 72%, 90% for t(15;17), 78% for inv(16), 68% for t(8;21), and 67% for *MLL* rearrangement. Comparison of 5-year EFS or OS between gene mutation-positive and mutation-negative patients in each genetic subtype of AML showed that neither gene mutation of RTKs nor gene mutation of RAS pathway had a significant influence on outcomes. *Conclusions.* Our results showed that 53.5% of pediatric AML with the 4 major recurrent genetic abnormalities harbored mutated genes involving RTK/JAK2/RAS pathways but presence of gene mutations did not have influence on outcomes.

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0199

MOLECULAR DETECTION OF CIRCULATING SEZARY CELLS IN PATIENTS WITH MYCOSIS FUNGOIDES: COULD IT PREDICT A FUTURE DEVELOPMENT OF SECONDARY SEZARY SYNDROME? A SINGLE MEDICAL CENTER EXPERIENCE

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Background. Mycosis fungoides (MF) is a primary cutaneous T-cell lymphoma and its prognosis heavily depends on clinical stage. While the majority of patients with early-stage disease have an excellent prognosis with life expectance similar to normal population, few cases progress to secondary Sezary syndrome (sSS), which carries a dismal clinical outcome. An early detection of sSS is crucial for clinical decision making, but a reliable test is currently not available. *Aims.* To investigate the PCR detection of blood T-cell receptor gamma gene (TCRG) rearrangement in patients with MF and its role in predicting clinical outcome with a special focus on cases with B0-1 of blood staging. *Method.* After the institutional approval of the study, 135 cases of MF/SS were identified in our medical informatics system. The clinical staging, skin histology, circulating Sezary cell count, flow cytometric analysis of blood sample and PCR detection of TCRG rearrangement in skin and blood specimens were retrospectively analyzed. Kaplan-Meier survival analysis was performed to study the follow up evaluations and patients' survivals. *Results.* Of 135 total cases, 74 (54.8%) are male and

61 (45.2%) are female. The median age is 60 years with range of 21-93. Fifteen cases (11.1%) fulfilled the diagnosis of SS and 120 were MF. The initial clinical staging includes T1 in 79 (58.5%), T2 in 41 (30.4%), and T3/T4 in 15 (11.1%) cases. In addition, 7 (5.2%) cases were staged as N1-N3 or Nx. No one showed evidence of visceral organ involvement (M0) at initial presentation. At the initial evaluation of blood, the median of Sezary-like cells is 4% of total lymphocytes with range from 0% to 70%. By flow cytometry, the median of percent CD4+/CD7- T-cells is 3.8 with range of 0-83, and the median for CD4+CD26- T-cells is 5.6 with range of 0-87. The median CD4:CD8 ratio is 2.1 with a range of 0.5-50. Of 131 cases with PCR/TCRG performed on blood, 44 (33.6%) are positive for clonal TCRG rearrangement and 87 (66.4%) are negative. When stratified by the diagnoses, the patients with MF showed a 26.5% (31/117) positive rate for blood T-cell clone, of which approximately 50% (10/20) had identical T-cell clone in skin. Follow up evaluation showed conversion into sSS in 50% (5/10) of the cases with positive blood T-cell clone (MF-1) (estimated mean interval=41.8 months) in comparison to none in the cases without (MF-2) (0/31) (P<0.0001) (Figure 1A). Interestingly, 4 of 5 cases with sSS had an identical T-cell clone in skin, while the remaining case did not have the test performed on skin for clonal comparison. Kaplan-Meier survival analysis demonstrated a poor clinical outcome in the group with blood T-cell clone in comparison with the one without in overall survival (P<0.0001) (Figure 1B1) and progression free survival (P<0.0001; HR=22.6) (Figure 1B2). *Conclusion.* The findings suggest a role of molecular detection of blood T-cell clone in predicting sSS. Due to amplification of non-neoplastic T-cell expansion in significant cases, comparison of blood T-cell clone with skin may have a confirmatory value for defining the clonal nature.

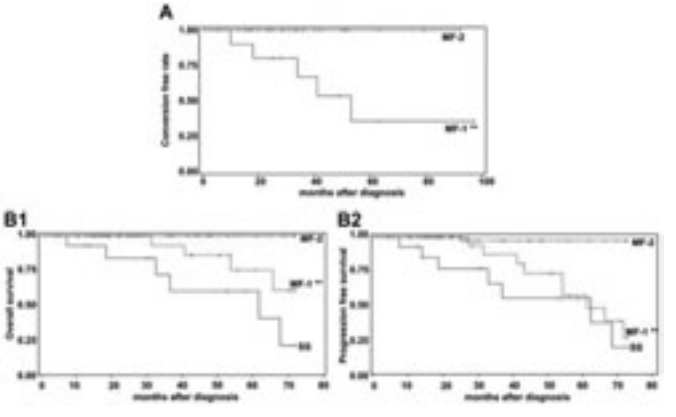

Figure 1. Kaplan-Meier survival analyses.

0200

BCR-ABL QUANTITATION: TO IS OR NOT TO IS, THAT IS THE QUESTION?

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Reverse-transcription real-time quantitative polymerase chain reaction (RQ-PCR) measurement of BCR-ABL oncogene expression is now a routine monitoring strategy for tyrosine kinase inhibitor (TKI) treated chronic myeloid leukemia. A major molecular response of at least a 3-log reduction in BCR-ABL expression is associated with favorable progression to disease free survival. Data from our UK NEQAS LI BCR-ABL quantitation programme has shown that there is an average 105% inter laboratory CV (range 78%-130%). Recently, to overcome this problem, a new International Scale (IS) for BCR-ABL measurement has been proposed. The aim of this study was to determine whether the use of the IS significantly reduces this variability and allows inter-laboratory comparison. UK NEQAS LI issued samples to over 100 laboratories worldwide for BCR-ABL quantitation. The consensus median (calculated from results returned) and delta values (difference between an individual laboratory's result and the median) were determined for each pre- and post- conversion percentage BCR-ABL/control gene result. This approach allowed us to determine whether IS conversion brought individual laboratory results closer to, or further, from the median value. Following analysis of 122 returned results from 8 different samples, it was apparent that many laboratories were inappropriately converting to IS values when BCR-ABL/control gene values were greater than 10% (note IS has not been validated for levels >10% due to the non- linearity of the BCR-ABL1 and ABL control gene relationship). For samples with a consensus median <10%, the number of results that moved closer to the median, post conversion, was 57% (n=33), whilst unexpectedly 43% (n=25) moved away, a fact that may relate to the failure to validate local conversion factors, as recommended by Branford *et al.* (2008).[1] In conclusion, this study has shown that the use of the IS has not reduced inter-laboratory variability but it has highlighted the need for further education and guidance on conversion factor determination/usage and IS application. Future development of reference reagents should help and hopefully lead to better internal quality control systems and facilitate comparative inter-laboratory studies.

References

1. S. Branford *et al*. Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patients response and comparison of response rates between clinical trials. Blood.2008;112:3330-3338.

0201

This abstract has been withdrawn.

0202

HIGH-RESOLUTION COPY NUMBER ARRAY IN THE MOLECULAR CYTOGENETIC DIAGNOSTICS OF PEDIATRIC MALIGNANT HEMATOLOGICAL DISORDERS

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Background and Aim. The importance of genetic characterization with cytogenetic and molecular genetics methods is well established in the diagnosis, prognosis prediction and determination of therapy in the management of malignant hematological disorders. The methods used today have limited detection sensitivity and resolution but are also relatively expensive. In order to validate the use of a genome-wide high resolution DNA copy number analysis in combination with conventional cytogenetic and molecular cytogenetic analysis, a high-density oligonucleotide based single nucleotide polymorphisms (SNP) array was performed on pediatric hematological malignancies. *Material and methods.* Bone marrow aspirates were consecutively collected from 56 children referred for diagnosis and treatment at The Queen Silvia Children's Hospital during the years 2006 to 2009. All patients were diagnosed with malignant hematopoietic disorders, including 24 patients with precursor B lymphoblastic leukemia (B-ALL), 11 with T-cell acute lymphoblastic leukemia (T-ALL), 15 with acute myeloid leukemia (AML), two with myelodysplastic syndrome (MDS), two with chronic myeloid leukemia (CML), one with acute promyelocytic leukemia (APL) and one with Burkitt lymphoma (BL). Genetic alterations were investigated with molecular allelokaryotyping (Human Mapping 250K Nsp Array) in combination with conventional and spectral karyotyping (SKY), flourescence in situ hybridization (FISH) analysis. *Results.* A total of nine balanced reciprocal translocations, 36 unbalanced translocations, 12 deletions and one inversion were detected by G-banding and/or SKY. FISH and RT-PCR confirmed all the known balanced translocations and the inversion. Eleven of the karyotypes in the material were complex, including four B-ALL, three T-ALL and four AML patients. The array analysis identified gains, losses and CNN-LOH in all the acute leukemia types, except for in the APL. The SNP information showed different patterns in the different leukemia entities. Gains dominated the B-ALL patients and losses the T-ALL and AML patients. Nearly all of the CNN-LOH was detected in the T-ALL patients. The SNP array was particularly helpful in detecting additional chromosome aberrations in the B- and T-ALL patients. The SNP array revealed the exact breakpoints in the unbalanced structural aberrations and small deletions were discovered. For example, loss of CDKN2A (9p21) was detected in four T-ALL patients but the array revealed ten losses. Also, the array verifies unbalanced structural rearrangements as well as confirming the balanced. None of the balanced translocations were identified by the array and small pathological clones detected by conven-

tional cytogenetics and /or FISH were not detected as the proportion of these cells was too small. *Conclusions.* Here, we conclude that the addition of SNP array based karyotyping combined with conventional cytogenetics improve the genetic characterization of pediatric hematological malignancies. The two techniques enhance the cytogenetic image and should not be contrasted as they meet distinct important roles. Since balanced translocation cannot be detected with the currently used SNP-arrays and tumor specific translocations are very important diagnostic and prognostic indicators we suggest that SNP-based array is a valuable adjuvant tool in the cytogenetic diagnostics of pediatric leukemias but cannot replace currently used techniques, i.e. G-banding and FISH.

0203

HSP 90 AS A POSSIBLE INDICATOR OF DISEASE DETERIORATION IN CHRONIC MYELOID LEUKEMIA

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Heat shock proteins 90 and 70 (HSP 90 and 70) are chaperones important for stability of various proteins. One of their client proteins is BCR-ABL kinase, fusion protein playing a crucial role in chronic myeloid leukemia (CML). The BCR-ABL/HSP90 complex stabilizes BCR-ABL kinase and decreases cell sensitivity to apoptosis (Shiotsu Y. Blood 2000; 96:2284-2291, Wu, L.X. Leukemia 2008; 22:1402-1409,). Moreover Src kinases and other proteins playing role in CML resistance to therapy are also clients of HSPs 90 and 70. The aim of our study was to find out whether the increasing levels of HSP90 and HSP70 might be associated with resistance to therapy and disease progression. We tested the expression levels of HSP90 and HSP70 in 41 CML patients with various responses to imatinib both on protein (western blots) and mRNA levels (real-time PCR). Eight more patients were examined during the course of therapy and development of resistance. Eight healthy donors and K562 cell lines were used as controls. By western blot analyses we found that the expression levels of Hsp70 did not markedly changed between different responses to therapy, between different disease stages or even between CML patients and healthy subjects. On the other hand, we found high variability in HSP90 protein levels. Patients at diagnosis and patients in good response to therapy (major molecular response) had low expression levels of HSP90 which were very similar to the HSP90 levels in healthy individuals. Patients in hematological relapse and in advanced CML phases (AP/BC) exhibited overexpression of HSP90. Very high HSP90 expression levels were also found in cell lines derived from CML blast crisis. Results indicate that HSP90 protein level well correlates with the disease status. In patients where the HSP90 protein expression was measured in course of disease, the increase of HSP90 level preceded relapse by 2 month at least. Thus the increasing HSP90 level seems to signalize arising resistance to therapy and disease progression. The amount of HSP90 also well correlated with the BCR-ABL transcript level in most cases. The only discordance was in samples from CML diagnosis, where BCR-ABL transcript level was high while HSP90 level was very low. This suggests different intensity of HSP90 action at CML diagnosis and blast crisis. In the same samples we also tested HSP90 expression on mRNA level by real-time RT-PCR. We quantified expression of two HSP90 isoforms - alpha and beta. The mRNA profiles showed high similarity with data obtained from western blot analyses. The analyses showed that increase in HSP90 levels associated with disease deterioration is probably mainly represented by HSP90beta isoform. These results suggest that HSP90 expression at protein as well as mRNA level may be an additional indicator of disease deterioration, a risk factor of poor therapy response. The advantage of such a non-specific general marker is in the possibility of monitoring the leukemic burden independently of the mechanism of developing resistance.

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0204

POLYPLOIDY DETECTION DURING CYTOGENETIC INVESTIGATION OF BONE MARROW FOR THE ASSESSMENT OF THE COURSE OF SOME HEMATOLOGICAL MALIGNANCIES

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Background. Polyploid metaphases are frequent findings during convenient cytogenetic investigation of bone marrow aspirate from patients with different hematological malignancies. However, they aren't recognized in most cases as a pathological feature, referring polyploid metaphases to the signs of normal megakaryocytes presence in the specimen. Nevertheless, endomitotic events in megakaryocytes are rare phenomena in normal bone marrow, as well as spontaneous polyploidization of hemopoetic cells, and contribute approximately 1% of endomitotic events at the same time in short cultures. *The aim of the Study.* was to evaluate the occurrence and the role of ploidy shift in dividing cells from the cytogenetic samples of bone marrow aspirate from patients with myeloid malignancies. *Materials and Methods.* The cytogenetic study was carried out for bone marrow aspirates from 76 patients: 35 of them had chronic myelogeneous leukemia (CML), 2 had primary myelofibrosis (PM) and 39 suffered from different forms of myelodysplastic syndromes (MDS). The standard 48h-method of cell cultivation was used. Karyotype analysis was performed using G-method of differential staining. Up to 25 metaphases were analysed from every aspirate. *Results.* olyploid metaphases was observed in 14 (35.9%) patients with MDS, and appeared in all cases as a mosaic feature (4.2-50.0% of whole amount of metaphases, in a given patient). Polyploidy as the only variation of karyotype was diagnosed in 6 patients; the other 8 had additional chromosome abnormalities. Polyploid cells were clonal in 3 (7.7%) patients with MDS, and constituted 12.5-50.0% of cytogenetically visible cell population of the specimen. The increased ploidy varied from 3n to 13n, but the most frequent (92.8%) were tetraploids in different proportions. Polyploid metaphases (tetraploids alone or in combination with triploids in 1 patient) were detected in 7 (20.0%) of CML patients and constituted 5.0-40.0% of all metaphases. Polyploids appeared after the beginning of treatment in 2 patients with CML, and were the sole change in the karyotypes, while the Ph-chromosome was already cytogenetically undetectable. Both 2 patients with PM had abnormal polyploid metaphases: 2 neartriploid clones in one patient and 2 variants of neartetraploids in another. *Conclusions.* The primary mutations in myeloid malignancies occur in multipotent cells-progenitors, which mean that such mutations could cause effect on both myeloid, erythroid and megakaryocytic lines. So, the explanation of an increased ploidy in the considerable amount of bone marrow cells may be a presence of pathologically changed megakaryocytes with increased endomitotic activity, which indirectly depict retention of residual malignant clone, even if proliferating blasts are eliminated. The other possibility is that discovered polyploids, especially those detected as a clonal change are blasts. Previous reports have shown that polyploid cells have decreased availability to proliferate. This could result in a small amount of polyploid blast cells during cytogenetic investigation. So, polyploid metaphases should be carefully detected, even if discovered in only one copy per sample, because such finding may show the presence of the residual pathological clone with low proliferative index, availability of several copies of oncogenes and enhanced heterochromatinization, which could cause together higher tolerance for injurious effects of antineoplastic agents.

0205

MUTATIONS OF NPM1, FLT3-ITD/TKD AND CEBPA IS VERY RARE IN MYELOID SARCOMA - FROM AN EVALUATION OF THE DIAGNOSTIC UTILITY OF CYTOGENETIC AND MOLECULAR GENETIC STUDIES IN ISOLATED MYELOID SARCOMA

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Background. Myeloid sarcoma (MS), an AML equivalent, refers to one or more tumor masses consisting of myeloid blasts occurring at an anatomical site other than the bone marrow (BM). MS is very rare consisting of 3-5% of AML, showing worse prognosis than other AMLs. Common chromosomal abnormalities in MS include *MLL* or *ETV6-RUNX1* rearrangement, monosomy 7 and trisomy 8. Few studies have

studied the incidence of mutations of *NPM1, FLT3* or *CEBPA* - the good prognosis factors of AML - in MS. The cytogenetic and the molecular genetic abnormalities were investigated using G-banding, FISH and mutation analysis. *Aims.* We identified the incidence of recurrent genetic changes of AML in MS and evaluated the diagnostic utility of cytogenetic and molecular genetic studies in detecting BM involvement of MS without morphological evidence. *Methods.* Fourteen cases (1.8%) of MS were selected based on pathological diagnosis out of 784 AML patients Seoul National University Hospital between Jan 1997 and Oct 2010. Serial specimens of primary tissue of MS and BM at diagnosis and in disease monitoring were included in the study. Interphase FISH technique was used to detect *ETV6-RUNX1, CBFB-MYH11, PML-RARA, MLL* or *BCR-ABL1* rearrangements on tissue and BM specimens. G-banding was performed on the BM specimen. Mutation studies including *NPM1, FLT3-ITD/TKD* and *CEBPA* mutation were also performed on tissue and BM specimens. *Results.* The incidence of MS was 1.8% (14/784, 5 males and 9 females, median age 31.1 years ranging from 1 month to 67 years). Most of them (85.7%, 12/14 patients) were presented as isolated MS without BM involvement at initial diagnosis. Affected sites include gingiva, neck lymph node, small bowel, abdominal wall, mediastinal lymph node, retromolar area, palate, uterus, supraclavicular lymph node, and buttock. Out of 12 with isolated MS, 6 patients developed BM involvement during the treatment (median interval, 5.3 months ranging from 2.7 to 14.0 months). Among 12 with isolated MS, 2 had *MLL* rearrangement, and one had *CBFB-MYH11*. The other 9 showed normal karyotype. In one patient with isolated MS, MLL rearrangement was observed in both the tissue of MS (abdominal wall, 91.0% of all nucleated cells in iFISH) and the BM section (5.0%) at the initial diagnosis, without the morphological evidence of BM involvement. The morphological evidence of BM involvement developed in 4 months with additional gain of 1q. Patients who developed BM involvement of GS during treatment showed *MLL* or *CBF-MYH11* rearrangement in both tissue and BM (2 cases), complex translocations involving chromosome 5, 4, 12, 15, 17 and 21, and loss of 7q- (2 cases). All 12 cases showed wild type of *NPM1, FLT3-ITD/TKD* and *CEBPA* genes. *Conclusions.* We confirmed that *MLL* and *CBF-MYH11* is relatively common cytogenetic abnormalities in MS. The mutations of *NPM1, FLT3-ITD/TKD* and *CEBPA* genes were not observed in MS in this study, which might be associated with the poor prognosis of MS than other AMLs. Our result suggests the potential diagnostic utility of cytogenetic/molecular genetic studies in detecting the occult BM involvement of MS without definite morphological evidence.

0206

MONITORING OF TOTAL WT1 EXPRESSION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML): WT1 AS A HELPFUL MARKER BESIDES BCR-ABL

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WT1 encodes for a transcriptional regulator which behaves as an oncogene in leukemias. Although the mechanism of WT1 oncogenic behaviour has not yet been uncovered, WT1 expression is already used as a marker for monitoring minimal residual disease of patients with acute myeloid leukemia. Concerning CML, there is still very limited information about WT1 expression, most of the studies focused on WT1 expression significance for early prediction of relapses following allogenic stem cell transplantation. Now, it is of interest whether monitoring of WT1 expression might be useful also for patients on tyrosin kinase inhibitors. In our study, we focused on WT1 expression in CML patients on imatinib. Altogether, we have examined 35 patients during the course of imatinib therapy: 4 patients exhibited optimal response, 8 suboptimal response and 23 therapy failure. Twenty % of those patients relapsed and the remaining patients stayed without relapse for more than 30 months (45,5 months in median). Real-time PCR was used according to Cilloni *et al*., 2004, B2M was applied as control gene. Predictive value of WT1 expression for development of haematological relapse was compared with that of BCR-ABL transcript and mutations in the BCR-ABL kinase domain. Before upcoming haematological relapse, WT1 expression was increased in about 60% of patients more expressively and in about 45% of patients even in median 2,3 months earlier as compared to BCR-ABL expression. As compared to BCR-ABL mutation analyses, WT1 increase predicted relapse earlier in about one third of patients. According to our experience, CML patients exhibiting suboptimal response and therapy failure represent a highly heterogenic group in terms of relapse emergence during the course of therapy. In our patients cohort, there were 70 % of suboptimal responders and 30 % of patients in therapy failure who did not relapse for more than 30 month of therapy despite of high levels of BCR-ABL (10 to 100%). Interestingly, exact WT1 levels even in the 12th month of therapy corresponded to relapses emergence during further course of the disease. Expression levels higher that 0,1 meant 90% probability of relapse. Taken together, our data indicate that WT1 might be a very useful marker for both relapses prediction during the course of therapy and patients stratification according to risk of relapse at the beginning of treatment. Monitoring of WT1 expression might be thus of advantage both for improving CML therapy outcomes and for further investigation of mechanism underlying different responses to therapy.

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Drug resistance and pharmacology

0207

PHARMACOGENOMIC PROFILE ASSOCIATED WITH HIGH SENSITIVITY AND LOW TOXICITY TO A COMBINATION OF GEMTUZUMAB OZOGAMICIN PLUS FLUDARABINE, CYTARABINE, IDARUBICIN IN CD33-POSITIVE ACUTE MYELOID LEUKEMIA

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Background. In acute myeloid leukemia (AML) the presence or absence of cytogenetic abnormalities allows the identification of favorable, intermediate and unfavorable subgroups. However, besides these specific subgroups, little it is known about the genetic variations influencing specific drug-related phenotypes. *Aims.* To perform an exploratory pharmacogenomic study that relates genetic variations in multidrug enzymes and transporters genes with the efficacy and toxicity to treatment. *Patients and Methods.* We analyzed 94 CD33-positive AML patients younger than 65 previously untreated and enrolled in a phase III multicenter clinical trial combining low dose of Gemtuzumab-ozogamicin (GO) with FLAI regimen (Fludarabine, Cytarabine, Idarubicin) as Induction chemotherapy (eudract: 2007-005248-26; ClinicalTrials.gov NCT00909168). The induction regimen (GO-FLAI) included fludarabine (25 mg/sqm) and Ara-C (2 g/sqm) on days 1-5, idarubicin (10 mg/sqm) on days 1, 3, and 5 and GO (3 mg/sqm) on day 6. Hematopoietic stem cell transplant was planned for all high risk AML patients in first complete remission (CR) after consolidation with intermediate doses of Ara-C and idarubicin. Cytogenetics, multidrug-resistance phenotype, FLT3 and NPM mutation status, as well as WT1 quantitative expression analyses were performed at diagnosis in all patients. Furthermore, high-resolution single nucleotide polymorphism (SNP) array analysis (Affymetrix, Inc. Santa Clara, CA, USA) was also performed. The allele frequencies of 1936 genetic variations of 225 absorption, distribution, metabolism and excretion were assessed using the new Affymetrix drug-metabolizing enzyme and transport (DMET Plus) genotyping platform (Affymetrix). All statistical analyses were performed using the R package 2.11.1. *Results.* Of the 94 patients, genotype results were evaluable for 91 cases. The median call rate was 99.48 (range, 96.32-100). Three samples were run in duplicate and results with "passed call rate"were compared across all the polymorphic sites, showing a repeatability of 99.99%. In an initial screening procedure, we tested the association among SNPs and response to the induction cycle (FLAI + Gemtuzumab-Ozogamicin). Therefore, the genotyping profile of 80 patients in complete (85%) and partial (3%) remission was compared to that of patients (12%) with no response. We found a highly significant difference ($p < 0.001$) in the allele frequency of 2 variants, in complete linkage disequilibrium, in the alcohol dehydrogenase enzyme (ADH1A). These variants were not associated with high risk AML, FLT3 and NPM1 mutations, but strongly influenced response to the induction phase also in a multivariate analysis. Since genetic polymorphisms may influence the toxicity of chemotherapy drugs, we stratified SNPs according to liver toxicity and a significant difference in the allele frequency of a member of the cytochrome P450 family which is involved in the alcohol metabolism (CYP2E1) was found to be associated with a grade I/II liver toxicity. *Conclusions.* A pharmacogenomic panel made up of 1 gene (ADH1A) associated with clinical outcome and 1 gene (CYP2E1) associated with toxicity was for the first time identified in AML patients younger than 65 years treated with a combination of GO and FLAI regimen.

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0208

CELLULAR INHIBITOR OF PROTEIN PHOSPHATASE 2A DETERMINES BORTEZOMIB-INDUCED APOPTOSIS IN ACUTE LEUKEMIA CELLS

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Background. Bortezomib has excellent antitumor activity against multiple myeloma and mantle cell lymphoma via its proteasome inhibition. The multiple cellular targets affected by proteasome inhibition implicate the potential role of bortezomib in enhancing antitumor activities in other hematological malignancies, despite that currently bortezomib is only approved in mantle cell lymphoma and multiple myeloma. Our previous study has shown that down-regulation of phospho-Akt (p-Akt) plays a key role in determining the sensitivity of hepatocellular carcinoma cells to bortezomib-induced apoptosis. *Aims.* In this study we aimed to examine the antitumor activity of bortezomib and to further explore the mechanism by which bortezomib induces apoptosis in acute leukemia cells, particularly focusing on target(s) regulating p-Akt (such as protein phosphatase(s)). *Methods.* Several acute leukemia cell lines were used for *in vitro* studies. Apoptosis was examined by both flow cytometry and Western blot. Signal transduction pathways in cells were assessed by Western Blot. Gene silencing was done by small interference RNA (siRNA). *Results.* We demonstrated bortezomib differentially induced apoptosis in acute leukemia cells (Fig 1b and 1c). Importantly, bortezomib showed the similar inhibition of the proteasome activity in both sensitive and resistant cells, suggesting that bortezomib-induced apoptotic effect may be independent of its proteasome inhibitory effect (Fig 1d). Furthermore, we found that a novel oncoprotein, cancerous inhibitor of pro-

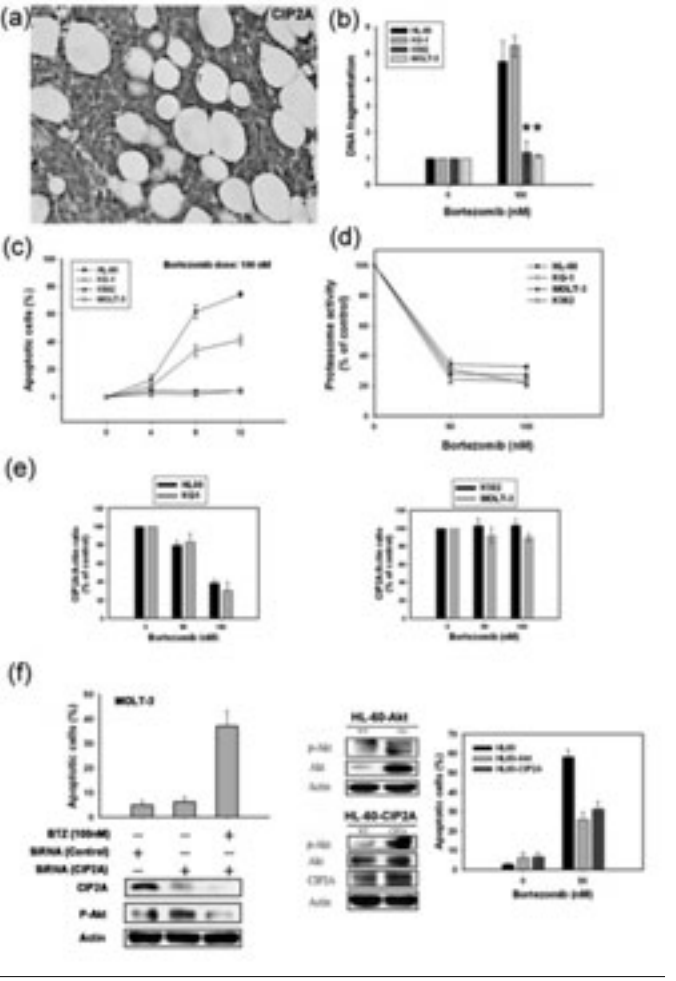

Figure 1. CIP2A in acute leukemia.

tein phosphatase 2A (CIP2A), a cellular inhibitor of protein phosphatase 2A (PP2A), mediated this apoptotic effect of bortezomib in acute leukemia cells. CIP2A expression is readily demonstrated in acute leukemic blasts (Fig. 1a). In accordance to our previous study on bortezomib, we showed bortezomib increases PP2A activity in sensitive acute leukemia cells (HL-60 and KG-1), but not in resistant ones (MOLT-3 and K562). We validated that bortezomib's down-regulation of CIP2A and p-Akt correlates with its drug sensitivity. Over-expression of CIP2A or Akt in HL-60 cells protected cells from bortezomib-induced apoptosis, and down-regulation of CIP2A by siRNA overcame the apoptotic resistance to bortezomib in MOLT-3 cells (Fig 1e and 1f). Furthermore, we indicated that CIP2A negatively regulates Akt-related PP2A activity. Importantly, bortezomib exerts *in vivo* antitumor activity in HL-60 xenografted tumors (in nude mice), but not in CIP2A overexpressed HL-60 tumors. Interestingly, HL-60 cells with ectopic CIP2A expression had increased cell proliferation and DNA synthesis, as well as a more rapid xenografted tumor growth. *Summary.* In conclusion, this study has identified CIP2A as a major molecular determinant of bortezomib's sensitivity on acute leukemia cells and that CIP2A may play an important role in leukemia biology. It is also implicated that focusing on interaction of oncoprotein and phosphatase and kinases could be a novel anti-leukemia strategy.

0209

THE MULTI-KINASE INHIBITOR TG02 DOWNREGULATES MCL-1 IN AML CELLS AND PREFERENTIALLY TARGETS CD34+CD38-CD123+ CELLS FROM SAMPLES WITH AN INTERNAL TANDEM DUPLICATION OF FLT3

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Background. In clinical trials, FLT3 inhibitors are reported to kill circulating AML blasts, but the bone marrow is protected. We have previously reported that niche-like conditions (fibronectin and a cytokine cocktail) significantly reduced the *in vitro* toxicity of the FLT3 inhibitor AG1296 to AML cells. Moreover, the toxicity of AG1296 to the quiescent, stem-cell-enriched leukaemic CD34+CD38-CD123+ subset was completely abolished under niche-like conditions. The novel multi-kinase inhibitor TG02 has selectivity against cell cycle and transcriptional CDKs and JAK2 as well as FLT3. TG02 has efficacy in *in vivo* models and induces apoptosis in primary AML cells including CD34+CD38-CD123+ cells. We have now evaluated the impact of FLT3 internal tandem duplications (ITDs) on the *in vitro* toxicity of TG02 under niche-like conditions. FLT3 ITDs are associated with over-expression of the survival molecule Mcl-1, particularly in the leukaemic stem cell compartment. We have therefore investigated the impact of TG02 on Mcl-1. *Methods.* The 48 hour toxicity of TG02 to primary AML blasts and to the MOLM13 cell line, which harbours a FLT3 internal tandem duplication (ITD), were studied by flow cytometric viable cell enumeration. Mcl-1, phosphorylated (active) STAT5 and phosphorylated RNA polymerase II serine 2 (RP2) were measured by flow cytometry. Phosphoproteome analysis was performed with a kit from R&D Systems. Phosphorylated p38 was measured by Western blotting. *Results.* In a cohort of eight FLT3-ITD and twelve FLT3-wildtype samples examined under niche-like conditions, 100 nM TG02 induced median decreases of 37% in bulk cells and 33% in CD34+CD38- CD123+ cells, whereas AG1296 (5μM) induced a median 20% decrease in bulk cells but no reduction in CD34+CD38- CD123+ cells. Cells with a FLT3 ITD were preferentially targeted by TG02 (p=0.05 for bulk cells and P=0.002 for CD34+CD38-CD123+ cells). TG02 might target Mcl-1 through inhibition of JAK/STAT pathways and/or through CDK9-mediated inhibition of RP2. In MOLM13 cells, a 20 hour incubation with 100 nM TG02 inhibited active RP2 to 4% of control values, and Mcl-1 protein was decreased by 73%, but STAT5 activity was not reduced. On treating primary AML samples with TG02, we found inhibition of STAT5 activity in the absence, but not the presence, of supportive niche-like conditions. However, RP2 is strongly inhibited by TG02 in primary samples, even in the presence of niche-like conditions and Mcl-1 protein expression is decreased, suggesting a role for CDK9 inhibition. Additionally, signalling through the stress kinase p38 is strongly inhibited by TG02, which may induce other changes in apoptotic regulatory circuits. These are currently under investigation. *Conclusion* . Under niche-like conditions TGO2 is able to target CD34+CD38-CD123+ AML cells, as well as bulk AML cells, particularly in samples characterised by a FLT3 ITD. TG02 toxicity in AML is associated with inactivation of RNA Polymerase II and p38 and downregulation of Mcl-1.

0210

POLYMORPHISM Q141K OF ABCG2 PROTEIN IS ASSOCIATED WITH POOR OUTCOME IN ACUTE MYELOID LEUKEMIA PATIENTS RECEIVING IDARUBICIN-BASED CHEMOTHERAPY

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Background. Single nucleotide polymorphisms (SNPs) in drug metabolism and transporters genes can explain the large inter-individual variability in response to therapy. Among naturally occurring SNPs reported in ABCG2 protein, a member of ABC transporter protein recently associated with negative prognosis in acute myeloid leukemia (AML), the Q141K variant is the most frequent in Caucasian population. Data from *in vitro* studies suggest that this polymorphism affects the protein function. modifying the resistance profile of many anticancer agents such as mitoxantrone, daunorubicin, topotecan and SN-38. However, *in vivo* effects are still largely unknown. *Aims.* We evaluated the prevalence of Q141K ABCG2 protein in a series of patients with AML referred to the Division of Hematology of Udine for diagnosis and/or therapy, with the purpose to assess its potential impact on response to chemotherapy. *Methods.* A total of 163 consecutive patients with non-promyelocytic AML were included in the study. ABCG2 expression was evaluated by flow cytometry; ABCG2 mRNA expression was normalized to the simultaneously analyzed GAPDH gene (assays on demand Applied Biosystems: ABCG2 Hs00184979 m1 and GAPDH Hs9999905 m1,). ABCG2 sequence was evaluated by the TaqMan SNP Q141K genotyping Assay (Applied Biosystems, C_15854163_70). One hundred twenty two of 163 patients received chemotherapy according to the Institutional protocol; induction regimen included fludarabine in all cases and idarubicin was the only anthracycline used through all the therapeutic program. *Results.* Q141K ABCG2 was detected in 29/163 (17.8%) patients, a percentage comparable to what is reported by the literature in Caucasian ethnicity. Membrane protein expression was variable among mutated patients: 18/29 (62%) overexpressed ABCG2. Seventy two out of the 122 (59%) treated patients responded to therapy, irrespectively of ABCG2 expression or protein polymorphism. Complete remission was affected by age, CD34 and CD56 expression. Relapse occurred in 27/72 (52%) patients; relapse risk was affected by ABCG2 overexpression, CD34 positivity and CD56 expression. Considering the whole population, a trend of a shorter leukemia free survival (LFS) was observed in patients with Q141K variant, but the difference did not reach statistical significance. However, stratifying patients in three groups, by high or low ABCG2 expression and by presence of Q141K polymorphism (regardless of ABCG2 expression intensity), we observed a significantly higher relapse probability in mutated patients, whose LFS was comparable to those with ABCG2 over-expression (chi squared 8.7; p=0.019). Median survival was 16 months (95% CI: 11-31 months). Overall survival (OS) was affected by age, CD34 and high ABCG2 expression. Again no differences were observed between wild type or mutated protein if the whole population was considered, but a significantly worse survival in Q141K group emerged after stratification in three groups (chi squared 6.8; p=0.032). High ABCG2 expression, Q141K polymorphisms, age and CD34 positivity maintained their negative impact also in multivariate analysis. *Summary/Conclusions.* This is the first observation of the negative impact of Q141K ABCG2 polymorphism on leukemia outcome. If confirmed in larger studies, our data suggest that patients-tailored post induction therapy should be planned in presence of Q141K ABCG2, including drugs not affected or positively affected by the mutated protein.

0211

REGULATION OF CYP3A4 AT NFSE IN HEMATOPOIETIC CELLS INVOLVES RNA AND EF1A

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Background. CYP3A4 is the most abundantly expressed hepatic cytochrome P450 and significantly contributes to drug metabolism, with substantial intra- and inter-individual variability. Its regulatory mechanisms are still enigmatic, with complex interactions in the 5'-flanking region. An A to G substitution in the nifedipine responsive element (NFSE) of the CYP3A4 promoter (termed CYP3A4*1B) is associated with a lower incidence of pediatric (Felix, PNAS, 1998) and adult (Rund, Leukemia, 2003) therapy-related AML. Previous studies by

others demonstrated binding of nuclear proteins at NFSE in hepatic cells and we have found such binding in hematopoietic cell lines and primary AML patient cells. NFSE regulation appears to be stress-responsive (heat shock) (David-Kalish, 2005). *Aims.* 1. To analyze expression of wild type and polymorphic NFSE sequences in hematopoietic cells. 2. To identify the proteins which differentially bind to the wild type and polymorphic sequences. 3. To determine if these proteins are regulated by stress (such as heat and chemotherapy), possibly via HSR1, an RNA molecule that activates stress response genes such as HSF-1. *Methods.* We constructed luciferase reporter plasmids (pGLE vector) driven by the CYP3A4 promoter, using polymorphic and wild type sequences. These plasmids were transfected into hematopoietic cell lines: K562 (CML blast crisis), CCRF (T cell ALL) and KG1a (myeloid leukemia). HepG2 (hepatoma) served as a control. In addition, electrophoretic mobility shift assays (EMSA) were performed using nuclear extracts from various cells using radiolabelled probes corresponding to the wild type and polymorphic sequences. A streptavidin-biotin system was used to isolate the proteins binding at NFSE. *Results.* CYP3A4 reporter gene studies demonstrated 20-30% lower activity in KG1a, CCRF and K562 using the polymorphic compared to the wild type sequence while HepG2 showed higher activity with the wild type. The results for HepG2 confirmed results reported by Rebbeck (2003). EMSA demonstrated NFSE binding using nuclear extracts from CCRF which increased following heat shock. In CCRF-Actinomycin resistant cells, the heat shock effect was stronger. The streptavidin-biotin system demonstrated that the DNA binding complex at NFSE was present in the same elution fractions containing HSF-1. Mass spectrometry demonstrated elongation factor 1a (EF1a). EF1a is known to be activated by an RNA molecule (Shamovsky 2006). Treatment with RNase enhanced binding to NFSE, suggesting that RNA interferes with binding of nuclear proteins to the CYP3A4 promoter. Addition of EF1a antibodies to nuclear extracts increased binding. NFSE complex formation was further enhanced when RNase and anti-EF1a were used together. The influence of RNase on the signal of the complex using the polymorphic NFSE probe was greater than its effect on NFSE wild type. Additional factor(s) may facilitate(s) assembly of the complex. The ALIBaBa database of transacting factors states high homology of the 5'11bp-NFSE-9bp 3' region with the consensus binding site of C/EBPb. C/EBPb itself was found involved indirectly, reducing the strength of the complex, possibly by binding to the C/EBPb element, found upstream to NFSE. *Conclusions.* Regulation of CYP3A4 at NFSE in hematopoietic cells appears to be stress-responsive, involving RNA as well as EF1a, not previously reported for other CYP genes.

0212

INTERACTION OF THE COMMON HOCT1 M420DEL AND M408V SNPS IS CRITICAL IN PREDICTING CLINICAL OUTCOME IN IMATINIB TREATED CHRONIC MYELOID LEUKAEMIA

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Background. The human organic cation transporter 1 (hOCT1) is responsible for the uptake of imatinib into chronic myeloid leukaemia (CML) cells. Its expression level and functional activity are powerful predictors of the clinical response to imatinib (Wang *et al.,* Clin. Pharm. Therapeut. 2008, White *et al.,* Blood 2007). Several single nucleotide polymorphisms (SNPs) in hOCT1 affect its expression and transport activity, contributing to inter-individual variation in clinical response. SNPs in hOCT1, particularly M420del (c.1260del, allele frequency 18.5% in European-Americans), can affect the action and pharmacokinetics of metformin, an anti-diabetic drug and a well known hOCT1 substrate (Shu *et al.,* Clin. Pharm. Therapeut. 2008). In addition, M408V is another common hOCT1 SNP with allelic frequency approximately 59.8% in European-Americans. *Aim.* Firstly to analyse the uptake activities of the common hOCT1 variants M420del and M408V in a CML cell line model and secondly to investigate the effect of these SNPs on the clinical response to imatinib treatment in a large cohort of newly diagnosed CML patients. *Methods.* KCL22 CML cells were selected for hOCT1-transfection due to their low basal hOCT1 expression. The specific SNPs were introduced to the pcDNA-hOCT1 plasmid by site-directed mutagenesis. Stably transformed cell lines with various combinations of M420del and M408V SNPs were generated. The uptake of 14C-radiolabelled imatinib into different cells was measured by scintillation counter. Genomic DNA samples were prepared from peripheral blood from 182 newly diagnosed chronic phase CML patients prior to imatinib therapy. Genotyping for the M420del and M408V SNP was performed by pyrosequencing and Sequenom MALDI-TOF Mass ARRAY. *Results.* Cell lines carrying the hOCT1 M420del and M408 showed a significant decrease in imatinib uptake compared with wild-type KCL22 cells with undeleted M420 and M408 (p=0.001). Conversely, KCL22 cells with undeleted M420 and V408 had an increased imatinib uptake compared with cells with undeleted M420 and M408 (p=0.05). In KCL22 cells with both M420del and V408, uptake did not differ from the wild type cells. These data imply that both M420del and M408V play a role in the uptake of imatinib by hOCT1 transporter, whereby M420del decreases uptake and V408 increases it. In clinical samples from 182 cases of newly diagnosed CML patients treated with imatinib, patients who carried the M420del allele (n=53) had a greater probability of imatinib resistance requiring a change of therapy (p=0.034) and treatment failure (i.e. resistance + intolerance; p=0.024, Kaplan-Meier log-rank test) than patients with undeleted M420. Patients carrying both M420del and M408 alleles (n=43) had a greater probability of imatinib resistance requiring a change of treatment (p=0.025) and treatment failure (p=0.014). However, patients with both M420del and V408 had comparable times to treatment failure to patients with undeleted M420 and M408 demonstrating that V408 can reverse the adverse effect of M420del. *Summary/Conclusions.* This study provides evidence on the functional significance of both M420del and M408V polymorphisms in hOCT1. It also demonstrates the importance of SNP interactions in determining clinical outcome. These SNPs may have potential use for patient-specific therapy in newly diagnosed chronic phase CML.

0213

PREDICTION OF IMATINIB THERAPY RESPONSE: A ROLE OF INTRACELLULAR TRANSPORTERS HOCT-1 AND ABCB1

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Background. The role of intracellular IMA transport in overall resistance and in treatment outcome prediction have been intensively studied since main influx (hOCT-1) and efflux (ABCB1) transport proteins have been described. Several studies that correlate the pretreatment activity/mRNA expression of IMA transporters with subsequent therapy response have been reported to date. However, different cell populations from patients who received various degree of pretreatment were used for these analyses. Therefore, several biases in the results and their interpretation may arise. *Aims.* In this report we demonstrate that the composition of analyzed material at the time of testing (i.e. percentage of different cell types) has critical impact on the resultant activity/mRNA expression of these transporters, and therefore it can significantly affect the overall correlation and data interpretation. *Methods.* We analyzed the differences in *hOCT-1* and *ABCB1* mRNA expression measured in peripheral blood (PB) leukocytes (LEU), polymorphonuclear cells (PMNC) and mononuclear cells (MNC) of PB LEU obtained from healthy volunteers and patients with *de novo* CML. Additionally, we analyzed the changes in *hOCT-1* expression during the first six months of IMA therapy and the relationship between the percentage of individual cells that comprise the total LEU count and *hOCT-1* and *ABCB1* mRNA expression assessed from the total LEU. Finally, we investigated the predictive value of the pretreatment mRNA expression levels of *hOCT-1* and *ABCB1* in selected cell populations with regard to the therapy response at six and twelve months of IMA therapy. *Results.* The *hOCT1* mRNA expression was significantly higher in PB PMNC compared to MNC. Expression in each analyzed group of cells was always significantly lower in IMA naïve *de novo* CML patients compared to healthy volunteers. This difference disappeared after the initiation of IMA therapy, suggesting that CML tumor burden and the degree of pretreatment at the time of monitoring were both influencing factors. Moreover we found the statistically significant relationship between *hOCT-1* mRNA expression and the percentage of immature myeloid cells as well as BCR/ABL transcript levels in PB (both as indirect markers of tumor burden). Considering *ABCB1* expression, it was significantly higher in MNC compare to PMNC. Similarly to *hOCT-1*, a

correlation with percentage of immature myeloid cell was obtained. All these results suggest that both *hOCT-1* and *ABCB1* mRNA expression level used as a prognostic factor should always be assessed in relation to the cell type in which expression was measured. Finally, regarding the therapy response prediction, no statistically significant relationship between the pretreatment levels of *hOCT-1* or *ABCB1* mRNA expression in different cell populations and therapy response, have been observed. *Conclusion.* The observed cell type dependence, tumor burden dependence, and other pre-analytical and analytical biases found in recent literature lead to the conclusion that the desired stratification of CML patients into responders/non-responders prior to IMA therapy is hardly to obtain and these parameters are not suitable for routine clinical practice.

0214

P-GLYCOPROTEIN AND BREAST CANCER RESISTANCE PROTEIN IN ACUTE MYELOID LEUKAEMIA CELLS TREATED WITH THE AURORA-B KINASE INHIBITOR BARASERTIB-HQPA

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Background. Aurora kinases play an essential role in orchestrating chromosome alignment, segregation and cytokinesis during mitotic progression, with both aurora-A and B frequently over-expressed in a variety of human malignancies. Over-expression of the ABC drug transporter proteins P-glycoprotein (Pgp) and Breast cancer resistance protein (BCRP) is a major obstacle for chemotherapy in many tumour types with Pgp conferring particularly poor prognosis in acute myeloid leukaemia (AML). Barasertib-hQPA is a highly selective inhibitor of aurora-B kinase that has shown tumouricidal activity against a range tumour cell lines including those of leukaemic AML origin. *Aims.* Using leukaemic cell lines and primary samples we aim to investigate the specificity of barasertib-hQPA with particular reference to their ABC transporter status. *Methods.* We analysed response in a panel of leukaemic cell lines and 37 primary AML samples by measuring phosphoHistone H3 (pHH3) expression, the biomarker for barasertib-hQPA activity. *Results.* In this study we report the creation of the cell line OCI-AML3DNR, which over-expresses Pgp but not BCRP or multidrug resistance-associated protein (MRP), through prolonged treatment of OCI-AML3 cells with daunorubicin. We demonstrate that Pgp (OCI-AML3DNR and KG-1a) and BCRP (OCI-AML6.2) expressing AML cell lines are less sensitive to barasertib-hQPA induced pHH3 inhibition and subsequent loss of viability compared to transporter negative cell lines. We also show that barasertib-hQPA resistance in these cell lines can be reversed using known Pgp and BCRP inhibitors. We report that barasertib-hQPA is not an inhibitor of Pgp or BCRP, but by using 14[C]-barasertib-hQPA that it is effluxed by these transporters. We measured Pgp and BCRP expression in 37 primary AML samples. 9/37 (24.3%) were positive for Pgp and 9/35 (25.7%) were positive for BCRP with a significant correlation (p=0.008) seen for co-expression. By measuring pHH3 expression in AML samples cultured for 1 hour with or without barasertib-hQPA we determined that Pgp and BCRP positive primary samples were less sensitive to barasertib-hQPA induced pHH3 inhibition (p=<0.001) than transporter negative samples. However, we found that IC50 inhibition of pHH3 by barasertib-hQPA was achieved in 94.6% of these samples after 1 hour drug treatment, in contrast to the resistance seen in the cell lines. *Conclusions.* We conclude that monitoring of Pgp, BCRP status and pHH3 down-regulation should be considered in patients treated with barasertib in order to establish whether transporter-mediated efflux is sufficient to adversely impact on the efficacy of the agent.

0215

THE ABCC3 EFFLUX TRANSPORTER CAN PREDICT CLINICAL OUTCOME IN CML PATIENTS AND IS AN IMPORTANT DETERMINANT OF DRUG FAILURE

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Background. Imatinib is a substrate for the ABC efflux transporters ABCB1 (MDR1) and ABCG2 (BCRP), whereas its uptake into the cells is highly dependent on the expression and/or the activity of the influx transporter SLC22A1 (OCT1). A recent study by Hu *et al*. (Clin. Cancer Res, 2008) showed that SLC22A1 expression was interrelated with the expression of ABCB1, ABCC3, ABCC4, ABCG2 and OATP1A2. Thus, they suggested that SLC22A1 expression may be a composite surrogate for expression of other transporters relevant to the intracellular uptake and retention of imatinib. *Aim.* To assess a panel of organic anion/cation and ABC transporters in CML patients to determine their contribution to the cellular transport of imatinib and to relate findings to the eventual patient outcome. *Materials and Methods.* Peripheral blood leukocytes were collected from 4 normal individuals and 48 newly diagnosed CML chronic phase patients prior to imatinib treatment, and 12 months after initiation of therapy. All CML cases were positive for the t(9;22) translocation at diagnosis and were classified into three response groups following 12 months of imatinib treatment; R (responders, complete cytogenetic response), NR (non-responder, complete haematological but no cytogenetic response) and blast crisis (BC). The TaqMan low density array (TLDA) and single TaqMan gene expression assays were used. The relative expression level of a particular gene of a given sample was calculated by the Comparative Ct method. KCL22, K562, KYO1 and Lama84 CML cell lines were included as controls. *Results.* A significant positive correlation was shown to exist between SLC22A1 (OCT1) and SLC22A4 (r=0.74, Sign at 0.000), ABCC3 (r=0.6, Sign at 0.000), SLCO3A1 (r=0.68, Sign at 0.000), SLCO1A2 (r=0.53, Sign at 0.000), SLC22A5 (r=0.48, Sign at 0.001), and ABCB1 (r=0.48, Sign at 0.001). Clinically, of the influx transporters, only SCL22A4 appeared to be important (but not to statistically significant levels R vs NR/BC p=0.059), while for the efflux transporters ABCC3 appeared to be more important than ABCB1 (R vs NR p=0.016; R vs BC p=0.027, respectively). Additionally, when patients' SLC22A1, ABCB1 and ABCC3 mRNA levels were compared it was apparent that patients with high ABCC3 expression were failing treatment irrespective of SLC22A1 expression levels. *Summary/Conclusions.* Our data clearly demonstrate the importance of the ABCC3 efflux transporter in imatinib treated CML patients and propose its potential use as a prediction marker of treatment failure. We are testing this idea within a larger clinical cohort as well as against the clinical response with other TKIs.

0216

MOLECULAR MECHANISMS OF NILOTINIB RESISTANCE AND REVERSAL OF RESISTANCE IN CHRONIC MYELOID LEUKEMIA CELLS

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Background. Chronic myeloid leukemia (CML) is a disease of hematopoietic stem cells, arising from a reciprocal translocation between long arms of chromosomes 9 and 22, known as the Philadelphia chromosome. This translocation causes a juxtaposition of ABL and BCR genes, resulting in a BCR-ABL fusion gene. Nilotinib disrupts the ATP-binding pocket of BCR/ABL oncoprotein and inhibits interaction, and thus, phosphorylation with the target proteins. However, despite high rates of hematologic and cytogenetic responses, intrinsic or acquired nilotinib resistance is still the main problem for the treatment of chronic myeloid leukemia patients. *Aims.* In this study we aimed to develop a nilotinib resistance cell line, to examine the molecular mechanisms of nilotinib resistance. Furthermore, we aimed to reverse nilotinib resistance by modulating bioactives phingolipids metabolism with using glucosyle ceramide synthase (GCS) and sphingosine kinase-1 (SK-1) inhibitors. *Methods.* Human Antiproliferative effects of nilotinib on K562 and 50 nM nilotinib-resistant K562 (K562/NIL-50) cells were determined by XTT cell proliferation assay. Changes in caspase-3 enzyme activity and mitochondrial membrane potential were determined by caspase-3 colorimetric assay kit and JC-1 mitochondrial membrane potential detection kit, respectively. Expression levels of Bcr/Abl, apoptosis related genes (bcl-2, bcl-xl, bax, caspase-3), ceramide synthase genes (CerS1-6), GSS and SK-1, drug transporter genes (mdr1, mrp1, bcrp, lrp) and Beta-actin as an internal positive control were assed by RT-PCR. *Results.* IC50 values of nilotinib were calculated as 42 and 385 nM for K562 and K562/NIL-50 cells, respectively indicating that K562/NIL-50 cells gain about 10-fold resistance to nilotinib as compared to parental K562 cells. Apoptotic mechanisms were repressed in K562/NIL-50 cells as determined by decrease in caspase-3 enzyme activity and increases in mitochondrial membrane potential. Expression levels of Bcr-Abl gene were upregulated in K562/NIL-50 cells as compared to parental sensitive cells. Nucleotide sequence analyses of ABL kinase gene revealed that there was no mutation in nilotinib binding site of BCR/ABL oncogene in resistant cells. There was also an increase in expression levels of MRP1 gene in resistant cells. Besides, apoptotic Bax and CerS1 genes were downregulated and

antiapoptotic GCS and SK-1 genes were upregulated in K562/NIL-50 cells. Inhibition of GCS and SK-1 by chemical inhibitors sensitized K562/NIL-50 cells to nilotinib as determined by cell proliferation and apoptosis analyses. *Summary/Conclusions.* Determination of genetics mechanisms of cellular resistance in response to nilotinib is very important. In conclusion, we determined mechanisms involved in nilotinib-resistance in CML cells. Our results also demonstrated that targeting antiapoptotic GCS and SK-1 genes, besides inhibition of BCR-ABL by nilotinib, may be a good way of treatment of CML.

0217

PHARMACOGENETICS IN CHRONIC MYELOID LEUKEMIA TREATMENT: A TOOL TO PREDICT THERAPY OUTCOME TO IMATINIB TREATMENT?

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Background. Imatinib mesylate (IM) is a selective tyrosine kinase inhibitor, that has achieved successful treatment outcomes and improved the life quality of chronic myeloid leukemia (CML) patients. However, some of the patients fail to achieve optimal response, and a substantial proportion of patients develop resistance to IM. Several determinants were known to be associated with the pharmacokinetics of imatinib with respect to absorption, distribution, and metabolism, influencing the systemic level or intracellular concentration of imatinib which might affect response to therapy. IM is a substrate for the adenosine triphosphate binding cassette (ABC) transporters, ABCB1 and ABCG2, whereas the active uptake of IM into cells is mediated by the human organic cationic transporter-1 (OCT1; SLC22A1). Also, IM is metabolized through first-pass drug metabolism by the cytochrome P450 - CYP1A2, CYP2C9, CYP2D6, CYP3A4 and CYP3A5. Genetic variations affecting genes involved in IM transport and metabolisms, however, role of single nucleotide polymorphisms (SNPs) in predicting therapy outcome remains to be established. *Aims.* The aim of present work is to correlate response to imatinib therapy in CML patients with common SNPs, alone or in combinations, in genes involved in pharmacokinetics of these drugs. *Methods.* We evaluated imatinib efficacy as: (a) molecular response: complete (CMoR; defined as disappearance of detectable BCR/ABL fusion gene transcripts by quantitative PCR) / major (MMoR; defined as =3 log reduction of BCR/ABL fusion gene transcripts) / absent (AMoR) after 18 moth treatment (b) cytogenetic response: complete (CCyR; 0% Ph+ cells in marrow by conventional cytogenetics) / partial (PCyR; 0-35% Ph+ cells in marrow) after 12 moth treatment. We analyzed 10 candidate gene SNPs: in 3 genes associated with imatinib transport (ABCB1, ABCG2 involved in drug extrusion and SLC22A1 involved in imatinib cell uptake) and 4 genes associated with drug metabolism (CYP1A2, CYP2C9, and CYP3A5). Genotyping was performed by Real Time PCR using TaqMan probe. ABCB1 phenotype was defined according to specific SNPs combinations as follow: Low Transporters (LT): patients carriers at least 3 polymorphic alleles; Intermediate Transporters (IT): patients carriers 2 polymorphic alleles and Extensive Transporters (ET): patients carriers no more that 1 polymorphic allele. *Results.* So far, 57 patients with CML treated with imatinib were enrolled: 11 (3M/8F; age 54±10 years achieved CMoR, 24 (14M/10F; age 54±17 years) MMoR and 22 (7M/15F; age 58±16) AmoR 51 patients (22M/29F; 55±15 years) shown CCyR and 6 (2M/4F; 56±16 years) PCyR. Cytogenetic response was not associated with genetic profile. However SNPs in ABCG2 (rs2231137), and in SLC22A1 (rs683369), but not in ABCB1 and cytochrome, were significantly associated with molecular response. Moreover, 4 (37%) patients with CMoM had ABCB1 LT phenotype, while only 2 (9%) patients with AMoR were LT, (CHI^2 test: P = 0.045). *Conclusions.* Preliminary results showed that the treatment outcomes, especially for molecular response, of imatinib therapy could be predicted using a novel, multiple candidate gene approach based on the pharmacogenetics of IM.

0218

ASPIRIN RESISTANCE IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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Background. Low-dose aspirin (100mg/die) improves the survival of patients with cardiovascular events; however, about 25% of the patients treated with aspirin present thromboembolic complications (aspirin resistance). Pharmacological resistance is defined as the inability of aspirin to inhibit platelet aggregation and thromboxane production. Patients affected by myeloproliferative neoplasms (MPN) are at risk for thromboembolic complications and low-dose aspirin are effective also in these patients in reducing their thromboembolic risk. At present, few is known about aspirin resistance in this set of patients. *Aims.* This study explores pharmacological aspirin resistance in patients with MPN, in particular with polycythemia vera (PV) and essential thrombocythemia (ET), evaluated with platelet aggregation and serum thromboxane (TxB2) assay. *Patients and Methods.* We studied 123 MPN patients (41 PV and 82 ET), 83 were treated with aspirin (100mg/die) (MPN-ASA) and 40 were not (MPN-basal). PV and ET were diagnosed in agreement with WHO criteria. As controls we studied 50 patients treated with aspirin (100mg/die) for secondary prevention of thrombosis (Controls-ASA) and 42 healthy subjects (Controls). Platelet aggregation under 1 mM arachidonic acid (AA) stimulus was evaluated with Born's method. Serum thromboxane B2 (TxB2) was measured with ELISA assay (Thromboxane B2 Express EIA kit-monoclonal, Cayman Chemical Company; USA). Comparison between categorical variables was performed by $\chi 2$test and the threshold of serum TxB2 has been defined with ROC curve considering Controls and Controls-ASA TxB2 levels and obtaining a cut-off value of TxB2 to define the "aspirin resistance". *Results.* All Controls-ASA (100%) had suppressed AA-aggregation (<10%), while 22 MPN-ASA (26.5%) had not (80 ± 16%). No statistical difference was found in serum TxB2 between Controls and MPN basal or between the MPN-ASA and Controls-ASA. TxB2 production was significantly reduced in Controls-ASA compared to Controls ($p < 0.0001$) as well as in MPN-ASA compared to MPN-basal ($p = 0.04$); however, MPN-ASA had significantly higher levels of TxB2 than Controls-ASA ($p < 0.0001$).

	Platelets $x10^9$/L	TxB2 pg/mL	TxB2 pg x 10^{-8} plts
Controls (42)	229±73	36451±5754	25085±6788
MPN basal (22)	584±120	45058±32515	32099±6650
Controls-ASA (21)	236±90	1095±170	441±65
MPN- ASA (77)	614±323	5759±2881	1432±194

Serum TxB2 948 pg/plts x 10-8 was the cut-off limit to distinguish patients with a reduced suppression of TxB2 by aspirin (aspirin resistant). None of Controls and MPN-basal had a TxB2 value lower than 948 pg/plts x 10-8, while 2 (9.5%) Controls-ASA and 37 MPN-ASA (48%) were "aspirin resistant". 18 MPN-ASA (25%) had both an AA-aggregation > 10% pattern and a reduced suppression of serum TxB2 production, despite the use of aspirin. *Conclusions.* Pharmacological aspirin resistance occurs more frequently in MPN patients than in general population. Platelet aggregation can identify aspirin resistance in about 25% of MPN patients while an incomplete inhibition of serum TxB2 identify aspirin resistance in about 50% of MPN patients.

0219

RELATIONSHIP BETWEEN METHYLENETETRAHYDROFOLATE REDUCTASE POLYMORPHISMS (C677T AND A1298C) AND METHOTREXATE METABOLISM AND TOXICITY

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Background. Pharmacogenetic is a promising tool for therapy personalization because there are several single nucleotide polymorphisms (SNPs) that can give rise to differing responses to drugs. Methotrexate (MTX) is a structural analogue of folic acid that blocks the enzyme dihydrofolate reductase, inhibiting purine metabolism and causing elevated homocysteine levels and in several cases, toxicity. The enzyme methylenetetrahydrofolate reductase (MTHFR) catalyses the irreversible conversion of 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate. Both C677T and A1298C MTHFR polymorphisms lead to decreased MTHFR activity and consecuently, to increased levels of blood homocysteine. *Aims.* Analyse the relationship between the presence of A1289C and C677T MTHFR polymorphisms and the metabolism and the toxicity caused by the MTX administration to haematologic patients. *Materials and Methods.* All included patients were treated according to standardized protocols (HD-MTX). We evaluated 67 MTX infusion courses administered to 17 subjects, aged 17 to 65 years (mean: 45 years). Five out of these patients were affected with ALL, 11 with NHL and 1 with myeloma. MTX concentrations were determined at 12, 36 and 60 hours post-drug infusion, and after, every 24 hours until undetectable MTX levels. We used a Dimension RXL

(Siemens). Delayed elimination was defined as MTX levels ≥ 1.0 mM at 36h or MTX levels ≥ 0.2 mM at 60 h. We applied the following hepatic toxicity definition: ALT levels higher than 200 U/L in any of measures obtained using a Dimension RxL. Genomic DNA was extracted from 200 mL EDTA-stabilized blood using the DNA kit Blood-Spin UltraCleanTM (MoBio). Using TaqMan technology in a real-time PCR platform (ABI Prism 7000, AB), allelic discrimination was applied for the MTHFR C677T and A1298C polymorphisms. Primers and fluorencence probes were designed by AB and the PCR cycling conditions were the 'universal amplification conditions'. *Results.* In our population, 11.1% of subjects were carriers of a wild genotype respect to MTHFR C677T and A1298C polymorphisms. Heterozygosity for C677T and A1298C were found in 33.3% and 11.1%, and homozygosity in 5.5% and 5.5% of patients, respectively. Moreover, we identified 27.8% of subjects with a heterozygous mixed genotype. The elimination rate of MTX was normal in 40 courses for 8 patients, while 27 courses of the remaining 9 patients met the criteria to be classified as delayed elimination. Relative risk (RR) for delayed elimination of heterozygous and homozygous respect to the other genotypes, was 2.2. Three out of 5 with ALT values exceeding 200U/L, were homozygous or mixed heterozygotes; RR for elevated ALT calculated for this group compared with the remaining patients was 2.1. RR for hepatotoxicity in patients with delayed elimination compared to patients with normal elimination was 1.3. *Conclusions.* Our results show that there are a relationship between the genotypic characteristics (C677T and A1298C MTHFR polymorphisms) and the response to MTX therapy, measured as drug elimination rate and of hepatic toxicity. We suggest that the genotyping of these polymorphisms is convenient to identify and monitor subjects with high risk of toxicity following administration of this therapy.

0220

ANALYSES OF MACROMOLECULES IN NILOTINIB RESISTANCE BY FOURIER TRANSFORM INFRARED SPECTROSCOPY

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Background. Nilotinib is a very efficient tyrosine kinase inhibitor used for the treatment of chronic myeloid leukemia. Although very high hematologic and cytogenetic responses were obtained for nilotinib, resistance to nilotinib observed in the beginning or during the treatment is the major problem in treatment of chronic myeloid leukemia patients. Fourier transform infrared spectroscopy is used to monitor molecular changes since it is a rapid, sensitive and nondestructive method which is widely used in the analysis of biological systems in any physical state. The method requires only minute amounts of samples and allows analysis of the data with many different digital manipulations. It is a valuable analytical technique in detecting the changes in the cellular components such as lipids, proteins, carbohydrates and nucleic acids in the level of functional groups simultaneously. The technique both qualitatively and quantitatively evaluates shifts in peak positions and changes in bandwidths and in intensities of the bands to obtain structural and functional information about the systems analyzed. In addition, FTIR spectroscopy provides information about the amount and chemical and physical nature of the groups in close vicinity. *Aims.* In the present work we examined the changes in macromolecules in nilotinib resistant K562 cells at the molecular level using Fourier transform infrared (FT-IR) spectroscopy. *Methods.* Human K562 CML cells were exposed to step-wise increasing concentrations of nilotinib, and subclones of K562 cells resistant to 50 nM nilotinib were generated and referred to as K562/NIL-50 cells. Antiproliferative effects of nilotinib were determined by XTT cell proliferation assay. Changes in macromolecules in parental and resistant cells were studied by FT-IR spectroscopy. *Results.* IC50 values of nilotinib were calculated as 42 and 385 nM for K562 and K562/NIL-50 cells, respectively. Nilotinib resistance caused significant changes which mainly indicated that the level of glycogen increased, the membrane/lipid order increased, the total amount of lipids did not change significantly but the relative proportion of cholesterol and triglycerides changed considerably and, the transcriptional status increased along with higher metabolic turn-over as revealed by the FT-IR spectra. In addition, changes in the proteome and structural changes in both proteins and nucleus were observed in the K562/NIL-50 cells. *Summary/Conclusions.* All these results indicate that changes in macromolecules may be an important regulator of nilotinib resistance. FT-IR technique provides a method for analyzing drug resistance related structural changes in leukemia and other cancer types.

Immune thrombocytopenia

0221

A PHASE II 6-MONTH EXTENSION STUDY OF THE EFFICACY, SAFETY AND TOLERABILITY OF E5501 (AKR501) IN SUBJECTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (CITP)

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Background. E5501 (AKR501) is a thrombopoietin (TPO) receptor agonist. In a previous 28-day, multicenter, randomized, double-blind, placebo-controlled Phase II study (501-CL-003), E5501 (once-daily) was well tolerated and, at higher dose, effective at increasing platelet counts in subjects with relapsed or refractory chronic immune thrombocytopenia (cITP). *Aim.* To evaluate the long-term efficacy, safety and tolerability of E5501 in subjects with relapsed or refractory cITP, in a 6-month extension study to 501-CL-003. *Methods.* Sixty four subjects entered the prior 28-day double-blind study randomized to treatment with placebo or E5501 (2.5, 5, 10, 20 mg). The 53 subjects who completed the study (501-CL-003) were enrolled in this multicenter, parallel-group, Phase II, 6-month extension study (501-CL-004) from 17 US centers. Subjects were classified as either previous responders or non-responders at Day 28 of 501-CL-003. Response was defined as an increase in platelet count by a minimum of 20 $x10^9$/L above baseline and a count of ≥50 $x10^9$/L at Day 28. In 501-CL-004, previous responders (n=25) continued to receive their E5501 blinded dose (2.5, n=2; 5, n=6; 10, n=7; 20 mg, n=10). Previous non-responders (n=28) received open-label 10 mg E5501 once-daily. In both groups, dose was titrated upwards in 10-mg increments every 14 days depending on subject response (to a maximum dose in non-responders, of 40 mg once daily; and in responders, blinded dose plus 20 mg once daily) for 24 weeks, followed by a 4-week follow-up period. Concomitant medication dose reduction/discontinuation was permitted based on platelet response. Safety and tolerability of E5501 were evaluated in all 64 subjects who had been treated with E5501 in both CL-003 and CL-004. Efficacy was evaluated in the 53 subjects who were treated with E5501 for an additional 6 months based on platelet counts. Efficacy endpoints included the proportion of patients who achieved, without rescue medication, a durable platelet response, defined as a response-level platelet count at least 75% of the time at ≥3 platelet count assessments during the last 14 weeks of the treatment period. The number and percentage of subjects who achieved a platelet response at

Table 1.

Proportion of subjects who achieved response-level platelet count by study visit

Time point in Study 501-CL-004	Responders (n=25) with response-level platelet count n (%)	Nonresponders (n=28) with response-level platelet count n (%)	E5501 Total (n=53)
Week 2	19 (86.4)	15 (53.6)	34 (68.0)
Week 4	19 (79.2)	12 (44.4)	31 (60.8)
Week 8	19 (90.5)	12 (46.2)	31 (66.0)
Week 12	12 (85.7)	13 (65.0)	25 (73.5)
Week 16	9 (81.8)	8 (47.1)	17 (60.7)
Week 20	9 (90.0)	8 (66.7)	17 (77.3)
Week 24	17 (81.0)	12 (70.6)	29 (76.3)

Table 2.

Serious treatment-emergent adverse events (TEAEs)

Serious adverse event	Subjects n (%)
Thrombocytopenia	5 (7.8)
ITP	1 (1.6)
Leukocytosis	1 (1.6)
Mitral valve incompetence	1 (1.6)
Myocardial infarction	1 (1.6)
Retinal artery occlusion	1 (1.6)
Vomiting	2 (3.1)
Diarrhea	1 (1.6)
Gastritis hemorrhagic	1 (1.6)
Nausea	1 (1.6)
Chest pain	1 (1.6)
Pyrexia	1 (1.6)
Pneumonia	1 (1.6)
Platelet count decreased	1 (1.6)
Back pain	1 (1.6)
Cerebrovascular accident	1 (1.6)
Hemorrhage intracranial	1 (1.6)
Lethargy	1 (1.6)
Transient ischemic attack	1 (1.6)
Hypotension	1 (1.6)
Pelvic venous thrombosis	1 (1.6)

each study visit were also evaluated. *Results.* A durable platelet response was achieved in 52.8% of all subjects, and this response rate ranged from 35.7% among previous non-responders in the CL-003 study up to 72% among previous responders. The platelet response rate ranged from approximately 60% to 80% at on-therapy visits, and was higher in previous responders than previous non-responders at all assessment timepoints during the study (Table 1). Among subjects using steroids, 54.2% decreased their use by >50%, including 33.3% who discontinued their use permanently. All 64 subjects experienced ≥1 treatment-emergent AE (TEAE); most were mild and transient, and resolved completely. The most common TEAEs were fatigue (37.5%), headache (32.8%) and epistaxis (25%). TEAEs leading to study discontinuation were reported in 10/64 (15.6%) subjects. Serious TEAEs were reported in 12/64 (18.8%) subjects (Table 2); only 4 (6.3%) were considered treatment-related. *Conclusion.* This study demonstrates a durable platelet response to long-term E5501 treatment in subjects with relapsed or refractory cITP.

0222

PHASE II RESULTS OF THE FIRST-IN-CLASS ANTI-RHD ANTIBODY MIXTURE, ROZROLIMUPAB IN PRIMARY IMMUNE THROMBOCYTOPENIA (ITP)

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Background. Rozrolimupab is a recombinant antibody mixture of 25 fully human monoclonal antibodies, designed to capture the natural diversity of the human antibody response to RhD as a modern counterpart to the plasma-derived anti-RhD immunoglobulins currently used in the treatment of ITP. *Aims.* To investigate the safety and efficacy of a single dose of rozrolimupab in RhD positive, non-splenectomized patients with ITP. *Methods.* Following informed consent patients were enrolled in this dose escalation (75 µg/kg - 350 µg/kg), multicentre, open label trial. Inclusion criteria included confirmed presence of thrombocytopenia with two individual pre-dosing platelet counts of < 30 x 109. The patients received a single iv dose of rozrolimupab, were followed for 6 weeks and evaluated for safety and efficacy. Response was defined as platelet count ≥ 30 x109/L and increase in platelet count from baseline by > 20 x 109/L at 7 days after dosing. *Results.* Four dose groups comprising 36 patients have been evaluated so far: 75 µg/kg (11 patients), 100 µg/kg (10 patients), 125 µg/kg (10 patients) and 150 µg/kg (5 patients). The four cohorts differed in a number of baseline characteristics including distribution by sex (3, 9, 6 and 4 females), median platelet count before trial entry (15, 26, 16 and 22 x 109/L) and median time from first ITP diagnosis (68, 20, 5 and 8 months). In the individual dose groups, up to 70% of patients responded including one of the seven patients who had baseline platelet counts below 10 x109/L. All reported adverse drug reactions (E=20), except for a severe event of headache in the 100 µg/kg dose group, were of mild or moderate intensity. The reactions included pyrexia (E=2), decreased haemoglobin (E=2) and more frequent (E=6) events of headache. Laboratory data showed that haemoglobin values decreased in all patients indicating biological activity but generally, the values reverted towards baseline during the course of the trial. Three patients had a haemoglobin decrease of ≥ 2 g/dL; the biggest drop (3.1 g/dL) was categorized as a serious adverse event possibly related to rozrolimupab. The event was mild and not associated with serious bleeding and the patient recovered without receiving treatment. *Conclusions.* Rozrolimupab is well tolerated with no unexpected toxicities and shows preliminary signs of clinical activity. Beneficial effect of this agent in patients with ITP should be further evaluated.

0223

INTERIM RESULTS FROM AN INTERNATIONAL, MULTI-CENTER, SINGLE-ARM STUDY EVALUATING THE SAFETY AND EFFICACY OF ROMIPLOSTIM IN ADULTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP)

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Background. Romiplostim is recommended for second- and third-line treatment of chronic ITP in adults. This study, conducted in Europe, North America and Australia, investigated romiplostim in adults with ITP of varying duration and severity. *Aims.* To expand the understanding of the safety and efficacy of romiplostim in adult ITP patients. *Methods.* Eligibility criteria were broad: patients ≥18 years of age, who had received prior ITP therapies (current amendment: ≥1, previous amendments: ≥3), with low platelet counts (current amendment: ≤ 30 x 10^9/L, previous amendments: ≤10, ≤20 x 10^9/L) or uncontrolled bleeding. The only excluded comorbidities were: hematological malignancy, myeloproliferative neoplasms, MDS and bone marrow stem cell disorder. Romiplostim was initiated at 1 µg/kg/week, with dose adjustments allowed to maintain platelet counts ≥50 x 10^9/L. Patients could continue on study until they had access to commercially available romiplostim. Rescue medications were allowed at any time; concurrent ITP therapies could be reduced when platelet counts were > 50 x 10^9/L. Primary endpoint was incidence of adverse events (AEs) and antibody formation. Secondary endpoint was platelet response, defined as either (1) doubling of baseline count and ≥50 x 10^9/L or (2) ≥20 x 10^9/L increase from baseline. *Results.* As of April 2009, 235 patients had enrolled and received at least one dose of romiplostim. Of these, 77% remained on study (121/235) or had completed the study (61/235); 23% (53/235) had

Table 1. Adverse events - Subject incidence.

		N=235 n (%)
All events		198 (84)
Most common events	*Headache, Fatigue, Arthralgia, Nausea, Contusion, Epistaxis, Diarrhoea*	66 (28), 53 (23), 45 (19), 38 (16), 37 (16), 37 (16), 35 (15)
Serious events		62 (26)
Treatment-related events		104 (44)
Serious, treatment-related events		8 (3)
Key events of interest	*Thrombotic/thromboembolic*	14 (6)
	Thrombocytosis	7 (3)
	Hemorrhage events	91 (39)
	Bone marrow fibrosis/reticulin	3 (1)

withdrawn, with withdrawn consent the most common reason (10/235 [4%]). Median (Q1, Q3) time from ITP diagnosis was 4.7 (1.2, 12.2) years, with 60% of patients splenectomised and 38% receiving baseline concurrent ITP therapies. Median (range) baseline platelet count was 12.0 (1.0-170.0) x 10^9/L. Median (Q1, Q3) treatment duration was 18 (7, 39) weeks (maximum 201 weeks), with a total of 7288 subject-weeks on study. Incidence and type of AEs were consistent with previous studies. Nine patients died; 2 deaths (haemolysis, aplastic anemia) were considered treatment-related. One event of mild (1+) reticulin fibrosis, occurring 3 weeks after last romiplostim dose, was considered serious and treatment-related. No neutralizing antibodies to romiplostim or TPO, or hematopoieitic malignancies or MDS events were reported. Approximately 90% of patients achieved each of the platelet response definitions [(1): 86%; (2): 91%]; median (Q1, Q3) time to response was 1 [(1): (1, 4); (2): (1, 2)] weeks for both. *Summary/Conclusions.* The data are similar to that reported for previous romiplostim studies, with romiplostim able to safely induce a rapid platelet response in adult ITP patients with low platelet counts or bleeding symptoms. Romiplostim is an important, well-tolerated, treatment option for adult ITP patients, which significantly increases and maintains platelet counts.

0224

BONE MARROW FIBROSIS, IMMUNOPHENOTYPING AND CYTOGENETICS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP) TREATED WITH THROMBOPOIETIC AGENTS

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Background. ITP is characterized by accelerated platelet destruction and suboptimal platelet production. Thrombopoietin receptor agonists (Tpo-RA) increase platelet counts in ITP through stimulation of thrombopoietin receptors. While an acceptable safety profile has been demonstrated for these agents, an important area of concern is bone marrow (BM) fibrosis, which has been reported in a few patients treated with these agents. The relation between megakaryocyte stimulation and fibrosis has been proved in animal models and is apparently mediated by cytokines. *Aims.* This study aimed to determine the extent of bone marrow fibrosis, immunophenotypic abnormalities and chromosomal aberrations in ITP patients on treatment with Tpo-RA. *Methods.* This retrospective study included ITP patients receiving treatment with Tpo-RA at the Platelet Disorders Center, The New York Presbyterian Hospital (NYPH), USA, having at least one BM biopsy (BMB) performed prior to or during treatment with Tpo-RA. All BMB and aspirates were performed as part of the standard follow-up practice of patients on Tpo-RA. BM morphology, cytogenetic and flow-cytometric (FCM) examinations were performed in the Pathology Department of NYPH. Histological sections were stained with H&E, Gomori stain for reticulin and trichrome stain for collagen; fibrosis was graded according to the European Consensus Classification into 4 grades. *Results.* 77 BMB were available from 49 ITP patients (median age 50 years; 29 females 59%) treated with various thrombopoietic agents (see table). Ten patients had a pre-treatment BMB, of whom eight had on-treatment biopsies. The grade of reticulin in these eight increased from grade MF-0 to MF-1 in 5 patients, decreased from MF-1 to MF-0 in one patient and remained unchanged (MF-1) in two, after initiation of Tpo-RA. Median time from Tpo-RA initiation to first BMB was 1.3 years (IQR 1.0-1.8). Thirty-eight of 45 patients (85%) had greater than MF-0; of these 4 (9%) had MF-2 and 3. Median duration of treatment to second BMB in 16 patients was 3.0 years (IQR 2.3-4.3). From first to second BMB, reticulin grading increased by at least one grade in 6, remained unchanged in 8, and decreased in 2 with ongoing treatment with Tpo-RA. Out of all on-treatment biopsies, 10 patients had BMB with grade 2 or (one) 3 MF-grade. Cytogenetic analysis was performed in 50 of the BM examinations; 2 had abnormal karyotypes (see table). All 36 FCM revealed normal immunophenotypes. *Summary/Conclusions.* At a median duration of 1.3 years on treatment with Tpo-RA the proportion of patients having reticulin deposition in their marrows was 85%, which appears to be higher than that reported in the literature in ITP patients unexposed to Tpo-RA (40%) (Ettrup *et al.* Am J Hematol. 2010; 85:930-934) and in pretreatment BMB in this material (50%). Further increment in reticulin was observed in >30% of patients who continued treatment. No serious cytogenetic of immunophenotypic abnormalities emerged during treatment with Tpo-RA. Based on these results, regular follow-up with BM biopsies is recommended during treatment with Tpo-RA.

Table 1. Pre- and on-treatment bone marrow examinations.

Pre- and on Treatment Bone marrow examinations					
	Pre-	On-treatment			
	BM	1st BM	2nd BM	3rd BM	4th BM
BM biopsy (n)	10	45	16	4	1
MF-0	5 (50%)	7 (15%)	1 (8%)	-	-
MF-1	5 (50%)	34 (76%)	9 (56%)	4 (100%)	1
MF-2	-	3 (7%)	6 (38%)	-	
MF-3	-	1 (2%)	-	-	-
Trichrome (n +/-)	0/8	1/41	0/9	0/3	0/1
Cytogenetic (n +/-)	-	2*/30	0/14	0/3	0/1
FCM (n +/-)	-	0/22	0/12	0/1	0/1

* Cytogenetic abberations were: 45,X,-Y[15]/46,XY[5] in an 80 years old male- a finding commonly associated with aging; 46,X,i(X)(q10)c[13]/45,Xc[7] in a female with Turner Syndrome.

0225

RITUXIMAB ADDED TO STANDARD THERAPY FOR NON-SPLENECTOMIZED PATIENTS WITH IMMUNE THROMBOCYTOPENIA: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Background. Early treatment with rituximab for patients with immune thrombocytopenia (ITP) may improve rates of disease remission; however, the feasibility of conducting a blinded randomized trial to address this question is not known. *Aims.* 1) To estimate event-free survival with rituximab added to standard ITP therapy; 2) To determine the feasibility of a blinded, randomized trial of rituximab in ITP. *Methods.* We performed a randomized, double-blind, concealed, placebo-controlled pilot trial in non-splenectomized patients with newly-diagnosed or relapsed ITP in 7 centres in Canada. Patients were randomly assigned to receive rituximab or placebo infusion adminis-

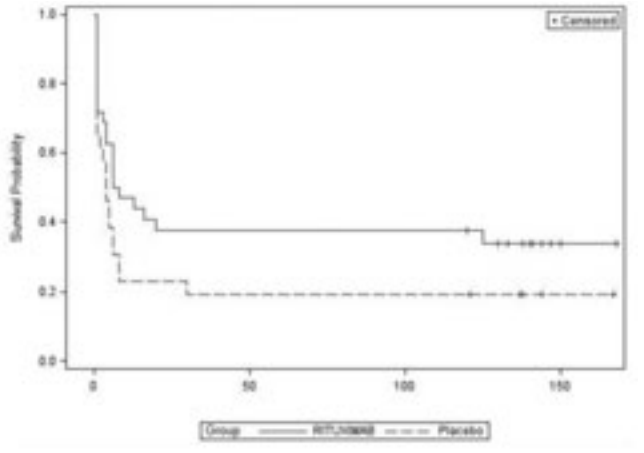

Figure 1. 6-month event-free survival (LogRank= 2.4; p= 0.1).

tered weekly for 4 consecutive weeks. Patients were 18 years of age or older, had primary ITP, a platelet count below 30 x10^9/L and had begun standard therapy chosen at the discretion of their physician. The efficacy outcome was 6-month event-free survival, where an event was any platelet count below 50 x10^9/L, the need for rescue treatment or grade 2 bleeding (Page *et al.* 2007) occurring after standard therapy was discontinued. Feasibility outcomes were rate of recruitment, protocol adherence and blinding integrity. Analysis was by intention-to-treat. *Results.* 33 patients were assigned to rituximab and 27 to placebo. All patients received standard ITP therapy consisting mostly of corticosteroids and/or intravenous immune globulin. Two patients (1 in each group) were lost to follow-up before receiving a single study infusion. Median age was 40 years [interquartile range (IQR), 30.5 - 59.0] and 58% were female. Median baseline platelet count was 15 x10^9/L (IQR, 9 - 23) and patients had ITP for a median of 1 year (IQR, 0 - 3.5) prior to randomization. Event-free survival at 6 months was 11/32 (34.4%; 95% CI 17.9 - 50.8%) in the rituximab group and 5/26 (19.2%; 95% CI 4.1 - 34.4%) in the placebo group (odds ratio= 2.2, 95% CI 0.7 - 7.4) (Figure). Fewer patients in the rituximab group had any platelet count below 50 x10^9/L [17/32 (53.1%) vs. 16/26 (61.5%)] or needed rescue treatment [14/32 (43.8%) vs. 17/26 (65.4%)] during the 6-month follow up period (differences were not statistically significant). Rates of grade 2 bleeding were similar between groups [7/32 (21.9%) vs. 6/26 (23.3%)]. Adverse events in rituximab-treated patients were minor. Recruitment was slow because 53 patients refused to participate and 13 failed screening. Refusals were mostly due to concerns about side effects of rituximab or an unwillingness to receive placebo. Blinding integrity was maintained for research staff after the administration of 4 study infusions; however, patients were able to infer group assignment more accurately than what could be attributable to chance. *Conclusions/Summary.* This study demonstrates promising effects of early rituximab administration on avoidance of thrombocytopenia and on the need for rescue treatment when given in conjunction with standard ITP therapy. A larger randomized trial of upfront rituximab for ITP powered on efficacy endpoints may be feasible; however, the widespread use of rituxamb in practice and difficulties in maintaining patient blinding would require careful consideration.

Funded by Hoffmann-LaRoche. All patients provided informed consent. ITP is not a licensed indication for rituximab.

0226

MARKERS FOR CHRONICITY IN IMMUNE THROMBOCYTOPENIA OF CHILDHOOD

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Background. Immune Thrombocytopenia (ITP), the term currently used for idiopathic or immune thrombocytopenic purpura, is known as a haemorrhagic state from Hippocrates's ages and myriads of studies have been published on it; however, there are still unanswered questions regarding diagnosis, clinical expression and therapeutic management, mainly based on the unavailability of markers that could predict a chronic course for ITP in children. *Aim.* To point out whether any differences in clinical or laboratory data at the onset of the disease between acute and chronic cases could predict the form of ITP. *Methods.* Data from the files of 795 children with ITP, followed up during a 30 years period (1974-2005), were retrospectively logged in a computerized program for statistical evaluation. Duration of thrombocytopenia (TP) <6 or >6 months was characterized as acute (aITP) or chronic (cITP) ITP. *Results.* A total of 728 children with ITP were included in the study; 60.6% had aITP, 32.8% cITP, 6.6% recurrent ITP. Children with a preceding viral infection or vaccination had a 2.4-fold and 2.5-fold probability, respectively, to run aITP versus cITP (CI 1.691-3.414, p< 0.01 and CI 1.181-5.171, p=0.013, respectively). In cITP, 55.2% were females (p< 0.01). Children with bleeding manifestations at the onset of ITP had almost 8.5-fold probability to have aITP [OR 8.38 (CI 5.14-13.64, p <0.001)]. Furthermore, although not all of the patients with severe TP (platelet count <5x109/L) bled, the probability of children with severe TP and hemorrhage to have aITP was twice as high (OR 1.92) than that of patients with severe TP without bleeding (p<0.05). Regarding therapeutic management, aITP patients who had received no therapy at the onset of the disease were more likely not to need therapy during the course of ITP [OR 2.97 (CI 1.75-5.05)]. On the contrary, cITP patients who had required therapeutic intervention at the onset of ITP had a 3-fold probability to receive additional treatment during the course of ITP [OR 2.946 (CI 1.540-5.636), p<0.01]. Recovered cITP patients had a lower platelet count at the onset of the disease in comparison to non-recovered patients (p=0.009). Splenectomy was applied in 15% of cITP children. Prior to splenectomy, children had bleeding manifestations twice as frequently than non splenectomized patients (p< 0.01), whereas therapeutic intervention was required 4 times more often than in non splenectomized children (p<0.01). Nevertheless, in non splenectomized children, TP at the onset of ITP was more profound than in splenectomized cITP cases (mean platelet count: 12 vs 28 x109/L, respectively). Finally, in multiple regression analysis, a higher probability for cITP was revealed in the absence of all three: preceding viral infection, vaccination and bleeding at the onset of the disease, in comparison to aITP (45.4% vs 25.1% of cases, respectively, p<0.05). *Conclusion.* Preceding viral infection, vaccination, severe thrombocytopenia coupled with bleeding manifestations at the onset of ITP were found to be involved in aITP. In contrast, the absence of any previous history related to ITP, mild clinical presentation and female predominance could be associated with cITP in childhood. Presenting author's email: eplatokouki@paidon-agiasofia.gr

0227

SPLENECTOMY IN IMMUNE THROMBOCYTOPENIA: RESULTS OF 73 CASES WITH A MEDIAN FOLLOW-UP OF 22 YEARS

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Background. Splenectomy is still acknowledged as the gold-standard treatment of refractory immune thrombocytopenia (ITP). Recently, new medical therapies (anti-CD20 and thrombopoietin mimetics) have entered into clinical practice, encouraging a generalized 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 the data of 73 ITP patients who underwent laparotomic splenectomy between 1963 and 1998 and have now a minimum follow-up of 10 years (median, 22 years; range, 10-47). *Results.* Overall, 73 ITP patients followed at our institution have now a minimum observation time of 10 years after splenectomy. Sixty-six percent were women; median time from diagnosis to splenectomy was 13 months (range, 0-254) and median age at splenectomy was 35 years (range, 6-65, with 12 patients younger than 16). Nine patients (12%) underwent splenectomy front-line; the other patients were splenectomized after failure of at least one course of medical therapy (prednisone alone or in combination with azatioprine and/or immunoglobulin). Overall, 66 patients (90%) achieved a complete response (platelet >100x109/L) after splenectomy and in 44 cases (66%), the response was stable at last contact. Twenty-two patients (33%) lost the response during the follow-up. Nine patients (41%) experienced a very early relapse (within 30 days from splenectomy), while in the remaining 13 patients, relapse occurred after a median time of 40 months (range, 4-256), for a relapse-free survival of 64% at 20 years (Figure 1). Three patients (4%) achieved a response (platelet count >50x109/L), while 4 patients (6%) were refractory. Overall, 25 patients (34%) needed further treatment after surgery. At last contact, 57 patients (78%) were in complete response (13 of whom, thanks to medical treatment post-splenectomy), 3 patients were in response and 13 patients had a platelet count <50x109/L. Eight patients (11%) remained in

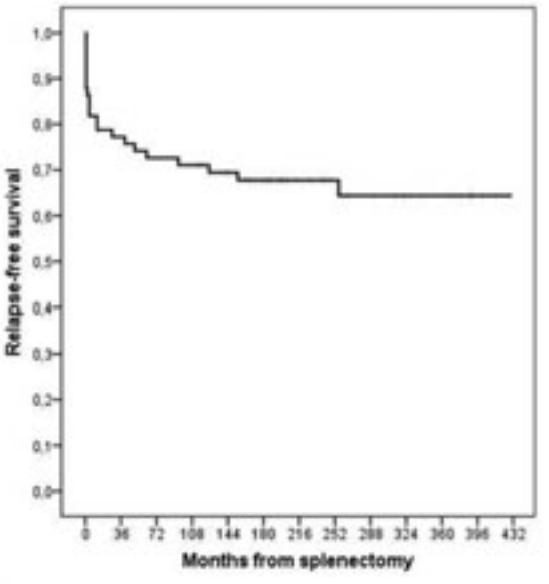

Figure 1. Relapse-free survival.

on-demand steroid therapy after a median time of 12 years (range, 3-24) from splenectomy. Forty-two hemorrhagic events (7 of which grade 3-4 WHO) were observed in 17 patients (23%), after a median time of 83 months. Fourteen patients (19%) experienced one or more infectious episodes after surgery, which were severe (pulmonary) in 9 cases and were observed late in the follow-up (median time from splenectomy to the event, 16.7 years, range 2.8-21.7). Nine patients died for causes unrelated to ITP (median age, 74 years; range 43-85). *Conclusions.* Splenectomy confirmed to induce a stable remission in 66% of ITP patients in the long-term. Relapse rate was higher in the first months, with sporadic relapses occurring even 20 years after surgery. The incidence of late severe infectious complications was not negligible, probably due to the fact that most patients did not receive prophylactic vaccinations.

0228

PREVALENCE OF DIAGNOSED ADULT IMMUNE THROMBOCYTOPENIA IN THE UNITED KINGDOM

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Background. Primary immune thrombocytopenia (ITP) is an autoimmune disorder most commonly characterized by decreased platelet count (< 100 × 10^9/L) resulting from autoantibody-mediated, peripheral platelet destruction and suboptimal platelet production. Data regarding prevalence of ITP are scant, and are derived mostly from North American and European population-based analyses. *Aims.* The aims of this study were to 1) estimate the unadjusted overall, age- and gender-specific prevalence of diagnosed adult (>=18 years of age) ITP in the General Practice Research Database (GPRD) in United Kingdom (UK) from January 1, 1992 to December 31, 2009 (18 year prevalence) and 2) estimate the overall age- and gender-adjusted prevalence (per 100,000) of diagnosed adult ITP adjusted to the 2009 UK population. *Methods.* This was a retrospective study using observational data from the GPRD, an electronic medical records database in the UK. Data from the GRPD are drawn from the computer systems of a representative sample of general practices throughout the UK and currently include information regarding diagnoses, prescriptions, referrals, outcomes and laboratory results, together with basic demographic information for approximately 6.4 million patients from over 480 centers. The GPRD is an excellent resource for estimating the prevalence of ITP because its population-based and representative of the age, sex and geographic regions of the UK. Prevalence of ITP was generated by accruing cases of adult ITP from 1992 through 2009 (unadjusted prevalence), then applying age- and gender-specific prevalence to the 2009 UK population (adjusted prevalence).Prevalent cases included all adult individuals (male and female) with at least one diagnostic code for ITP identified by the OXMIS and Read codes (42P2.11; D313.12; D313000; D313012; 2871C) in their medical records, enrolled any time in the 18 year study period (Jan 1, 1992- Dec 31, 2009), and with at least one year of prior up-to-standard follow-up in the GPRD. The overall prevalence (per 100,000 patients) of ITP the GPRD in 1992-2009 was calculated as the total number of identified adult ITP cases between January 1, 1992 and December 31, 2009, divided by all adults in the GPRD database. The age- and gender-specific prevalence was calculated using the same methodology. Confidence intervals (CIs) were calculated based on the binomial distribution. *Results.* The study results are presented in the Table. ITP prevalence was lower in adults aged 18-49 years of age than in older adults 50-64 years of age or 65+ years of age. Prevalence was higher among females vs. males. As expected due to the aging of the population, prevalence in the GPRD rose over time. *Summary/Conclusions.* This recent analysis of the general practice in UK provides most robust prevalence estimates of diagnosed ITP among adults. Prevalence of ITP was higher in older females and gradually increased over years in the GPRD.

Table 1. GPRD ITP prevalence.

Population	Prevalence per 100,000 patients	95% CI
Overall, unadjusted	50.3	48.5, 52.1
Overall, age-gender adjusted	50.0	49.2, 50.9
Ages 18-49	30.1	28.3, 31.9
Ages 50-64	58.2	53.9, 62.6
Ages 65+	93.8	88.8, 98.8
Females	59.3	56.6, 62.0
Males	40.7	38.4, 43.0
Year 1992	16.2	13.7, 19.0
Year 2000	36.7	34.5, 39.3
Year 2008	56.0	53.5, 58.8

0229

AN OPEN-LABEL EXTENSION STUDY EVALUATING THE SAFETY AND EFFICACY OF UP TO 3.5 YEARS OF ROMIPLOSTIM IN THROMBOCYTOPENIC JAPANESE PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA (ITP)

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Background. Chronic ITP is characterized by increased platelet destruction and decreased platelet production. The peptibody romiplostim increases platelet counts by binding to and activating downstream signaling of the thrombopoietin receptor. *Aims.* To examine the safety and efficacy of long-term romiplostim use in Japanese patients with chronic ITP. *Methods.* Patients from a phase 2 open-label study and a phase 3 randomized study could choose to enroll in an open-label extension study. If patients enrolled within 12 weeks of the previous study and had a platelet increase ≥20x10^9/L from baseline once during the 13-week treatment period in the previous study, the romi-

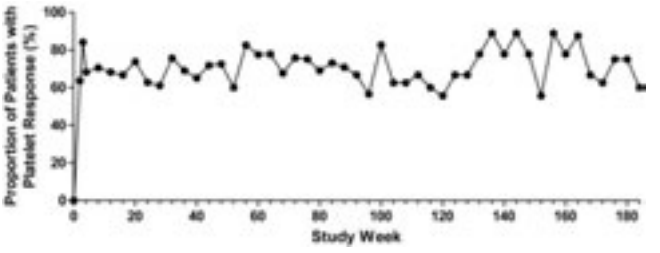

Figure 1.

plostim dose remained the same. Otherwise, the initial dose was 3 μg/kg romiplostim per week, which could be titrated up to 10 μg/kg to maintain platelet counts between 50-200x10^9/L. *Results.* As of April 2010, 44 patients had enrolled; 71% women, median (min, max) age of 55.5 (25, 81) years. At data cutoff, 5 patients (11%) discontinued study drug due to patient request (2), administrative decision (2), or other (1). The median (Q1, Q3) treatment duration was 100 (70, 113) weeks (maximum 184 weeks), and the median (Q1, Q3) average weekly dose was 3.8 (2.0, 6.4) μg/kg. The most frequent adverse events were nasopharyngitis (2.1/100 patient-weeks), headache (0.7/100 patient-weeks), back pain (0.3/100 patient-weeks), contusion (0.3/100 patient-weeks), and malaise (0.3/100 patient-weeks). Nine patients (21%) had a total of 14 serious adverse events (0.31/100 patient-weeks); the only one considered treatment-related was mouth hemorrhage. There were no life-threatening adverse events, and no patients died or withdrew from the study due to adverse events. A total of 50 hemorrhagic adverse events were reported in 20 patients (46%), with 3 patients (7%) having serious hemorrhagic adverse events, one patient with epistaxis and hemorrhagic anemia, one with melena and subcutaneous hematoma, and one with mouth hemorrhage. The most common hemorrhagic adverse events were contusion (0.3/100 patient weeks), epistaxis (0.2/100 patient-weeks), purpura (0.1/100 patient-weeks), and conjunctival hemorrhage (0.1/100 patient-weeks). No events were observed of hematopoietic malignancy, myelodysplastic syndrome, thrombocytosis, or bone marrow reticulin/collagen fibrosis (bone marrow biopsies were performed at investigator discretion). The only thromboembolic event was a serious adverse event of transient ischemic attack, which was not considered treatment-related. No patients tested positive for neutralizing antibodies to romiplostim or to TPO. Ninety-six percent of patients had a platelet response (doubling of platelet count and platelet count ≥50x10^9/L). Median platelet count was ≥50x10^9/L from Week 2 onwards. Of the 25 patients receiving concurrent ITP therapy at baseline, all reduced or discontinued the therapy: 11 with >25% reduction in ≥1 concurrent therapy, 5 with >50% reduction in ≥1 concurrent therapy, and 9 discontinued all concurrent therapies. Nine patients (21%) received rescue medications. *Summary/Conclusions.* Administration of romiplostim for up to 3.5 years was well tolerated in Japanese patients with chronic ITP with a platelet response that was maintained during this long-term extension study. The reported incidence of adverse events was similar to that seen in other romiplostim studies and did not increase over time. No new safety concerns were identified.

0230

EPIDEMIOLOGIC PROSPECTIVE STUDY TO EVALUATE PRIMARY IMMUNE THROMBOCYTOPENIA CHARACTERISTICS

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Background. Primary immune thrombocytopenia (ITP) affects a heterogeneous group of patients, whose initial diagnosis is often difficult and entails the exclusion of other diseases. Although ITP has been a well-known hematologic disorder for more than two centuries, some aspects regarding the etiologic factors, clinical behaviour or efficacy of the most frequently used treatments still remain unclear or even unknown. *Aims.* To describe the clinical characteristics of patients with ITP, compare with the available clinical evidence and define their therapeutic management and response. *Methods.* This is a Spanish multicentre observational epidemiological study in patients with recently diagnosed ITP. Patients were followed every 6 months for 1 year. Results from interim analysis of data between baseline and month 6 are presented. *Results.* The database was locked on 25 January 2011, with a deviation from the planned recruitment of +4.5%. A total of 315 patients from 98 Spanish hospitals were evaluated, with a median age of 53 years (range 27.4-73.8). Platelets at baseline: 11x109(range 4-28)/L. The most commonly performed diagnostic tests included: antibody testing (hepatitis B serology, 90.8% [10.3% positive]; hepatitis C serology, 90.1% [3% positive]; antinuclear antibodies, 78.2% [13.8% positives]; antiphospholipid antibodies, 49.3% [4.7% positives]; antiplatelet antibody, 20.3% [6.1% positives]), bone marrow study (38.7% [1.7% abnormal]), and H. pylori determination (35.7% [9.7% positive]). Clinical manifestations were observed in 235 patients, which were mainly skin bleeding (86%), oral mucosa bleeding (37.9%) and epistaxis (23.8%). Two hundred seventy-seven patients received first-line treatment. Most frequent first-line treatments were: corticosteroids (48.1%), corticosteroids+IV immunoglobulin (39.8%) and IV immunoglobulin alone (7.5%). Response was evaluated in 173 patients, with an 89% response rate. Data at month 6 were available in 132 patients. Median platelet counts at month 6 was 121x109(range 67-217)/L. At this time, only 13 patients experienced clinical manifestations: skin bleeding (69.2%), oral mucosa bleeding (38.5%) and metrorrhagia (23.1%). Thirty-seven patients underwent a second-line treatment. Mean time between lines was 26.5±50.8 days. The most frequent second-line treatments included rituximab (24.3%), romiplostim (18.9%) and corticosteroids (10.8%). The data was insufficient to assess the response. Splenectomy occurrence was observed in later lines. *Conclusion.* This interim analysis, which includes a greater recruitment than expected, shows heterogeneity in diagnostic procedures to exclude secondary etiology in patients with thrombocytopenia among the participating hospitals. Moreover, although the first-line treatment was homogeneous and showed a remarkable response rate, the second-line treatments seem not to be universally accepted. Further analyses will confirm that more homogeneous protocols are needed for the management of ITP patients.

0231

THE COMBINATION OF THREE DEXAMETHASONE CYCLES AND RITUXIMAB YIELDS HIGH RESPONSE RATE IN PREVIOUSLY TREATED IMMUNE THROMBOCYTOPENIA (ITP)

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Introduction. ITP is characterised by an immune-mediated destruction of platelets and impaired platelet production. The aim of treating ITP is to achieve sustained elevation of platelet counts (PC). Both dexamthason and rituximab are effective in elevating PC and are widely used in the treatment of ITP. Sustained response rates (RR) achieved by multiple cycles of dexamethasone given upfront looked superior to single cycle (85 % vs 45%), while RR for rituximab in previously treated patients is about 60% with 40 % of complete responses (CR). Recently up-front treatment rituximab plus one cycle of dexamethasone was shown to be superior to dexamethasone alone with respective RR of 63% vs 36% at 6 months (Zaja *et al.,* Blood, 2010). The combination of multiple cycles of dexamethasone and rituximab in previously treated ITP has not been reported previously. *Aims.* This pilot study aimed to determine the efficacy and safety of combining three cycles of dexamethasone to rituximab in previously treated patients with ITP. *Methods.* A retrospective study that enrolled patients with persistent, chronic or refractory ITP who were intended to be treated with the combination of 4 weekly rituximab infusions (375mg/m2) plus 3 dexamethasone cycles (40 mg or adjusted for weight on days 1-4, 15-18, and 29-32). The primary endpoint was CR (platelet count >100x109/L) at last follow-up; secondary endpoints were RR (platelet 30-100 x109/L with at least doubling of baseline PC) and safety defined number of greater > grade 2 adverse events (AE) during first 6 months of treatment. The study was approved by the IRB at Weill Cornell Medical College. *Results.* The table shows characteristics of 21 patients treated with rituximab and dexamethasone (R&D). Median duration of follow-up from first rituximab infusion was 6 months (IQR 4.9-8.5). Median pre-treatment and post-treatment PC registered at last follow-up were 35 X 109/L (IQR

Table 1. Patients' charachteristics.

Patients' Characteristics	
	N=21
Median age in years (IQR)	20 (13-39)
Female gender	14 (66%)
Median number of previous treatments (R)	2 (1-6)*
Median duration of ITP in years (IQR)	3.6 (0.6-6)
Treatment	**Number of patients**
Number of Rituximab infusions	4 in 19; 3 in 1; 2 in 1
Number of Dexamethsone cycles	3 in 15; 2 in 4; 1 in 2

*Only one patient was splenectomized. R= Range; IQR= interquartile range

20-49) and 133 X 109/L (IQR 35-277) respectively. At the last follow-up, 11 patients (52%; 95%CI 30-74) were in CR and 2 (9.5%; 95%CI 1-30) were in PR resulting in total RR of 62% (95%CI 38-82). There was no statistical difference in the CR rates in relation to the disease duration of <1 or> 1 year. During the first 6 months, four grade-3 and one grade-4 AE's occurred three patients. In addition 11 bleeding episodes were recorded in four patients, three of whom were non-responders. *Summary/Conclusion.* In previously treated patients with persistent, chronic and refractory ITP, the suggested combination yielded CR rate of 52%, which is comparable to that reported in previously untreated patients by Zaja *et al*. Four grade 3/4 AE occurred in 2 (10%) patients. 75% of the patient adhered to the designated treatment regimen. The retrospective nature, small sample and short follow-up are the main limitations of this trial. In conclusion, a regimen of rituximab and three 4-days cycles of dexamethasone appears effective, safe and tolerable. This combination merits further exploration in a prospective clinical trial.

0232

EVALUATION OF BASELINE LABORATORY PARAMETERS POTENTIALLY ASSOCIATED WITH THROMBOEMBOLIC EVENTS IN PATIENTS WITH CHRONIC ITP ENROLLED IN A CLINICAL TRIAL OF ELTROMBOPAG

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Background. It has been proposed that immune thrombocytopenia (ITP) may have pro-thrombotic characteristics and low platelet counts may prevent a higher incidence of thrombotic events (TEEs).[1-3] In the UK General Practice Research Database, incidence rates for TEEs were 1.35/100 patient years (PYs) (95% CI [0.99, 1.79]) for patients with ITP vs 1.16/100 PYs (95% CI [0.99, 1.35]) in patients without ITP.1 Similar results were found in a US claims database study.[4] Despite increasing acceptance of this premise, the reason why patients with ITP are prone to thrombotic events is not well understood. *Aim.* To describe the frequency of potential laboratory predictors of thrombophilia in patients with chronic ITP. *Method.* Adult patients with chronic ITP[5] were enrolled in a phase 4, open-label, 2-year study to evaluate potential changes from baseline in bone marrow reticulin, associated to long-term treatment with eltrombopag. Patients with a prior history of a TEE and ≥2 risk factors for thrombosis were not eligible for the study. A "thrombophilia panel"collected at baseline comprised multiple known or suspected laboratory predictors of thrombosis. Patients could not have been treated with a TPO-R agonist in the 6 months prior to enrollment. All patients provided signed informed consent prior to study initiation. *Results.* In this ongoing study, baseline thrombophilia panels were available for 80 patients as of the date of this evaluation (Feb 2011). Mean age was 41 years; 70% (n=56) were female; mean BMI was 24.9 (range, 15.5-40.5). The median time since ITP diagnosis was 4 years (range, 0.2-45.7 years). 5/80 patients had prior exposure to eltrombopag. Most patients did not report a family or personal history of TEEs (n=75). Five patients reported clinical risk factors potentially associated with thromboembolic events (atrial fibrillation [n=1], arterial HTN [n=3]), and cardiac insufficiency [n=1]). The majority of tests in the thrombophilia panel were normal; however, many patients (Table 1) had abnormal levels of well known or suspected predictors of thrombosis or markers or activation of the coagulation cascade: ß2-Glycoprotein 1 (22/80), d-dimer (29/80), Factor VIII (46/80), Lupus anticoagulant (15/80), and Protein S activity (decreased, 14/80). Two patients in the study reported a TEE: 1 DVT (study day 31) and 1 infective thrombophlebitis requiring anticoagulation (study day 27). Both patients had baseline increases in d-dimer, Factor VIII, and Lupus anticoagulant (detected and confirmed). *Summary/Conclusions.* To our knowledge this is the only published report of a thrombophilia profile in a cohort of patients with chronic ITP. The multiple baseline abnormalities in possible predictors of thrombophilia may support the theory that ITP is a pro-thrombotic disease. The potential correlation of such abnormalities with TEEs will be the topic of further reports as the study progresses.

Table 1.

Patients with Abnormalities in the Thrombophilia Panel

	N (%)
Total patients	80 (100)
≥1 abnormality	69 (86)
1 abnormality	16 (20)
2 abnormalities	15 (19)
3 abnormalities	14 (18)
4 abnormalities	11 (14)
5 abnormalities	5 (6)
6 abnormalities	6 (8)
7 abnormalities	2 (3)

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0233

THE CLINICAL SIGNIFICANCE OF DETECTION OF CIRCULATING B CELLS SECRETING PLATELET-SPECIFIC ANTIBODY AND PLATELET GLYCOPROTEIN IIB/IIIA IN THE PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA

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Background. Primary immune thrombocytopenia (ITP), is characterized by a low platelet count, which is the result of both increased platelet destruction and insufficient platelet production. The development of autoantibodies against platelet glycoproteins remains central in the pathophysiology of ITP, The diagnosis of ITP is still a puzzle problem. *Aims.* Througn detecting the circulating B cells secreting platelet-specific antibody, the platelet glycoprotein IIb/IIIa and platelet-specific antibody in patients ITP, evaluate their roles in the diagnosis of ITP and their clinical significance. *Methods.* The frequencies of circulating B cells secreting platelet-specific antibody, the positive rate of platelet glycoprotein IIb/IIIa and platelet-specific antibody in 64 ITP patients 33 non-immune thrombocytopenia patients and 31 healthy controls were measured with ELISPOT,MAIPA and FCM respectively. *Results* Compared with the controls and non-immune thrombocytopenia patients, the frequencies of circulating B cells secreting platelet-specific antibody and the positive rate of platelet glycoprotein IIb/IIIa in ITP patients were significantly increased and notably decreased, respectively(P 0.05). The ELISPOT and FCM had a sensitivity of 70.69% 58.7% a specificity of 90.91% 93.8% for the diagnosis of ITP, the sensitivity were higher than that of modified MAIPA's (43.10%) P 0.05 . However there was no apparent difference of ELISPOT and FCM for the diagnosis of ITP patients P 0.05). *Conclusion.* Detecting the frequencies of Circulating B Cells Secreting Platelet-Specific Antibody and the platelet glycoprotein IIb/IIIa in the patients with idiopathic thrombocytopenic purpura, which can reflect the pathogenesis of idiopathic thrombocytopenic purpura and have higher sensitivity and specificity than modified MAIPA, could be able to improve the diagnosis and guide clinical therapy in some degree.

0234

CYTOKINES AND CHEMOKINES IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (ITP) TREATED WITH TPO-RECEPTORAGONISTS (TPO-RA) COMPARED TO HEALTHY CONTROLS

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Background. Thrombopoietin receptor agonists (TPO-ra) are new treatment modalities for chronic ITP, exerting their effect by stimulating platelet production. Treatment with TPO-ra has been preliminarily shown to increase suppressive activity of regulatory T-cells, but much remains to be learned about the influence of TPO-ra on the immune system. *Aims.* In this study, the production of a panel of inflammatory cytokines and chemokines in chronic ITP patients treated with TPO-ra was investigated and compared to healthy controls. *Methods.* Cytokines and chemokines in EDTA-plasma samples from 22 ITP patients treated with TPO-ra (16 females, median age 51 years, IQR 33-58 years, median platelet counts $59x10^9$/L, IQR 30-$100x10^9$/L) and 7 healthy controls (2 females, median age 35 years, IQR 21-38 years) were analysed by immuno-bead-based multiplex assay. *Results.* Elevated levels of soluble CD40 ligand (sCD40L) (median 411 vs. 222 pg/mL, p=0.003), tumor necrosis factor alpha (TNFα) (median 4.0 vs. 0 pg/mL, p=0.000), interleukin-1-receptor antagonist (IL-1ra) (median 915 vs. 325 pg/mL, p=0.001), chemokine (C-X-C motif) ligand 10 (CXCL10) (median 55 vs. 16 pg/mL. p=0.000), CXCL11 (median 52 vs. 23 pg/mL, p=0.000), chemokine (C -C motif) ligand 4 (CCL4) (median 30 vs. 8 pg/mL, p=0.008) and IL-8 (median 3.6 vs. 1.7 pg/mL, p=0.016) were found in ITP patients, with no differences in IL-6, CCL-2 or CCL5. However, a positive correlation between the increase from pre-treatment to on-treatment platelet counts and levels of CCL5 was observed (p=0.01). Correspondingly, a negative correlation between the increase in platelet counts and levels of CXCL10 (p=0.047) was observed. No other correlation between increase in platelet counts and cytokine levels were detected in this series. We also found a 3-fold increase in the median ratio of the Th1-associated chemokine CXCL10 compared to the Th2-associated chemokine CCL2 (median ratio 0.56 vs. 0.19, p = 0.001). *Summary and Conclusion.* The inflammatory mediators sCD40L, CXCL10, CXCL11, CCL4 were significantly increased as compared to healthy controls. This was also observed for TNFα and IL-8, although concentrations were low. CCL5 and IL-6, often associated with acute inflammatory reactions, were not elevated in this series. However, levels of CCL5 correlated to the increased platelet counts following TPO-ra treatment. Increased CXCL10/CCL2 ratio have previously been reported in treatment-naïve ITP patients, and is associated with a Th1-predominant active immune response. In our series, ITP patients had higher CXCL10/CCL2 ratios than healthy controls, despite succesfull treatment with TPO-agonists. Interestingly, levels of CXCL10 inversely correlated to the increase in platelet counts obtained by treatment with TPO-ra. Platelets are associated with inflammatory processes involving both the innate and the adaptive immune compartment. It can be hypothesized that TPO-agonists, i.e. by increasing platelet production, exert a larger than expected effect on the immune compartment in these patients. In this study, we found elevated levels of a range of inflammatory mediators in TPO treated ITP patients compared to healthy controls. Further studies are needed to fully understand the apparent influence of TPO agonists on the immune system in patients with ITP.

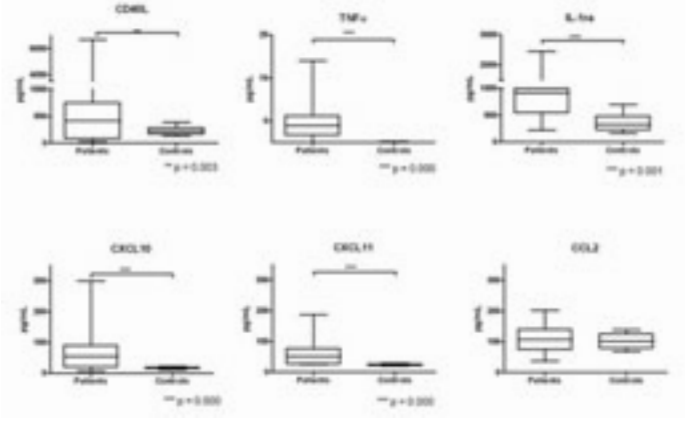

Figure 1. Cytokine and chemokine levels. Medians, IQR, range.

0235

ROMIPLOSTIM THERAPY IN CHILDREN WITH REFRACTORY CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA

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Background. Romiplostim, a thrombopoiesis-stimulating peptibody, represents a new therapeutic option in adult refractory chronic immune thrombocytopenic purpura (ITP).There are lacking studies about romiplostim use in pediatric chronic ITP. *Aim.* This study aimed to assess the short term efficacy and safety of romiplostim in children with refractory chronic ITP. METHODS:Eight patients with chronic ITP refractory to standard lines of therapy were recruited from the Pediatric Hematology Unit, Children's Hospital, Ain Shams University, Cairo, Egypt. One patient was initially excluded because of increased bone marrow reticulin.Therapy was initiated in 7 patients, aged 3.4 years-15.2 years(median 5.5 years),and the disease duration ranged from 13 months-7.3 years (median 2.4 years), none was splenectomized. Romiplostim dose was started as 1µgm/kg/week and dose was escalated by 1 µgm/kg/week according to platelet count. The duration of therapy varied between 1 week to 22 weeks (median 12 weeks). *Results*. Results revealed that 4 out of the 7 patients achieved variable response. Four patients demonstrated rapid increase in platelets counts when pulse steroid therapy was added, achieving rapid control of serious bleeding in three patients. Most reported adverse events were mild and transient including acute nasopharyngitis in 2 patients, epistaxis in 1 patient, but one patient developed moderately severe wheezy chest necessitating hospitalization. *Conclusions.* These results revealed variable response rate in children with chronic ITP to romiplostim therapy; addition of steroids especially in emergency bleeding situations could potentiate romiplostim thrombopoietic effect even in patients initially refractory to steroids. Romiplostim safety and efficacy in pediatric ITP needs further long-term studies.

0236

MORTALITY DURING CLINICAL STUDIES OF ELTROMBOPAG IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA

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Background. Disease- and treatment-related mortality in adult patients with chronic immune thrombocytopenia (ITP) is the subject of ongoing investigation. Reports suggest a rate of 0.3%-12%,[1] increasing to 8%-16% in patients with refractory disease[2] and to 15.7% for refractory patients following splenectomy failure.[3] Cohen reported an incidence of fatal bleeding events of 0.0162-0.0389/patient year (PY); life expectancy was reduced for patients with persistent thrombocytopenia and for older patients, with 5-year rates of fatal bleeding events of 2% and 47.8% for patients <40 years and >60 years, respectively.[4] Similar findings were reported by Portielje, who noted that the most common causes of death were bleeding and infection in almost equal proportions.[5] Eltrombopag is an oral thrombopoietin receptor agonist approved for chronic ITP. *Aim.* To describe mortality in adult patients with chronic ITP in eltrombopag clinical studies. *Methods.* Mortality among 494 adult patients with chronic ITP exposed to placebo or eltrombopag were analyzed from 5 clinical trials: two 6-week, placebo-controlled studies (TRA100773A/B: eltrombopag, n=164; placebo, n=67); RAISE, a 6-month, placebo-controlled, phase 3 study (eltrombopag, n=135; placebo, n=62); REPEAT, an open-label study of 66 patients treated intermittently with eltrombopag in three 6-week cycles; and EXTEND, an ongoing extension study with 299 patients from prior eltrombopag trials receiving eltrombopag for up to 3 years. *Results.* Eight patients (1.6%) have died (eltrombopag, n=7; placebo, n=1), with an overall exposure period of 584.4 PYs for eltrombopag and 35 PYs for placebo. The overall mortality rate for patients who died on therapy or within the subsequent 8 weeks was 0.0077/PY (95% CI 0.0025, 0.018). Two patients died 107 and 199 days following the last dose of study medication and were excluded from the calculation. Of the 8 patients who died, 5 were from EXTEND, and 1 each were from TRA100773A, RAISE, and REPEAT. Ages ranged from 40-77 years, with 3 patients >60 years. The deaths were not considered by investigators to

Table 1.

Causes of Death in Patients Exposed to Eltrombopag

Study	Age	Gender	Cause of Death	Study Drug	Exposure (Days)	Time from Last Dose to Death (Days)
TRA100773A	66	M	Cardiac failure - respiratory failure	Eltrombopag	21	5
RAISE	43	F	Brain stem hemorrhage	Placebo	7	2
REPEAT	71	M	Pancreatic cancer	Eltrombopag	126	199
EXTEND	56	F	Motor vehicle accident	Eltrombopag	86	On treatment
EXTEND	44	M	Hypovolemic shock/GI hemorrhage	Eltrombopag	46	55
EXTEND	59	F	Unknown (autopsy not performed; investigator opinion: Acute brain hemorrhage vs. myocardial infarction vs. arrhythmia and cardiac arrest vs. sepsis in a patient with fever on steroids)	Eltrombopag	529	On treatment
EXTEND	40	F	Subarachnoid hemorrhage	Eltrombopag	412	107
EXTEND	77	F	Multiple organ failure/pulmonary sepsis	Eltrombopag	218	42

GI, gastrointestinal.

be related to study medication. Fatal hemorrhage occurred in 3/494 patients: two, treated with eltrombopag, had never achieved a response and died 55 and 107 days following their last dose of eltrombopag; a third patient, treated with placebo, died of an intracranial hemorrhage. Other deaths include one each of cardiorespiratory failure, multi-organ failure, motor vehicle accident, pancreatic cancer, and unknown (died at home with no autopsy). *Conclusions.* No discernible pattern in cause of death is apparent, other than fatalities due to bleeding events in patients with persistently low platelet counts. The mortality rate across the eltrombopag trials is lower than that reported in the literature. These data need to be interpreted cautiously as patients enrolled in clinical trials are subjected to specific monitoring schemes not necessarily implemented in clinical practice.

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0237

SUSTAINED HEMOSTATIC PLATELET COUNTS IN ADULT PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) FOLLOWING CESSATION OF ROMIPLOSTIM - FOUR EUROPEAN CASE STUDIES

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Background. Romiplostim is recommended for second- and third-line treatment of chronic ITP in adults. While often perceived as a chronic, long-term treatment, previous data suggest some patients maintain hemostatic platelet counts when treatment stops. *Aims.* To describe individual cases of patients maintaining hemostatic platelet counts following cessation of romiplostim. *Methods.* We describe four patients with long-term (6-49 years), chronic ITP, and refractory to previous treatment options, including splenectomy, corticosteroids, IVIg, anti-D and rituximab. All received romiplostim in clinical trials, initiated at 1-3 µg/kg/week and with dose adjustments allowed to maintain platelet counts above 50 x 10^9/L. Romiplostim was withheld when counts rose above a pre-specified threshold (400 or 450 x 10^9/L), and reinitiated at a lower dose when counts fell below 200 x 10^9/L. Following completion of the relevant trial, patients were managed as per routine clinical practice. *Results.* Patient 1 completed a 24-week trial, during which platelets counts increased to normal levels. Following cessation of romiplostim, counts fell slightly and a single dose of IVIg was administered. Thereafter, hemostatic platelet counts were maintained without any ITP therapy for over 4 years. Patient 2 experienced high platelet counts after 8 weeks of dose titration, and romiplostim was withheld for 11 weeks. Following a single count <200 x 10^9/L and one dose of romiplostim 3 µg/kg, counts were again high and romiplostim was withheld for the remaining 7 months of the study. Thereafter, hemostatic platelet counts were maintained without any ITP therapy for 2 years, until death from ventricular fibrillation. Patient 3 experienced highly variable platelet counts, with romiplostim withheld on several occasions due to high values. After 3 years dosing at 1 µg/kg/week, counts remained >200 x 10^9/L and romiplostim was withheld for the remaining 3 months of the study. Patient 4 experienced a high platelet count after 1 year of romiplostim treatment and romiplostim was withheld. At the last recorded contact, patients 1, 2 and 4 had maintained platelet counts >50 x 10^9/L for 9 months-4.5 years in the absence of any ITP therapy or bleeding episodes. *Summary/Conclusions.* Dose adjustment rules allow romiplostim to be discontinued when hemostatic platelet counts are reached. This report of sustained hemostatic counts following romiplostim cessation provides evidence that romiplostim can be a short-term treatment in some adult ITP patients, including those with long-term, chronic disease. While the exact incidence of such cases is unknown, additional anecdotal evidence may provide more insight.

Table 1. Patient data.

Patient	1	2	3	4
Age (yrs) / sex	49/female	73/male	69/female	65/female
ITP duration (yrs)	6.5	6.5	9	49
Splenectomy	Yes	No	No	Yes
Prior ITP therapies	7	4	5	3
Baseline platelet count	3.0	6.0	27.0	13.9
Romiplostim starting dose	1	3	1	3
Romiplostim maximum dose	15	6	4	4
Duration of romiplostim treatment	6 months	5 months	4.5 years	1 year
Platelet count before last rom. dose	510	167	371	683
Duration platelet count > 50 after last rom. dose	4.5 years	3.6 years	16 months	9 months

Platelet counts, x 10^9/L; romiplostim dose, µg/kg/week

0238

INTRAMUSCULAR USE OF ANTI-D GAMMAGLOBULIN IN PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA: A SINGLE CENTER EXPERIENCE

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Background. Treatment guidelines support the intravenous administration of anti-D gammaglobulin as an option in Rh positive patients with a diagnosis of immune thrombocytopenic purpura (ITP). However, in Spain this drug is only available for intramuscular (IM) injection. This is the reason why we used the anti-D immunoglobulin intramuscularly in our patients. *Aims.* In this study we show our experience with IM anti-D gammaglobulin, when indicated, in the treatment of ITP patients. Our purpose was to evaluate the efficacy and safety of this therapeutic option. *Methods.*We have analyzed 41 patients with ITP treated with IM anti-D gammaglobulin in our institution over the past 20 years. The unitary dose was 900 micrograms repeated on days 1, 2 and 4 as the initial dose (loading dose). After one week, another loading dose was administered if necessary, and eventually further single doses as maintenance. The current consensus guidelines published in 2009 by the IWG were used to asses the response to treatment, especially the time to achieve a response and a complete response, and the number of doses required to obtain them. *Results.* Thirty of 41 patients were evaluable (median age 47 years, range 16-85; 19 women and 11 men). The exclusion criteria were a diagnosis other than ITP or the discontinuation of treatment before completing at least one loading dose. In most cases, anti-D gammaglobulin was administered as second or third line treatment, with median prior lines 2 (range 0-4). Corticosteroids were the initial therapy in 28 patients, most often associated with intravenous immunoglobulins (17 cases). The indications for starting anti-D were: relapse after other lines of treatment in 14 patients (47%), refractoriness to initial therapy in six cases (20%), and as maintenance approach in eight (27%). One patient received anti-D as first line therapy and another one because of toxicity to previous treatments. The mean platelet count before starting anti-D was 67 x 109/L. The number of patients who achieved complete response criteria (platelet count > 100 x 109/L) was 23 (77%), while two patients failed to reach it. Complete response criteria were not applicable in five cases because the initial platelet count was above 100 x 109/L. No adverse effects related to drug (especially any bleeding complication associated with the intramuscular route of administration) were registered. *Conclusions.* Although current guidelines do not include the IM administration of anti-D gammaglobulin, our experience demonstrates that this is an effective, safe, and economical option, with the added advantage of an outpatient administration.

Myelodysplastic syndromes - Biology

0239

THE INTERNATIONAL MULTICENTRIC COOPERATION CYTOGENETIC SCORING SYSTEM EFFECTIVELY PREDICTS DISEASE OUTCOME IN DE NOVO MDS

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Background. In 2010 an International Multicentric Cooperation proposed a new, comprehensive cytogenetic scoring system for primary MDS (Haase *et al.,* EHA 2010). *Aims.* The present study was aimed at assessing whether this new cytogenetic scoring system could improve the prognostic stratification of 631 consecutive de novo MDS patients, who were observed in the period January 1990-January 2010. *Methods*. Inclusion criteria were: primary MDS, age >=16 years, bone marrow blast cell percentage <20%, supportive therapy only. Univariate and multivariate analyses concerning overall survival (OS) and MDS/AML progression were performed. Median follow-up was 19.6 months (mo.) (Inter-quartile range, IQR, 7.3-46.3). At the time of the analysis 160 pts (25.3%) had died after a median follow-up of 18.7 mo. (IQR 8.4-38.1) and 137 pts (21.7%) had experienced MDS/AML progression after a median time of 14.6 mo. (IQR 5.9-34.2). *Results.* There were 249 females and 382 males whose median age was 65.3 yrs (IQR 56.6-72.5). According to WHO 70 pts (11.1%) were RARS, 122 (19,3%) RA, 25 (3,9%) RCMD with ringed sideroblasts (RCMDS), 149 (23,6%) RCMD, 38 (6,0%) 5q- syndrome, 9 (1,4%) unclassifiable MDS (u-MDS), 102 (16,1%) RAEB-1 and 116 (18,4%) RAEB-2. According to IPPS 177 pts (28,1%) were low-risk, 255 (40,4%) int-1 risk, 139 (22%) int-2 risk and 60 (9,5%) high-risk. Three-hundreds fifty-three (55.9%) pts presented an abnormal karyotype: 260 (41.2%) carried a single chromosomal defect, 46 (7,3%) carried two defects and 47 (7,4%) >=three defects. Based on the new cytogenetic scoring system, 14 pts (3,8%) were considered very good risk, 392 (62,1%) good risk, 162 (25,6%) intermediate risk, 29 (4,6%) high-risk and 34 (5,3%) very high-risk. Two-years OS was 91.0% (95% CI: 50.8-98.7) for the very good-risk category, 87.0% (95% CI: 82.7-90.6) for the good-risk category, 72.0% (95% CI: 63.2-80.0) for the intermediate-risk category, 54.8% (95% CI: 29.7-74.1) for the high-risk category and 9.4 % (95% CI: 0.6-32.9) for the very high-risk category (p<0.0001). Multivariate analysis, which compared each category to the very-good, resulted in a Hazard Ratio of 1.7 for the good-risk category; 2.8 for the intermediate-risk category; 4.0 for the poor-risk category and 16.3 for the very-poor risk category (p<0.0001). Two-years PFI was 90.9% (95% CI: 50.8-98.6) for the very good-risk category, 79.0% (95% CI: 74.0-83.3) for the good-risk category, 54.7% (95% CI: 45.3-63.2) for the intermediate-risk category, 46.1% (95% CI: 22.7-66.7) for the high-risk category; no patient of the very high-risk category was surviving at this time (p<0.0001). Multivariate analysis resulted in a Hazard Ratio of 2.0 for the good-risk category; 3.9 for the intermediate-risk category; 4.4 for the poor-risk category and 15.5 for the very-poor risk category (p<0.0001). Six multivariate models were compared by means of Akaike Information Criterion (AIC). To predict OS, the best models included age, peripheral cytopenias, WHO classification, and either new cytogenetic categories or number of chromosomal abnormalities (AIC=1631 and 1622); to predict PFI, the best models included the same variables (AIC=2454 and 2448). *Conclusion.* The new cytogenetic scoring system is effective in improving the prognostic stratification of pts with de novo MDS.

0240

GERM-LINE GATA2 T354M MUTATION IN FAMILIAL MDS WITH MONOSOMY 7 SYNDROME WITH RAPID ONSET AND POOR SURVIVAL

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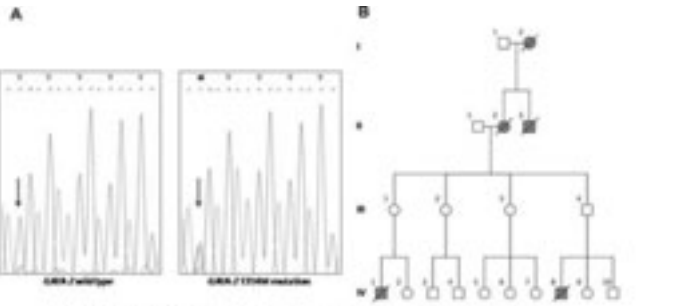
Background. While the majority of MDS/AML cases are sporadic, rare familial cases occur and have provided insight into the molecular basis of leukemogenesis. The best defined familial MDS/AML syndromes result from inherited mutations in *RUNX1* or *CEBPA*. More recently, a novel germline mutation in *GATA2* was reported. *Methods.* Individuals from families with ≥ 1 first-degree relative with MDS/AML were selected for investigation. Informed consent was obtained. Genomic DNA was used to PCR amplify exon 5 of the *GATA2* gene, sequencing performed by bidirectional Sanger sequencing, with mutations confirmed from 2 independent PCR amplicons. *Results.* Individuals from 6 families with a history of familial MDS/AML and wildtype *RUNX1* and *CEBPA* were investigated. A *GATA2* T354M mutation (Figure 1A) was observed in samples (2 tumour, 1 remission) from 2 first-degree cousins in a single pedigree. This same missense mutation was reported by Scott *et al.* in 3 of 4 previously described pedigrees and was not observed in 268 sporadic MDS/AML samples (ASH LBA-3). Material was not available from other family members. Figure 1B demonstrates the genogram. The proband IV-1, presented at age 23 with cytopenias and no past medical history. A bone marrow biopsy demonstrated trilineage dysplasia with 17% myeloblasts, consistent with a diagnosis of RAEB. Cytogenetic analysis demonstrated isolated monosomy 7. He underwent chemotherapy and obtained a CR but with persistence of dysplasia. The bone marrow at CR revealed normal cytogenetics. Seven months later, he relapsed with re-emergence of the monosomy 7 clone, and died early of transplant-related complications following a haplo-identical transplant. IV-8, the first cousin of IV-1, presented 1 week after his cousin at age 19 and was similarly diagnosed with RAEB and monosomy 7. His bone marrow revealed 7% myeloblasts and monosomy 7. He underwent a haplo-identical HSCT but died from relapsed disease. None of the individuals in the preceding generation have hematological abnormalities. The *GATA2* mutation was observed in MDS samples from IV-1 and IV-8 and was absent from the remission sample of IV-1. The presence of this presumed germline mutation suggests that III-1 and III-V are asymptomatic obligate carriers who have avoided development of MDS, confirming that the *GATA2* mutation is not sufficient alone to cause overt MDS/AML. This was also a feature of previous *GATA2* pedigrees. Monosomy 7 in association with pure familial MDS/AML has been previously reported in more than 12 pedigrees and typically presents at young age with autosomal dominant transmission. Non-preferential deletion of parental chromosomes has been demonstrated in several sibling pairs suggesting that the predisposing locus is not located on chromosome 7. The presence of monosomy 7 in both affected individuals suggests that germline *GATA2* mutations may be a cause of the "Familial MDS with monosomy 7 syndrome". *Conclusions.* These findings confirm the recent report that germline *GATA2* mutations predispose to familial MDS/AML and that outcomes after diagnosis of MDS/AML are uniformly poor in these patients. Our investigations also suggest that monosomy 7 may be a recurrent cytogenetic abnormality in these families, providing the necessary 'second hit' for development of overt MDS/AML.



A Illustrated is the GATA-2 T354 mutation and the corresponding wild-type sequence. The change of the ACG codon to ATG leads to a threonine (T) – methionine (M) substitution at the protein level. B. Genogram for GATA2-mutated pedigree. The squares denote males and circles denote females. The grey boxes mark the individuals with a history of MDS or AML.

Figure 1.

0241

REPRODUCIBILITY OF THE WHO 2008 CLASSIFICATION FOR MYELODYSPLASTIC SYNDROMES

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Background. Myelodysplastic syndromes (MDS) are a group of clonal hematologic neoplasms with different risk for progression to acute leukemia and survival. The current 2008 WHO classification for MDS

was built in an attempt to improve the prognostic value of the previous FAB classification. The 2008 WHO classification is based on the percentage of blast cells in bone marrow (BM) and peripheral blood (PB), the type and degree of dysplasia, the proportion of ring sideroblasts, and number of cytopenias. However, the reproducibility of WHO classification is still unclear. The aim of this study was to analyze the interobserver concordance between four experienced morphologists from three different centers and to define potential diagnostic difficulties. *Methods.* Smears from PB and BM samples (stained by May Grunwald-Giemsa and iron) from 50 patients with well established diagnosis of MDS were blindly analyzed by 4 observers. The assessment of myelodysplasia and blast cell enumeration was performed as recommended by WHO guidelines (Swerdlow *et al.*, 2008). The degree of correlation between observers in the percentages of blast cells in BM and PB and ring sideroblasts was analyzed by Pearson correlation and inter-observer agreement index in the diagnostic morphological subtype (7 categories) and degree of dysplasia (3 categories; <10%, 10 - 39%, ≥ 40%) was studied by using the generalized kappa statistic for multiple raters. *Results.* The degree of correlation between observers for the proportion of blast cells in BM (R, .44 - .89; *P*, .002 - <.0001) and PB (R, .31 - .72; *P*, .03 - <.0001) and ring sideroblasts in BM (R, .77 - .94; *P*, <.0001) was statistically significant in all instances. The kappa coefficient for dysgranulopoiesis, erythroid, and megakaryocyte dysplasia was .40, .18, and .41 respectively. The kappa value for morphological subtype was .39, ranging from .17 for refractory cytopenia with unilineage dysplasia to .66 for mixed MDS/myeloproliferative disorders (chronic mylomonocytic leukemia subtype). *Conclusions.* These results suggest that there is a good correlation between observers in quantification of the percentage of blast cells in PB and BM and ring sideroblasts in BM. Second, the degree of concordance in the assessment of dysplastic changes is adequate for the granulocytic and megakaryocytic lineages but inadequate for erythroid lineage. However, the apparently accurate concordance for most parameters evaluated did not translate in a good agreement in the final diagnosis classification for several subtypes. This absence of agreement could be due to the arbitrary cut off points in the percentage of blast cells and dysplastic features that determine the different subtypes.

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0242

ANTI-LEUKEMIC EFFICACY OF THE EGFR-INHIBITOR ERLOTINIB IN MDS/AML IS AT LEAST PARTIALLY CONVEYED BY OFF-TARGET EFFECTS ON THE SRC-KINASE LYN

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Background. The EGFR-inhibitor erlotinib (Erlo), currently evaluated in phase-I/II clinical studies (NCT00977548, NCT01085838), demonstrated acceptable toxicity and anti-leukemic efficacy in higher risk MDS patients having failed hypomethylating agents (Komrokji *et al*., abstract 1854, ASH 2010). Nevertheless, due to the EGFR-negativity of these entities, the molecular determinants conveying erlotinib's off-target effects remain to be defined (Boehrer, Blood, 2009). *Methods.* Hypothesizing that erlotinib functions by inhibiting one or several tyrosine kinases (TK) deregulated in MDS/AML (notably Lyn and Syk), we incubated erlotinib-sensitive KG-1 cells with biochemical inhibitors (+/-erlotinib) of individual kinases (PP2, Piceatannol, BAY61-3606), and quantified the phosphorylation/activation status by immunoblot and flourocytometric analysis. Biological consequences were determined by evaluation of apoptosis (DioC3(6)/PI staining), cell cycle progression (PI staining), and autophagy (by LCII/I quantification using immunoblot and immunoflourescence analysis, as well as electron microscopy, EM). Erlo's impact on colony-forming capacity was evaluated in patient-derived MDS-/AML cells (n=5). *Results.* Erlotinib decreased aberrant activation of the TK Lyn in KG-1 cells and patient-derived AML-cells. In addition, biochemical inhibition of Lyn (by PP2) recapitulated erlotinib-induced anti-proliferative effects, i.e. 10μM of PPI induced the same degree of G1-arrest at 24h as 10μM of erlotinib (+10% as compared to controls). The observation that this anti-proliferative effect was not increased by concomitant incubation of KG-1 cells with PP2 and Erlo indicates that both drugs work by employing the same pathway. In contrast, none of the Syk-inhibitors (Piceatannol, 10μM and BAY 61-3606, 50nM) had an effect on cell cycle progression. Whereas Lyn-inhibition alone (by PP2) did not induce apoptosis in KG-1 cells, it considerably increased apoptosis upon combination with Erlo, providing evidence that anti-proliferative and pro-apoptotic effects of erlotinib are conveyed by different signaling pathways. Assessment of phosphorylation status confirmed that PP2 (but not Piceatannol or BAY 61-3606) diminished aberrant activation of Lyn to the same degree as erlotinib, and that this dephosphorylation was not increased by concomitant incubation with both drugs. Since the critical role of aberrant activation of Lyn and subsequent deregulation of mTOR signaling has been previously identified in AML (Dos Santos *et al*., Blood, 2008), we next tested if correction of Lyn activation by Erlo also impacts on mTOR-signaling: 4h of incubation with Erlo indeed significantly diminished phosphorylation of the mTOR-targets p-p70S6K(Thr389) and p-4E-PB1(Thr70). Assessment of LC3-II, as well as the cytoplasmic accumulation of autophagosomes and autolysosomes, both correlates of autophagy, provide evidence that erlotinib's inhibition of aberrant mTOR-signaling results in autophagy. Finally, we confirmed the antiproliferative activity of Erlo by demonstrating its capacity to decrease colony growth of patient-derived CD34-positive myeloblasts *Conclusions.* These results provide evidence that anti-proliferative effects of Erlo in EGFR-negative blasts are obtained by antagonizing aberrant Lyn-activation, therefore providing a mechanistic explanation for the therapeutic benefit of Erlo in high-risk MDS patients. In addition, we provide further evidence that the biological effects (notably on cell cycle progression and apoptosis) elicited by Erlo are conveyed by different signaling pathways.

0243

ACTIVATION OF COMPENSATORY PATHWAYS ARE RESPONSIBLE OF AML SECONDARY TO HIGH-RISK MDS TREATED WITH 5-AZACYTIDINE

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Background. Approximately one-third of Myelodysplastic syndromes (MDS) patients progresses to acute myeloid leukemia. Several studies have shown increased rates of programmed cell death (apoptosis) in marrow cells of patients with low grade/early stage MDS, and a changing interplay of pro-apoptotic and anti-apoptotic signals is central to disease progression. 5-azacytidine (5-AZA) is a DNA methyltransferase inhibitor used in treatment of high-risk MDS. A large clinical trial showed its beneficial in achieving better clinical outcomes and quality of life when compared to supportive care. Despite 5-AZA can delay the progression of MDS to acute myeloid leukemia (AML), by preventing the inactivation of tumor suppressor genes, high-risk MDS mortality for progression to AML is still too high. *Methods.* We analyzed 17 patients affected of high risk MDS, treated for 4 months with 5-azacytidine (median age 71 years, M/F=12/5). In 5 cases the sample after 4 cycles of therapy was matched to the sample collected before the therapy start. Bone marrow mononuclear cells were obtained using density gradient centrifugation (Fycoll) under sterile conditions, at the beginning of treatment and after 4 cycles of therapy. All samples were lysed at the same time into 40 uL of lysis buffer containing a 1:1 mixture of 2x Tris-Glycine SDS sample buffer (Invitrogen Life Technologies) and Tissue Protein Extraction Reagent (Pierce) plus 1.0% betamercaptoethanol for 5 min at 100oC. Reverse-phase protein microarray (RPMA) is a reproducible, high-throughput system for protein signal pathway profiling. RPMA were used to quantitatively map 45 cell signaling pathway endpoints, including survival, proliferation, drug resistance, apoptosis, and autophagy. *Results.* All patients were evaluable for response one month after the 4th cycle. 2 patients were refractory, progressing to AML under treatment, 1 progressed with a concomitant monoclonal gammopathy, and 1 was a late responder (documented response after 7cycles). 5/35 protein endpoints were linked together in the induction of a compensatory pathway induced after the treatment with 5-azacytidine, independently from the quality of achieved response. PLC-y-1-Tyr783 (p=0.0017), and its upstream SrcTyr416 (p=0.002) and downstream target STAT5Tyr694 (p=0.0017) were increased, without affecting proliferative pathways, such as AKT activation status on Ser473 and Thr308 or mTORSer2448. Comparing pool of samples at diagnosis with the pool of samples after 4 cycles we found an increase of the three main proteins considered markers of autophagy: ATG5 (p<0.0001), Beclin 1 (p=0.0056) and LC3B (p=0.0124), independently from the achieved quality of response. The activation occurred downstream mTOR pathway, since mTORSer2448, AktSer473, AktThr308, ERKThr202Tyr204 were not affected. *Conclusion.* We identified at protein level two compensatory pathways induced by

5-azacytidine. Since STAT5 is involved also in the transcriptional regulation of H3/H4, herein we provide the molecular rationale for the development of a combination based upon 5-azacytidine+ inhibitors of HDAC. Similarly, autophagy activation can be considered an escape pathway of survival in neoplastic cells. The observation that 5-azacytidine does not affect proliferative pathways, identified as new potential target in acute myeloid leukemia, suggests the need to combine 5-azacytidine to anti-proliferative agents, such as Rapamycin or RAD001, in order to target proliferation of neoplastic cells.

0244

METHYLATION OF WNT ANTAGONISTS AND EFFECTS OF AZA TREATMENT ON WNT PATHWAY IN MDS CELLS

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Background. The implication of the Wnt pathway in self-renewal and proliferation of hematopoietic stem and progenitor cells suggests its involvement in the pathogenesis of haematopoietic neoplasias. This signalling cascade is controlled by different Wnt antagonists that interfere with ligand-receptor activating interactions. Among them, members of the DKK and sFRP family, whose activation induces the accumulation of non-phosphorylated β-catenin (NPBC) in the cell nucleus. Interestingly, aberrant methylation of these Wnt antagonists has been demonstrated in some haematological malignancies (acute myeloid leukemia and chronic myeloid leukemia). *Objectives.* 1. To study the methylation status of the Wnt antagonists: sFRP1, sFRP2, sFRP4, sFRP5 and DKK1 in primary MDS cells 2. To evaluate the correlation between methylation of Wnt antagonist and activation of the Wnt pathway, analyzing the expression of NPBC using bone marrow MDS cells before and after treatment with azacytidine (AZA). *Methods.* We studied bone marrow cells from 24 patients diagnosed with MDS according to the WHO classification: 10 RCMD, 5 RAEB-1, 3 RA and 6 RARS. Median age was 73 yr and M/F was14/24. After bisulphite treatment, methylation was evaluated by methylation specific PCR (MSP) using primers specific for the methylated and unmethylated alleles of the genes. Bone marrow cells of MDS patients were grown in RPMI 1640 medium supplemented with FBS and treated with AZA 1μM during 48 hours. Expression of β-catenin was studied by confocal microscopy using antibodies against total β-catenin and NPBC. *Results.* Hypermethylation of the gene promoters was observed for all Wnt antagonist genes. Among the 24 cases, methylation frequencies were as follows: 75% sFRP2, 75% DKK1, 61% sFRP1, 29% sFRP5 and 16% sFRP4. Most MDS patients (92%) showed methylation of at least one gene, ranging from one to 5 methylated genes. Bone marrow cells of the MDS cases with sFRP2 and sFRP2 together with sFRP4 methylated, respectively, were cultured *in vitro*. After AZA treatment, a reduction of DNA methylation level of sFRP2 and sFRP4 was observed, indicating the decrease on the. Additionally, confocal microscopy showed a reduction of NPBC in the cell nucleus, clearly indicating that inactivation of the Wnt pathway was provoked by the treatment with AZA. *Conclusion.* Hypermethylation of Wnt antagonists is a frequent event in MDS and seems to be associated with activation of the Wnt pathway, as demonstrated by the relocation of NPBC in the cell compartments after AZA treatment.

0245

ALTERED CELL CYCLE PROFILES OF SPECIFIC COMPARTMENTS OF BONE MARROW (BM) CELLS FROM MYELODYSPLASTIC SYNDROME PATIENTS IS ASSOCIATED WITH PROGNOSTIC FEATURES OF THE DISEASE

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Background. Information concerning the cell cycle distribution of different bone marrow (BM) cell compartments in myelodysplastic syndromes (MDS) remains limited and most studies reported have either been restricted to the overall BM cellularity or to the CD34+ BM cell subset. *Aims.* In the present study, we provide a comprehensive analysis of the cell cycle distribution of different BM hematopoietic cell compartments in a series of 117 MDS patients at different disease stages versus both normal and cytopenia-associated reactive BM. *Methods.* The proliferation index (PI; S+G2/M phase cells) was analyzed among the BM compartments of CD34+ hematopoietic progenitor and precursor cells (HPC), maturing neutrophils, maturing monocytes, mature lymphocytes, eosinophils and nucleated red blood cells (NRBC) from a cohort of 117 patients with MDS and 94 normal (n=47) and cytopenia-associated reactive BM samples (n=47), using multicolor flow cytometry immunophenotyping and the DRAQ5 DNA-dye. *Results.* Overall, our results show similar proliferation profiles in normal (n=47) vs reactive (n=47) BM, except for a slightly decreased PI among non-lymphoid CD34+ HPC (p=0.02), and a higher PI among CD13hi/CD11b+ neutrophils and monocytic cells in the latter group (p=0.03). Regarding MDS, significantly different proliferation profiles were detected in low- vs high-risk MDS. Overall, abnormally increased PI were found among low-risk MDS patients for the non-lymphoid CD34+ HPC (p 0.001), maturing and mature neutrophils (p 0.005) and NRBC (p=0.01); conversely, high-risk MDS cases showed lower PI for the non-lymphoid CD34+HPC (p<0.001), the more immature neutrophil compartments (p 0.004), monocytic cells (p≤0.05) and NRBC (p<0.001). Upon grouping patients according to their PI, a clear association was observed between a lower PI of the non-lymphoid CD34+ HPC (PI: <10% vs ≥10%) and other adverse disease features: >2 cytopenias (79% of cases vs 56%; p=0.03), thrombocytopenia (71% vs 35%; p=0.004) and increased LDH serum levels (57% vs 11%; <0.001). Similarly, a lower NRBC PI (<24% vs ≥24%) in MDS was associated to detection of >2 cytopenias (86% vs 53%; p=0.03), thrombocytopenia (86% vs 30%; p<0.001), increased LDH serum levels (46% vs 17%; p=0.05), anemia (86% vs 53%; p=0.03), transfusion dependence (90% vs 28%; p=0.001) and transformation into acute leukemia (41% vs 9%; p=0.01). Interestingly, those subjects with intermediate/poor cytogenetics according to the IPSS displayed an abnormally increased PI of CD13hi/CD11b+ neutrophils (3.5±3.4% vs 1.3±1%; p=0.005) associated with a lower PI of NRBC (24±5% vs 31±9%; p=0.004) and eosinophils (5±4% vs 6±5.5%; p=0.004) vs cases with normal or favourable karyotypes. From the prognostic point of view, assessment of the PI of NRBC emerged as an independent prognostic factor for overall patient survival. *Summary*. The assessment of the PI of different compartments of BM cells from patients with MDS revealed differential proliferation profiles in low vs high-grade MDS which might contribute to the prognostic stratification of the disease.

0246

GLOBAL DNA METHYLATION IN BONE MARROW CELL LINEAGES IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background. Epigenetic aberrations are now well recognized as very frequent and also as early events in the process of malignant transformation. It is very often reported that gene-specific hypermethylation occurs in the context of global hypomethylation. The clinical responses of MDS to drugs that reverse aberrant hypermethylation, such as 5-aza-2-deoxycytidine and 5-azacytidine, suggest that aberrant hypermethylation plays a causative role. *Aims.* We investigated global DNA methylation by immunohistochemistry in bone marrow trephine biopsy specimens in a cohort of 132 MDS patients comprising all subgroups. Results were compared to an age-matched control group of 47 healthy subjects and to a group of de novo (36) and secondary (20) AML patients. We applied a double staining procedure to discriminate the cell lineage of positive cells. *Methods.* Immunohistochemistry was performed on paraffin-embedded sections using anti-5-methylcytosine/5mc antibody. Scoring of immunohistochemistry was evaluated with a four-points scale for both the number of positive tumor cells and their intensity of immunoreactivity. Double immunostainings were performed on histological section 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) by using EnVision® G|2 Doublestain System,

Rabbit/Mouse (Dakocytomation): cells showing double stainings showed brown nuclei and red cytoplasms or cell membranes. *Results.* Normal bone marrows showed a low number of cells reactive for 5mc: with double stainings they were recognised as intermediate myeloid MPO-reactive and early erythroid glycophorin-C-reactive precursors accounting for less than 10% of the entire series. Segmented and polymorphonucleated granulocytes and orthochromatic erythroblasts were not stained with 5mc. CD34+ precursors with double stainings were hardly visualised in sections. Compared to normal bone marrows, MDS and AML cases showed respectively a moderate and a marked increase of positivity for 5mc. Primary AML were characterized by the highest percentage of 5mc+/CD34+ and 5mc+/MPO+ cells, including also more mature cells like segmented granulocytes and bands; 5mc+/glycophorin-C+ cells were few in this group of cases. Secondary AML showed a percentage of 5mc+/MPO+ and 5mc+/glycophorin-C+ cells and 5mc+/CD34+ cells higher than MDS cases and lower than primary AML. Unilineage and multilineage MDS without excess of blasts showed a mild increase of 5mc+/MPO+ and a significant increase of 5mc+/glycophorin-C+ precursors compared to normal bone marrows. MDS with excess of blasts exhibited a slight increase of 5mc+/CD34+ precursors compared to MDS without blasts and normal bone marrows. Differences were statistically significant between AML and MDS cases and between AML and normal marrows. In MDS the 5mc+/CD34+ and 5mc+/MPO+ percentage correlated significantly with the risk score according to the International Prognostic Scoring System. Factor VIII+ megakaryocytes were frequently reactive for 5mc in all conditions and we were not able to find any difference among different groups of patients. *Summary.* These results may provide a molecular explanation for the success in treating MDS patients with hypomethylation-inducing agents. Future studies have to analyse whether the determination of global methylation levels may serve as a new predictive marker for therapy response.

0247

OVEREXPRESSION OF THE ILDR1 GENE IN MYELODYSPLASTIC SYNDROMES

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Background. Myelodysplastic syndromes (MDSs) are a heterogeneous group of hematopoietic stem cell disorders characterized by ineffective hematopoiesis and a propensity to progress into acute myeloid leukemia. In de novo MDS, chromosomal lesions consist mainly of unbalanced rearrangements and numerical defects whereas balanced structural rearrangements are rare, being observed in fewer than 5% of patients and their prognostic impact remains unknown. *Aims.* We aimed to characterize the t(3;11)(q13;q14) rearrangement in MDS at the genomic, molecular and functional level. *Methods.* One-hundred and fifteen MDS patients at diagnosis were analyzed by conventional cytogenetic analysis. FISH analyses were performed using BAC and fosmid probes selected according to the University of California Santa Cruz database (http://genome.ucsc.edu/). Quantitative real-time PCR (qRT-PCR) experiments were performed using the ABI Prism 7300 Sequence Detection System. Statistical analysis of the relative expression results was performed with the Relative expression software tool (REST). *Results.* Two (1.7%) cases showed a t(3;11)(q13;q14) translocation. FISH experiments detected the presence of the same breakpoints in both patients. UCSC database querying showed that no known gene was located on the chromosome 11 breakpoint, whereas 3 genes (CD86, ILDR1, and CASR) with known function were mapped next to the chromosome 3 breakpoint. qRT-PCR experiments showed ILDR1 upregulation in the patients by a mean factor of 13.775 (p=0.02). Bioinformatic analysis of the chromosome 11 breakpoint region showed the presence of a promoter (892+)#2 and a CpG island (CpG 172) at a distance of about 220 Kb from the breakpoint region. *Conclusions.* We reported a novel t(3;11)(q13;q14) rearrangement associated with overexpression of the immunoglobulin-like domain-containing receptor (ILDR1) gene in MDS patients. We hypothesize that the gene upregulation could be mediated by the juxtaposition of regulatory elements next to the ILDR1 gene as a consequence of the chromosomal translocation. ILDR1 expression has been related to the development and/or progression of cancer, as it has been detected in the transformation of a low grade follicular lymphoma to a high-grade diffuse large B cell lymphoma. The question whether there is a functional link between the clinical features and the ILDR1 gene dysregulation and whether it may have a potential prognostic significance in MDS remains to be established.

0248

UP-REGULATION OF SUBSET OF MIRNAS LOCATED IN 14Q32 DOMAIN IN CD34+ BONE MARROW CELLS FROM PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background. MicroRNAs (miRNAs) are small non-coding RNAs functioning as key regulators of many cellular processes including hematopoiesis. Differential miRNA expression patterns and a number of causative aberrations in miRNA genes have been detected in various pathological processes originating from hematopoietic stem cells. However, limited information is available regarding the importance of miRNAs for the development and progression of myelodysplastic syndrome (MDS), a heterogeneous group of clonal preleukemic conditions with high risk of transformation into secondary acute myeloid leukemia (AML). Recently, 46 potential miRNA genes located in the human imprinted 14q32 domain were suggested to play a role in various developmental or oncogenic processes, including AML. *Aims.* To expand the understanding of MDS etiology, here we focused on miRNA expression levels of 14q32 miRNA domain in patients with MDS using microarray techniques. *Methods.* Expression levels of miRNAs were measured in CD34+ bone marrow cells of 39 patients with MDS and AML evolved from MDS using human v2 MicroRNA Expression Profiling Kit (Illumina), and obtained data were compared with the group of 6 samples of healthy donors. Presence of cryptic chromosomal aberrations was tested in 23 MDS patients by the whole genome SNP-array (HumanCytoSNP-12 BeadChip; Illumina). All tested subjects signed the informed consent and the Institutional Review Board approved the study. *Results.* We defined a set of 9 miRNAs (miR-299-3p, miR-299-5p, miR-323-3p, miR-370, miR-409-3p, miR-431, miR-432, miR-494 and miR-654-5p) within 14q32.2 region significantly ($p<0.001$) upregulated in patients compared to controls; additionally, all miRNAs of this cluster whose expression was detected in our array experiment (n=35) displayed higher expression in patients compared to controls (24 miRNAs (i.e. miR-127) reached $p<0.01$, the others (n=11) displayed apparent but statistically non-significant increase in approx. 80% of patients). To examine a presence of some cryptic chromosomal aberrations (copy number variation (CNV) and uniparental disomy (UDP)) in this region which could be responsible for increased miRNA expressions, we applied SNP-microarray based karyotyping but there was no evidence for the chromosomal defect in this region; this rather suggest change in the methylation or acetylation status of the region or aberrant characteristics of some transcriptional regulator. *Conclusions.* Several publications suggested implication of miRNA members of 14q32 cluster in various developmental or oncogenic processes regarding mainly brain, placenta, and gastrointestinal tract tissues (e.g. miR-370 or miR-494). Interestingly, miR-127, another up-regulated member of 14q32 miRNA cluster, is involved in B-cell differentiation process through post-transcriptional regulation of BLIMP1 and XBP1; moreover, we detected negative association of another miR-127 target, BCL6, in MDS patients. Proto-oncogene BCL6, mediating aberrant cell survival, functions as a transcriptional regulator required for B- and T-lymphocyte terminal differentiation. The over-expression of miR-127 may thus represent a key event in lymphomagenesis by blocking the B-cell differentiation process. In the context of MDS, over-expression of miR-127 may be associated with previously documented down-regulation of genes involved in B-cell lineage differentiation. This is an early report describing distinctive miRNA expression profiles in 14q32 miRNA cluster in MDS CD34+ cells, likely reflecting the disease-specific regulation.

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0249

ABERRANT BNIP3 EXPRESSION IN MDS CELLS: A POSSIBLE DECITABINE TARGET

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Introduction. Myelodysplastic syndrome (MDS) encompasses a group of clonal hematopoietic stem cell disorders characterized by in-

Table 1.

Patient characteristics

	Low Risk (n°)	High Risk (n°)
FAB	RA (14) RARS (7) LMMC (1)	RAEB (9) RAEBt (3)
WHO	RCUD (1) RARS (7) RCMD (12) MDS 5q (1)	RAEB-1 (6) RAEB-2 (3) AML with myelodysplasia-related changes* (3)
IPSS	Low (10) Int-1 (16)	Int-2 (5) High (1)
Cytopenia	0 (4) 1 (11)	2 (12) 3 (7)

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.

effective hematopoiesis and a tendency to progress towards acute myeloid leukemia (AML). MDS progression is characterized by changes in protein functions that confer the ability of proliferation, impaired cell differentiation and decrease in apoptosis. BNIP3 is a hypoxia-induced enhancer of cell death and is associated with various tumors. BNIP3 expression is reduced in hematopoietic cell lines and primary leukemia cells and this result was associated to aberrant methylation and histone deacetylation of the transcription start site. BNIP3 expression is also reduced in myeloproliferative neoplasia, indicating that BNIP3 may play a role in the disturbed apoptosis observed in these diseases. Furthermore, BNIP3 was related to the regulation of erythrocyte production through modulation of apoptosis. Nevertheless, there are as yet no studies of the expression or function of BNIP3 in MDS. *Aims.* The aim of the present study was to characterize *BNIP3* expression levels in bone marrow cells from MDS and AML patients and normal donors, and the expression levels of *BNIP3* in MDS cells submitted to treatment with 5-aza-2'-deoxycytidine (DAC). Moreover, we evaluated the expression levels of *BNIP3* transcripts during erythroid differentiation of $CD34^+$ cells from normal donors and MDS patients. *Methods.* Bone marrow aspirates were obtained from thirty-four patients with MDS and twenty-eight patients with AML. Samples were collected from patients at the time of diagnosis. Twenty samples from normal donors were used as controls. MDS patients were grouped in low-risk and high-risk according to FAB, WHO and IPSS (Table 1). Gene expression was evaluated by quantitative PCR in total cells. Mononuclear cells from four MDS patients were isolated with Ficoll Hypaque density gradients and treated with 5μM DAC for seventy-two hours. Erythroid-differentiation was performed in $CD34^+$ bone marrow cells from 4 normal donors and 4 MDS patients. *Results.* We observed a significant decrease in *BNIP3* expression of AML and MDS cells compared with normal hematopoietic cells (0.52 [5.27-0.00]; 0.52 [5.25-0.02] versus 1.09 [6.04-0.18], respectively; $p<0.05$). In MDS, *BNIP3* expression was lower in both low-risk and high-risk patients according to FAB and WHO classification, IPSS and number of cytopenias, when compared to normal subjects. Interestingly, among the mononuclear cells submitted to DAC treatment, *BNIP3* expression was increased by three fold after DAC exposure in the cells from the two patients that showed lower *BNIP3* expression. *BNIP3* expression was not modulated during erythroid differentiation in normal and MDS cells. *Conclusion.* The downregulation of *BNIP3* in MDS cells may play a role in the dysregulation of apoptosis in hematopoetic cells leading to ineffective hematopoiesis. The increase in *BNIP3* expression after DAC treatment indicates that this gene may be epigenetically inactivated by methylation in this disease and might be a target for DAC treatment. Although it is difficult to explain the pathophysiology of the MDS only by *BNIP3* gene modulation, the study of different pathways is important in order to identity new prognostic markers or therapeutic targets in this disease.

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0250

SELECTION BY SEVERE HYPOXIA OF REPOPULATING PROGENITOR CELLS IN PRIMARY MDS BONE MARROW CELL CULTURE

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Myelodysplastic Syndromes (MDS) are clonal disorders. However, whether the transforming event occurs in a myeloid committed cell or in earlier progenitor (stem cell?) it is still not ascertained. Evidence have been accumulated in both senses, but certainly, MDS initiating cells must be capable of sufficient repopulating capacity to perpetuate the disease. *Objectives.* To evaluate the repopulating ability of hypoxia selected cells in primary MDS bone marrow cultures and characterize the "stemness"of MDS maintaining cells. We evaluated 12 patients with different WHO subtypes of MDS (RCMD 5, RAEB-I 3, RAEB-II 3, 1 RA) . Mononuclear bone marrow cells were isolated after gradient centrifugation and grown in RPMI 1640 medium supplemented with 20% FBS and a cocktail of cytokines (TPO, FLT3-L, SCF,IL-3). Cells were incubated and selected in Ruskin Concept 400 anaerobic incubator, in severe hypoxia conditions by flushing with a performed gas mixture (0,3 % O2, 5%CO2, 95% N2). Cells were cultured under hypoxic conditions for 10-13 days (LC1), daily counted (Trypan blue) and then recovered from culture. The stem and progenitor cell potential of these cultures at different times of incubation was explored by transferring cells to growth-permissive secondary cultures in normoxia (LC2), with SCF, G-CSF, IL-6, IL-3, according to the Culture-Repopulating Ability assay methodology (Leukemia, 14:735-9, 2000). The phenotype of hypoxia selected cells was evaluated by determining the expression of CD34,CD38,CD117,CD133 and the frequency of early progenitor cells CD34posCD38negCD133 positive was compared to that present before hypoxic culture and to the Culture-Repopulating ability. Colony forming ability in semisolid medium was also evaluated in parallel in the presence of the same cytokine cocktail. The hypoxic culture system allowed selection of a minute cell population: in 12/12 cases viable cell number was decreased of one log after 10-13 days. In one case we observed, after hypoxia selection, a reduction of CD34 positive cells (0,15% against 9,44%). This population was enriched with CD 133 positive cells (91,8% against 83% before selection), and CD38positive cells were also increased (77% against 45%).Only 2/10 cases showed a significant repopulating ability at day 17 of LC2. In the other 10/10 cases, repopulating ability was apparently absent. Surprisingly, MDS cases presenting blasts in the bone marrow did not show more repopulating cells after selective hypoxic conditions. Although our results are preliminary, we demonstrate that it is possible to select by severe hypoxic conditions primary MDS progenitor cells endowed with repopulating ability. Further characterization (phenotypic and molecular) of these selected progenitors will allow a deeper insight into the biology of this heterogenous group of diseases.

0251

UNSUSPECTED CHROMOSOMAL LESIONS ARE REVEALED BY FISH IN KARYOTYPICALLY NORMAL MDS PATIENTS

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Background. In MDS the cytogenetic pattern is the most important parameter to predict overall survival (OS) and the risk of MDS/AML evolution, but 40-50% of patients are not informative being chromosomally normal. *Aims.* Our study evaluated whether probes derived from aCGH studies were truly able to unmask cryptic lesions in chromosomally normal MDS patients and influenced OS and disease evolution. *Methods.* The 73 chromosomally normal MDS patients analysed came to our observation in the period January 2005-December 2010. They were twenty-five females and forty-eight males, their median age was 64 years (range 22-77). According to WHO classification, 5 patients were classified as RARS, 27 as RA, 5 as RCMD, 22 as RAEB-1 and 14 as RAEB-2. Considering IPSS score, 24 patients were considered low-risk, 28 intermediate-1 risk and 15 intermediate-2 risk and 6 as high-risk. Median follow-up was 21 months (range 1-66). At the time of the study no patient has died. Overall, 9 transformed into a more advanced MDS subtype and 11 into AML. FISH probes were chosen based on the frequency of their involvement and their Mb position (UCSC genome browser on Human Mar. 2003 assembly). They were obtained from BACPAC Resources Center at C.H.O.R.I. (Oakland, USA), labelled and

applied as previously described. We used the following probes: RP11-912d8 (19q13.2); RP11-196p12 (17q11.2); RP11-269c4 (14q12); RP11-351o1 (10q21.3); RP11-144g6 (10q11.2); RP11-122a11 (7q34); RP11-951k18 (5q13.1); RP11-100m20 (4p14); RP11-544h14 (2q33). For i-FISH, cut-off values, obtained from the analysis of 300 nuclei from ten normal samples, were fixed at 10%. Results An abnormal FISH pattern was revealed in 26 patients (35.6%). A single defect was revealed in 19 patients (73.0%) and more than two defects in 7 (26.9). Sixteen patients (61.5%) presented a 19q13.2 deletion, 7 (26.9%) a deletion of band 14q13.2, 4 (15.4%) a deletion of band 17q11.2, 4 (15.4%) a deletion of band 5q13.1-q13.2, 4 (15.4%) a deletion of band 4p14, 3 (11.5%) a deletion of band 10q11.22, 3 (11.5%) a deletion of band 7q34, 2 (7.7%) a deletion of band 10q21.3 and one a deletion of band 2q33.1-q33.2. An abnormal FISH pattern was observed in 1/5 RARS, in 7/27 (25.9%) RA, in 3/5 RCMD, in 8/22 (36.4%) RAEB-1 and in 7/14 (50%) RAEB-2. Considering the IPSS, at least one defect was observed in 4/24 (16.6%) low-risk, in 12/28 (42.8%) intermediate-1 risk and in 7/15 (46.6%) intermediate-2 risk 3/6 (50%) high-risk patients. Disease evolution occurred in 2 RA patients, in 3 RAEB-1 and in 4 RAEB-2 patients with an abnormal FISH pattern. Seven of these patients presented at least two chromosomal deletions. In contrast, disease evolution occurred in one RARS, in two RA, 3 RAEB-1 and in 5 RAEB-2 with a normal FISH pattern. In conclusion our data suggest that FISH: i) reveals novel unsuspected chromosomal lesions in about 36% of chromosomally normal MDS patients; ii) these chromosomal lesions mostly consist in gains/losses, whereas balanced rearrangements are very rare; iii) an abnormal FISH pattern with more than two deletions seems to correlate with disease progression.

0252

NF-KB REGULATES FAS GENE EXPRESSION IN MYELODYSPLASTIC SYNDROMES

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Background. Overexpression of death receptor Fas and its ligand FasL, plays a major role in the activation of extrinsic pathway of apoptosis in MDS. TNF-a, a potent activator of the NFκB pathway, upregulates Fas in normal myeloid cells. FAS gene expression is controlled by NFκB in T cells and hepatocytes, while FasL is regulated by FOXO3A which nuclear translocation is inhibited by NFkB. *Aims.* This study aims at investigating the role of NFkB in the regulation of Fas and FasL in MDS/AML cells. *Methods.* Localization of p65 NFkB subunit by IF and WB on nuclear fractions, FAS transcript by RT-PCR and Fas and FasL proteins by flow cytometry were analyzed in P39 and HL-60 cell lines and in bone marrow-derived MDS/AML mononuclear (n=11) or CD34+ cells (n=3) maintained in culture with FLT3-L, TPO, IL-6, SCF. Binding of p65 NFkB subunit to the FAS promoter was evidenced *in vitro* by pull-down of p65-transfected 293T cells on 5'biotinylated-oligonucleotides mimicking consensus NFkB sequences of FAS promoter and *in vivo* by ChIP. NF-kB pathway was inhibited by the IKKa inhibitor BAY11-7082 (5 µM) or lentiviral expression of IkSR, a non-degradable form of IkBa. *Results.* The P39 cell line which exhibits a strong expression of the p65 NFkB subunit in the nucleus, express Fas at plasma membrane. Fas is inducible in HL-60 cells treated with TNF-a, which provoked p65 nuclear translocation. Apoptosis of MDS/AML CD45lo/CD34+ blasts is significantly lower than in earlyMDS. p65 is in the nucleus of 60 -100% MDS/AML mononuclear or CD34+ cells and this correlates with the lack of FasL and the expression of Fas at plasma membrane. *In vitro*, p65 is trapped on the 4 NFkB sequences of the FAS promoter by oligoprecipitation. *In vivo*, we demonstrate by ChIP that p65 is able to bind to the FAS promoter in myeloid cells. BAY11-7082 decreases nuclear expression of p65 NFkB subunit and expression of Fas transcript and protein in P39 cell line and MDS/AML mononuclear or CD34+ cells. In 4 MDS cases, lentiviral expression of IkSR in CD34+ cells both inhibits the nuclear expression of p65 and the expression of Fas in the GFP+ cell population corresponding to infected cells. *Conclusion.* NFkB pathway is implicated in the regulation of Fas expression in MDS/AML cells. Induction of Fas together with downregulation of FasL lead to the inhibition of Fas-dependent apoptosis, a mechanism by which NFkB may contribute to the progression of the disease.

MDS and other bone marrow failure syndromes - Clinical 1

0253

EVALUATION OF DYSPLASTIC FEATURES IN MYELODYSPLASTIC SYNDROMES: PROPOSAL FOR A STANDARDIZED MORPHOLOGICAL PANEL

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Background. WHO proposal for myelodysplastic syndrome (MDS) classification introduced uni- versus multilineage dysplasia as a diagnostic criterion in MDS with <5% bone marrow (BM) blasts, increasing the prognostic value of this classification. Cytopenias generally correspond to dysplasia, but discordance may be present. However, a structured and reproducible approach for the precise recognition of BM dysplasia is still missing, and the relationship between cytopenia and dysplasia needs to be clarified. *Aims*. The aims of this study were to identify a panel of reproducible morphological criteria associated with MDS useful for a correct application of WHO classification and to evaluate the prognostic relevance of the single morphological abnormalities and of the total lineage dysplasia as well as of the degree of dysplasia. *Methods*. We retrospectively examined the cytological features of BM smears from 429 MDS patients previously classified according to FAB criteria, 214 patients with hyporegenerative anemia and 74 healthy subjects. By counting 100 cells for the erythroid and granulocytic lineages and at least 20 megakaryocytes and classifying them for their dysplastic changes, a panel of dysplastic features showing a better sensitivity and specifity for MDS identification was developed. The morphological panel including 26 dysplastic features (12 erythroid, 8 granulocytic and 6 megakaryocytic) was employed, in association with the evaluation of blast and sideroblast percentages, to reclassify MDS patients by 2008 revised WHO proposal using the 10% threshold to record dysplasia in the erythroid and granulocytic lineages and the 25% threshold for dysmegakaryopoiesis, with a between-investigators and within-investigator agreement of 92% and 95% respectively. *Results*. Three hundred and one MDS cases were correctly reclassified, 45 were unclassifiable for inadequate BM smears and 83 belonged to other hematopoietic neoplasms. On univariate analysis, increase of BM blasts, multilineage dysplasia and two or more cytopenias were associated with worse outcome but multivariate analysis failed to confirm the prognostic value of cytopenias. In MDS without an increase of BM blasts, Kaplan Meier estimates of overall survival (OS) and leukemia-free survival (LFS) showed that all patients with multilineage dysplasia had a significantly worse outcome, independently of the number of cytopenias (P=0.03 and P=0.0005 respectively). Some morphological abnormalities, i.e. erythroblast irregular nuclear edges or multinuclearity, granulocyte hypo-agranularity, small binucleated megakaryocytes, were associated with poor outcome, and total granulocytic or megakaryocytic dysplasia showed a significant independent unfavorable prognostic value (P=0.0004 and P<0.0001 respectively). Also the degree of granulocytic or megakaryocytic dysplasia, estimated based on percentage of dysplastic cells, was found to have a significant effect on OS (P=0.002 and P=0.0003 respectively). *Conclusions*. The definition of BM dysplasia with a standardized morphological panel that improves the objectivity and reproducibility of microscopic analysis is useful for a correct application of WHO classification as well as for the differential diagnosis between MDS and other cytopenias. Our data confirm the significance of multilineage dysplasia correlation with high-grade MDS. Prognostic systems including the evaluation of the degree of BM dysplasia should be adopted for clinical decision-making and selection of MDS patients for new effective targeted therapies.

0254

LONG TERM OUTCOMES IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) WITH SUSTAINED ECULIZUMAB TREATMENT

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Background. PNH is a chronic, life-threatening disease associated with increased risk of thrombosis (TE), end organ damage, often poor quality of life, with premature mortality. TE accounts for 40-67% of PNH-related-deaths; anticoagulation (AC) management may not be effective in PNH, as TE rates in AC-treated PNH patients remain elevated. The complement inhibitor eculizumab reduces chronic hemolysis rapidly and significantly; it also leads to reductions in TE events pulmonary hypertension and improvements in chronic kidney disease (CKD) and quality of life. *Methods.* All patients (N=195) in the PNH eculizumab clinical trials and subsequent Extension studies were evaluated for safety, sustained outcomes, and patient survival. Median age was 40 yrs, 54% female, 29% with history of aplastic anemia and 1.5% with history of myelodysplasia. TE was reported in 32% (63/195) of patients prior to eculizumab treatment. *Results.* Median eculizumab treatment duration was 29 mo (1-66;IQR:23-32m); total eculizumab exposure was 467.1 patient-years. LDH was rapidly and significantly reduced from baseline of 2,293U/L (~10x ULN) to 310U/L post 1 month treatment (P<0.0001) and was sustained through 36 months (P<0.0001). TEs were significantly reduced from 52 pre-treatment to 10 trial events by matched-time analysis (P<0.0005). Of the 7/195 patients who experienced a TE, 5 had history of TE and 2 were concomitantly treated with AC. Of patients treated with AC, 58/98 experienced at least 1 TE prior to treatment. In 11 patients who discontinued AC while on eculizumab, no TEs were reported during or following AC discontinuation. Chronic Kidney Disease (CKD) was reduced from 69% at baseline to 31% (n=29) 36 months post-treatment. Significant increases in hemoglobin were sustained over 36 months treatment (mean increase over baseline at 36 months: 9.5g/L; P<0.0001; range: -31,68), despite significant and sustained reductions in transfusion requirements. Of the 87/195 patients receiving at least 36 months of eculizumab, 29% (25/87) became transfusion independent and maintained transfusion independence for the entire treatment period. Eculizumab was well tolerated; 90% (175/195) of patients completed the parent and extension trials. Twenty patients (10%) did not complete the trial including 9 patients following a reported adverse event (AE). In 16 week follow-up, TE was reported in 3 of these 20 patients, including 1 death. Most AEs (91%) were mild or moderate in severity. There were 2 cases of meningococcal sepsis; both were successfully treated without sequelae. There were 4 patient deaths: 3 not related and 1 possibly related to eculizumab, per investigator. Kaplan-Meier analysis (fig.1) showed probability of overall survival was 97.64% at 3 years and was maintained through 5.5 years of ongoing treatment. Patient survival compares favorably to a predicted survival rate previously reported in historical controls of 65% at 5 years. *Conclusion.* Long term reduction of chronic hemolysis in PNH patients treated with eculizumab is associated with significant improvements in the incidence of TE, CKD, and other PNH-associated symptoms. Long term treatment with eculizumab also results in a high probability of survival, which is maintained over 5.5 years of ongoing treatment.

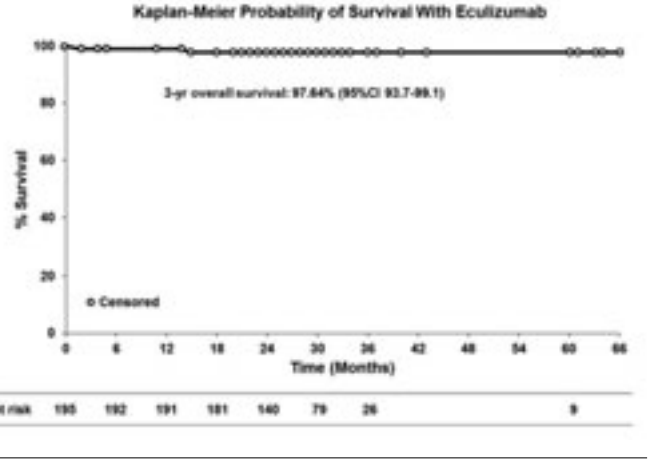

Figure 1.

0255

ALEMTUZUMAB FOR APLASTIC ANEMIA AND RELATED IMMUNE-MEDIATED BONE MARROW FAILURES: LONG-TERM FOLLOW UP OF A PILOT STUDY

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Background. Immunosuppression (IS) is a worthy treatment option for patients suffering from aplastic anemia (AA) or other lineage-specific immune-mediated marrow failure, such as pure red or white cell aplasias (PRCA and PWCA). *Aims.* We have previously described results from a pilot phase II prospective trial (NCT00895739) investigating the anti-CD52 monoclonal antibody alemtuzumab in combination with low-dose cyclosporine A (CyA; Risitano, BJH 2010;148:791). Here we report the long-term follow up of the study. *Methods.* Twenty-eight patients were enrolled in the study: 13 SAA, 13 PRCA and 2 PWCA; 15 (6 SAA, 9 PRCA and 1 PWCA) had not received any previous IS. Median age was 51 years (range 25-87). The patients received alemtuzumab as subcutaneous injection of 3-10-30-30-(30) mg (total dose 103 mg for SAA, 73 mg for PRCA and PWCA), in consecutive days, followed by oral CyA 1 mg/kg). Given the major concerns about infectious risk, all patients received adequate prophylaxis, including valganciclovir (for 3 months) and bactrim. *Results.* All patients completed the treatment without serious adverse events; lymphocyte depletion was immediate and complete in all patients, with delayed immune reconstitution (especially for CD4+ T-cells). With a median follow up of 33 months, infectious events were infrequent and clinically mild, with exception of 1 fatal sepsis and 1 progressive multifocal leukoencephalopathy (PML, occurring in a PRCA patient relapsed with metastatic thymic carcinoma, during salvage chemotherapy). No CMV disease nor EBV-related disease or lymphoproliferative disorder was observed, even if 5 patients developed asymptomatic CMV reactivation (promptly cleared by pre-emptive valganciclovir). The response rates were 77% (38.5% CR) in AA and 84.5% (61.5% CR) in PRCA patients; both PWCA achieved long-lasting CR. Current stable remission were achieved in 38.5% of AA and 23% of PRCA; the majority of long-term responders have received an additional dose of alemtuzumab to sustain the hematological response. Long-term treatment failures were due to refractory relapses (15% for AA and 7.5% for PRCA) or to clonal evolution (15% for AA, both myelodysplastic syndromes with chromosome 7 abnormalities, and 23% for PRCA, with 2 acute megakaryoblastic leukemia and 1 5q- syndrome). Overall survival in AA was 72%, even due to effective salvage therapies; all deaths were due to refractory disease. Unexpectedly, overall survival was only 20% in PRCA; the causes of death were disease evolution (3 leukemias and 1 AA), PRCA-associated morbidities (1 thymoma complicated with PML, 1 refractory connective tissue disease) or unrelated cardiovascular comorbidities. *Conclusions.* Long-term follow up of patients treated with alemtuzumab confirms that this agent has a remarkable efficacy for the treatment of immune-mediated bone marrow failures, with response rates comparable to that of standard IS. Treatment-related long-term toxicity was acceptable, with a low risk of infectious complications; as with standard IS, late treatment failures were mainly due to relapse (alternative maintenance strategies might be considered) or clonal evolution. Even in consideration of the recent data showing the inferiority of rabbit ATG (Scheinberg, ASH 2010;116:LBA-4), our results suggest that alemtuzumab is a worthy alternative to standard IST by horse ATG, possibly deserving appropriate head-to-head comparison.

0256

PROGNOSTIC VALUE OF IRON PARAMETERS AT DIAGNOSIS IN MYELODYSPLASTIC SYNDROME PATIENTS: ANALYSIS OF 643 PATIENTS FROM THE PIEDMONT MDS REGISTRY

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Background. The role of serum ferritin (SF) value as prognostic factor in patients affected by myelodysplastic syndrome (MDS) is still controversial. Increased SF levels mainly due to transfusion requirement has been associated with lower overall survival (OS) (Malcovati, 2007) and increased risk of leukemic transformation (Sanz, ASH 2008; de Swart ASH 2010). Conversely, Park and colleagues (ASH 2010) failed to identify a negative prognostic value of SF higher than 300 ng/mL at baseline in a cohort of low risk untransfused MDS patients. Little is known about the impact on survival of other iron parameters such as transferrin saturation (TS). *Aims.* We evaluated the prognostic significance of SF and TS at diagnosis in MDS patients analyzing data collected in the MDS Piedmont Registry. *Methods.* Data from 1558 patients from 37 centres included from 1999 to 2010 were analyzed. We evaluated OS according to iron parameters including the following variables: haemoglobin (Hb), neutrophils and platelets counts, IPSS, serum EPO (sEPO), C reactive protein (CRP), comorbidities, age, cytogenetic risk, transfusion dependence (TD), first line treatment. *Results.* SF at diagnosis was available in 643 patients (274 females, 369 males), who are the object of the present analysis. Median age was 74 (range 25-106). WHO classification was as follows: 177 RA (with 16 of them classified as 5q- syndrome), 46 RARS, 96 RAEB-1, 148 RCMD, 49 MDS-U, 1 atypic cronic myeloid leukemia, 86 RAEB-2. In addition 30 CMML and 10 RAEB-t registered before 2003 according to FAB classification were included. IPSS stratification was as follows: 15% low risk, 47% Int-1, 15% Int-2 and 23% high risk. Median Hb value was 9,5 g/dL (range 2,8-17; mean 9,8), median SF was 282 ng/mL (range 1-3496; mean 396), median TS was 36% (range 2,1-100; mean 43,5). In univariate analysis, SF >500 ng/mL was positively associated with increased TS (>45%) (p=0,001) and with male gender. Furthermore, increased TS was positively associated with increased sEPO (p=0,004) but negatively with CRP levels. OS by Kaplan-Meyer method was analyzed according to basal SF and TS values in the whole population. No statistically significant difference was found in OS of MDS patients according to SF lower or higher than 500 ng/mL also in the subgroup of non TD patients. Conversely, patients with TS <45% showed worse outcome in the whole population (p= 0,007) and in the non TD group (p=0,014). The prognostic significance of TS is confirmed in the group non TD low and Int-1 IPSS patients: median OS of 40 vs. 53 months (p=0,02). *Conclusions.* In our cohort of 643 patients from the MDS Piedmont Registry, increased SF >500 ng/mL at baseline does not impact on OS, conversely transferring saturation lower than 45% is strongly associated with worse OS in low risk non TD patients. Further evaluations of comorbidities, chronic inflammation and oxidative stress parameters should be evaluated in order to clarify the meaning of our findings.

0257

VALIDATION OF A FLOW CYTOMETRIC SCORING SYSTEM FOR THE PROGNOSTICATION OF THE MYELODYSPLASTIC SYNDROMES; A RETROSPECTIVE COHORT STUDY

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The International Prognostic Scoring System (IPSS) and WHO-based prognostic scoring system provide prognostic information in myelodysplastic syndromes (MDS). However, even if patients are allocated in the same risk category their clinical course remains heterogeneous. Previous studies demonstrated that flow cytometry (FC) and specifically a flow cytometric scoring system (FCSS) is emerging as a valuable technique for predicting outcome in MDS. The FCSS is a scoring system that allows for a numerical display of immunophenotypic aberrancies in the (im)mature myelo-monocytic lineage (Wells, Blood 2003). Scores are generated by enumerating abnormalities; high scores reflect high number of aberrancies. The current study aimed to validate the FCSS for identification of prognostic subgroups in MDS. We analyzed aberrancies in (im)mature myelo-monocytic cells by FC in BM of 107 MDS patients, including 48 MDS patients from a previous cohort. The diagnoses according to WHO 2001 classification were RA(RS) n=20, RCMD(RS) n=60, RAEB-1 n=14, RAEB-2 n=13 and age-matched healthy volunteers (n=39) were included. The FCSS in RA(RS) (median=3, range 1-6) patients was significantly higher compared with healthy controls (median=1, range 0-2, p<0.001). In contrast to our previous results the FCSS for RA(RS) and RCMD(RS) patients did not differ. This is a remarkable finding, since by morphology RA(RS) patients show only unilineage dysplasia, in contrast to flow cytometric findings, where 85% of the RA(RS) patients had two or more aberrancies in the (im)mature myelomonocytic compartment. Patients with a high FCSS experienced a significantly worse overall survival (OS) compared with patients with an intermediate FCSS (p=0.001, median OS 28 and 89 months, respectively). Remarkably, the FCSS was not correlated with IPSS cytogenetic risk categories low, intermediate and poor. However, within the good risk category according to IPSS, the FCSS identified different prognostic subgroups. Patients with good cytogenetics and a high FCSS have a worse OS compared to those with intermediate FCSS (p=0.001, median OS 39 and 164 months, respectively). Transfusion data was available in 77 patients. Interestingly, the majority of MDS patients who were transfusion dependent or progressed into AML, had aberrant expression of CD5, CD7 and/or CD56 on myeloid progenitors compared with patients without aberrant marker expression (50% vs 24%, respectively; p=0.012). In conclusion, the FCSS and detection of aberrant myeloid progenitors can provide refined prognostication within currently used classification and prognostication systems. The FCSS can identify patients at risk for transfusion dependency and adverse clinical outcome, independent of current classification systems.

0258

FMNL1 EXPRESSION IN CELLS OF MDS PATIENTS AND THEIR CD4:CD8 T-CELL RATIOS

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Background. Myelodysplastic syndromes (MDS) are a heterogeneous group of disorders characterized by impaired peripheral blood cell production and a tendency to develop leukemia. Evidences have shown that T-cell mediated marrow suppression is the cause of the cytopenia in approximately 20-30% of MDS patients. Human FMNL1 belongs to a conserved family of formin-related proteins, and is restrictedly expressed in hematopoietic cells, including lymphocytes, and overexpressed in chronic lymphocytic leukemia and lymphoma samples as well as in malignant cell lines. Moreover, FMNL1 participates in the regulation of the cytoskeleton in activated T lymphocytes and in the control of the polarization of centromeres, required for the cytotoxic function of these cells. *Aims.* Characterize *FMNL1* expression in peripheral blood $CD3^+$ lymphocytes and in total bone marrow cells of patients with MDS and normal donors; compare *FMNL1* expression levels among low-risk, high-risk MDS (according to FAB classification and number of cytopenias) and normal donor cells; correlate the CD4:CD8 T-cell ratios of patients with the three disease classifications (FAB, WHO and IPSS) and number of cytopenias. *Methods.* A total of seventy-one patients with a diagnosis of MDS, receiving no treatment, were included in the study; twenty-two samples from normal donors were used as controls. Patients were grouped into low-risk and high-risk disease, according to FAB, WHO, IPSS and number of cytopenias (Table 1). This study was approved by the National Ethical Committee Board, with the signed informed consent of all patients. *FMNL1* expression levels from $CD3^+$ cells (obtained by Ficoll-Hypaque followed by magnetic selection) or total bone marrow cells were determined by quantitative PCR (q-PCR). $CD3^+$ cell counts and CD4:CD8 ratio was determined by flow cytometry. *Results. FMNL1* expression was significantly higher in MDS $CD3^+$ peripheral lymphocytes when compared with normal donor cells (P=0.01), although there was no change in $CD3^+$ cell number between those two groups. In the bone marrow samples, *FMNL1* expression was higher in low-risk compared to high-risk MDS according to FAB (P=0.07) and number of cytopenias (P=0.01). CD4:CD8 T-cell ratios were significantly higher in high-risk MDS group when compared to normal donor cells according to FAB, WHO and IPSS classification (P<0.01), and this is due to a tendency of decrease in the number of $CD8^+$ cells in the peripheral lymphocyte pool of those patients. Interestingly, we observed a higher CD4:CD8 ratio in MDS high-risk group when compared with the low-risk group, based on

Table 1.

Patient characteristics

	Low Risk (BM/CD3+)	High Risk (BM/CD3+)
FAB	RA (18/22) RARS (04/04)	RAEB (11/06) RAEBt (06/00)
WHO	RCUD (05/07) RARS (01/02) RCMD (15/16)	RAEB-1 (09/05) RAEB-2 (06/02) AML with myelodysplasia-related changes* (3/0)
IPSS	Low (09/14) Int-1 (20/15)	Int-2 (08/02) High (02/00)
Cytopenia	0 (04/05) 1 (13/11)	2 (14/14) 3 (07/02)

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.

the number of cytopenias (although not statistically significant). *Conclusions.* During the early stages of MDS, one mechanism contributing to hypercellular marrow and peripheral blood cytopenia is the significant increase of apoptosis in haematopoietic cells. The higher expression of *FMNL1* in MDS $CD3^+$ lymphocytes and bone marrow cells may be related to clonal or oligo-clonal T cell activation, since FMNL1 is important for the cytotoxic function of these cells. The CD4:CD8 imbalance could reflect an alteration in the immune regulation, which could contribute to the cytopenia in some MDS patients. Further studies are required to test these hypotheses. Supported by FAPESP, CNPq and INCT do Sangue.

0259

THE PRESENCE OF ABERRANT MYELOID PROGENITORS PREDICTS OVERALL SURVIVAL IN INTERMEDIATE-2 AND HIGH RISK MYELODYSPLASTIC SYNDROMES UPON TREATMENT WITH AZACITIDINE

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One of the major challenges in MDS is the prognostication and selection of the most suitable therapeutic options. Flow cytometric analysis of bone marrow cells in low and int-1 risk MDS patients can identify distinct subgroups within validated risk groups and predict response to growth factor treatment. (Westers *et al*., Blood 2010) In this study we investigate the predictive value of flow cytometry (FC) for prognosis and selection of int-2 and high risk MDS patients for response to azacitidine. Bone marrow aspirates were analyzed by FC in 25 constitutive MDS patients before treatment and after every third cycle of azacitidine. A flow score was calculated using the flow cytometric scoring system (FCSS; Wells *et al*., Blood 2003). The FCSS is a scoring system that allows for a numerical display of immunophenotypic aberrancies in (im)mature myelo-monocytic cells (0-1 no flow cytometric dysplasia, 2-3 mild dysplasia, ≥4 severe dysplasia); e.g. high scores (≥4) reflect a high number of aberrancies. Response was evaluated using IWG-2006 criteria. The WHO 2008 categories were 2 RCMD, 3 RAEB-1, 9 RAEB-2, 7 AML with multilineage dysplasia and 4 CMML. Flow cytometric follow up was available in 17 patients. Median follow up time after initiation of the first cycle was 9.6 months. Four patients achieved complete remission (CR), 7 stable disease (SD) and 6 had progressive disease (PD). The median FCSS at baseline was 7 (range: 3-8). Patients who achieved CR showed a significant decrease in the FCSS after 3 cycles as compared to patients with SD and PD (median FCSS 1.5, 5 (p=0.009) and 6.5 (p=0.003), respectively). At baseline, 16/25 patients had aberrant marker expression (AME) (i.e. CD5, CD7, CD11b and/or CD56) on myeloid progenitors and/or monocytes. These patients had significantly worse overall survival as compared to patients without AME (p=0.004; relative risk (RR) of death in patients with AME was 6 times higher than without AME). Absence of AME in patients with SD was strongly associated with erythroid response (HI-E). Our data indicate that patients with SD and AME are less likely to show HI-E upon azacitidine treatment (RR=4). In conclusion, persistent high FCSS during treatment and/or presence of AME at baseline is of prognostic value and identifies int-2 and high risk MDS patients who are unlikely to achieve CR or HI-E and with worse overall survival as compared to patients without AME.

0260

CLINICAL CHARACTERISTICS OF THERAPY-RELATED MYELODYSPLASTIC SYNDROME

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We identified 438 patients who have had one or more malignancies prior to the diagnosis of MDS evaluated at MDACC between 1997 and 2007. To select therapy-related myelodysplastic syndrome (Tx-MDS), we removed patients who have diagnosed as MDS by FAB classification but have not included in WHO classification, and we selected patients who have undergone chemotherapy (CTx) and/or radiation therapy (RTx) for prior malignancies (Fig 1). Finally 281 patients were included for the analysis of Tx-MDS. Male sex was 165 (58.7%) and median age at diagnosis of MDS was 66.2 years (range 13.4-89.4). Patients were classified by WHO classification as follows: RA (n=63, 22.4%), RARS (n=29, 10.3%), RCMD/RCMD-RS (22, 7.9%), RAEB-1 (n=90, 32.0%), RAEB-2 (n=67, 23.8%), MDSu (n=10, 3.6%). IPSS was low in 30 (11%) patients, INT-1 in 87 (31.0%) patients, INT-2 in 120 (42.7%) patients and high in 35 (12.5%) patients. The most common cytogenetic abnormality was -5 and/or -7 (n=149, 53.1%). Seventy five patients (26.7%) were diploid. Prior cancers included: head and neck (n=7, 2.5%), thyroid (n=3, 1.1%), lung (n=7, 2.5%), breast (n=32, 11.4%), gastrointestinal (n=13, 4.6%), prostate (n=34, 12.1%), other genitourinary or gynecological (n=16, 5.7%), melanoma/skin cancers (n=5, 1.8%), sarcomas (n=8, 2.8%), other solid cancers (n=2, 0.7%), lymphoma (n=102, 36.3%), CML/CLL (n=6, 2.1%), AML/ALL (n=5, 1.8%), multiple myeloma (n=11, 3.9%) and multiple cancers (n=30, 10.7%). Prior Tx was CTx only (n=107, 38.1%), RTx only (n=73, 26.0%) or both CTx and RTx (n=101, 35.9%). The treatments of Tx-MDS were categorized as follows: supportive care/cytokine therapy in 126 (44.8%); non-cytotoxic drugs in 77 (27.4%); cytotoxic chemotherapy in 65 (23.1%); hematopoietic stem cell transplantation (HSCT) in 13 (4.6%). A total of 54 patients had received HSCT (autologous, n=52 (18.5%); allogeneic, n=2 (0.7%)). Univariate analyses for survival revealed that presence of hepatomeglay (no hepatomegaly vs. hepatomegaly; p=0.023), cytogenetics (8+, 20q-, Y-, normal vs. others; p<0.001), types of MDS by WHO classification (RA, RCMD, MDSu vs.

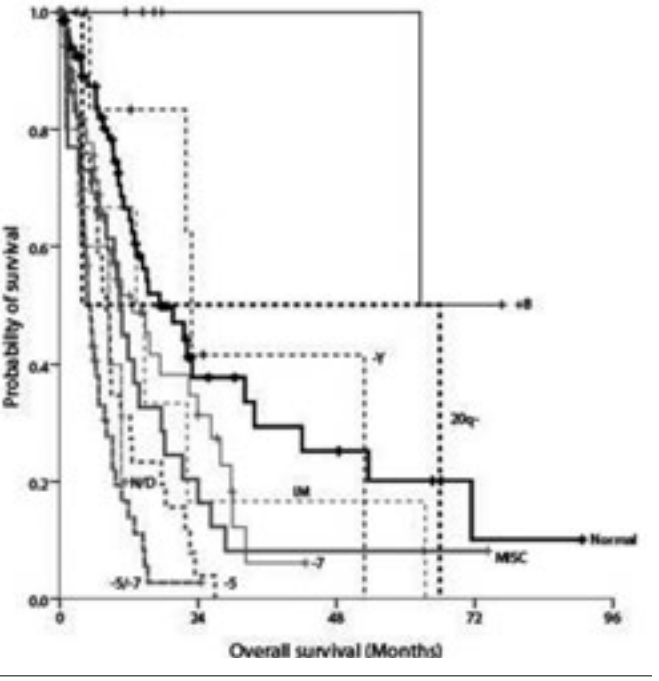

Figure 1. Overall survival by chromosomal abnormalities.

others; p<0.001), time from Tx to MDS (≤5 vs. >5Y; p=0.027), number of line(s) of therapy (1 vs. ≥2; p=0.011), serum albumin (≥4 vs. <4g/dL; p=0.005), serum beta2 microglobulin (≤3 vs. >3mg/L; p=0.015), serum creatitine (≤1 vs. >1mg/dL; p=0.061), ECOG performance status (0-1 vs. ≥2; p<0.001) as significant. Age (≤65 vs. >65; p=0.109), sex (male vs. female; p=0.862), prior Tx (CTx vs. RTx only; p=0.471), prior malignancies (hematological vs. solid cancer; p=0.650), prior lymphoma (lymphoma vs. non-lymphoma; p=0.958), prior HSCT (ASCT vs. alloHSCT vs. none; p=0.691) and serum ferritin level (≤600 vs. >600ng/mL; p=0.420) were not significant. The events of leukemic evolution were not consistent with the risk groups: 3 (10.1%) in low, 12 (13.5%) in INT-1, 9 (8.3%) in INT-2 and 9 (25.0%) in high by IPSS. High risk group in IPSS showed high possibility of leukemic evolution (p=0.039).

0261

CYTOGENETIC CHARACTERISTIC OF MYELODYSPLASTIC SYNDROME IN HUMAN IMMUNODEFICIENCY VIRUS INFFECTED PATIENTS: HIGH INCIDENCE OF POOR PROGNOSTIC KARYOTYPE AND CHROMOSOME 7 ABNORMALITIES

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Background. Human Immunodeficiency Virus (HIV) infection can often cause myelodysplastic features in bone marrow. However bona fide myelodysplastic syndrome (MDS) is not common in this population. Data are lacking whether HIV associated MDS has distinct clinical features from non-HIV MDS. *Aims.* The aim of this study was to compare the clinical characteristics of HIV associated MDS and non-HIV MDS. *Methods.* This is the retrospective cohort study with 91 patients who were diagnosed with MDS in our allied three teaching hospitals in New York City from 2005 to 2010. Their clinical history, pathological data, cytogenetic studies, and laboratory data were obtained through electronic medical records. HIV diagnosis was confirmed based on serological testing of enzyme-linked immunosorbent assay (ELISA) with Western Blot confirmation as well as HIV-1/2 viral PCR. MDS diagnosis was confirmed with bone marrow morphology, laboratory data and cytogenetic studies and was classified both according to the French-American-British (FAB) classification and World Health Organization (WHO) criteria. Research protocol was approved by our Institutional Review Board. *Results.* Within the cohort of 91 patients with MDS, 9 patients carried diagnosis of HIV and 82 patients were non-HIV. Karyotype abnormalities were more associated with HIV related MDS (88% vs 39% p <0.01). Additionally, poor prognostic karyotype abnormalities were more associated with HIV related MDS according to the International Prognostic Scoring System (IPSS) (66% vs 16%, p<0.01). Number 7 chromosome abnormalities which are also considered to be poor prognostic marker, was highly involved in HIV related MDS cohort (77% vs 15.6%, p<0.01). Within the cases of HIV related MDS, 8 out of 9 patients had a follow up data. Their median survival after diagnosis of MDS was 9.3 months and rate of transformation to acute leukemia was 62.5% (n=5). Median survival after transformation was 3.6 months. *Conclusions.* HIV related MDS has higher incidence of poor prognostic karyotype abnormalities compared to non-HIV MDS cohort and has high rate of transformation and poor survival. This result suggests that HIV infection itself, antiretroviral therapy, or immunodeficiency state might be associated with clonal mutagenesis in bone marrow.

0262

THE QUANTITY OF P15INK4B METHYLATION IS LOWER IN PEDIATRIC MDS THAN IN ADULTS, BUT CORRELATES WITH BONE MARROW BLAST PERCENTAGE AND SURVIVAL

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Background. The inactivation by promoter hypermethylation of *p15INK4b* is believed to contribute to the initiation and progression of MDS in adults. Contrary to demonstrated benefits of demethylating agent in adults, the use of demethylating agent has not been reported for childhood MDS. *Aims.* To evaluate the potential of the use of demethylating agent in childhood MDS, we performed a quantitative analysis on *p15INK4b* promoter methylation. *Methods.* The study included 41 childhood MDS (22 RCC, 14 RAEB and 5 JMMoL) and12 healthy bone marrow donors as controls. Pyrosequencing was performed using PSQ96MA system (Biotage, Uppsala, Sweden). The mean % of methylated cytosine (methylation level: MtL) in childhood MDS was compared with that of adult MDS in the previous study. *Results.* The MtL of *p15INK4b* was lower in children than in adults both in control group (1.62% vs. 4.30%, p=0.001) and in MDS group (2.57% vs. 8.76%, p=0.001). The childhood MDS patients with >5% BM blasts showed higher MtL than those with <5% BM blasts (3.28% vs. 1.92%, p=0.098). MtL>2.50% was a poor prognostic factor in childhood MDS (p=0.075, univariate analysis). *Conclusions.* To our knowledge, this is the first study which quantitatively analyzed *p15INK4b* methylation in childhood MDS. In conclusion, the methylation quantity of p15INK4b of children is lower than that of adults both in controls and in MDS patients. The methylation quantity of *p15INK4b* of children MDS is higher than that of normals. The high methylation quantity of p15INK4b is associated with BM blasts and shorter mean survival in childhood MDS.

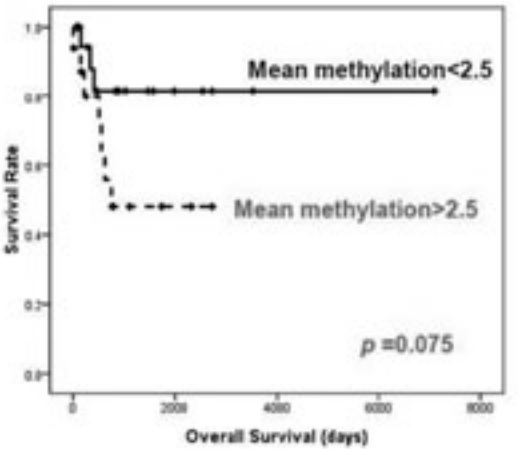

Figure 1. Prognostic impact of p15INK4b methylation.

0263

COMPARABLE OUTCOME OF IMMUNOSUPPRESSIVE THERAPY WITH RABBIT ANTI-THYMOCYTE GLOBULIN (ATG) PLUS CYCLOSPORINE A (CSA) TO ONE WITH HORSE ATG PLUS CSA IN REFRACTORY CYTOPENIA IN CHILDHOOD

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Background. Refractory cytopenia in childhood (RCC) is the most common subtype of pediatric myelodysplastic syndrome (MDS). In

addition to allogeneic hematopoietic stem cell transplantation (HSCT), immunosuppressive therapy (IST) has recently been introduced as a treatment modality. *Aims.* Because horse anti-thymocyte globulin (h-ATG) is currently not available in Europe, rabbit ATG (r-ATG) is widely used. However, the information on the efficacy of rabbit ATG in IST is limited. Here we report the results of IST with ATG and cyclosporine A (CSA) in patients (pts) with RCC, comparing r-ATG and h-ATG. *Patients.* Ninety-one pts (52 boys /39 girls) with RCC were given IST as first line treatment between 1999 and 2009. At diagnosis, the median age was 9.8 (1.2-18.1) years. The median absolute neutrophil count (ANC) was 315 (0-3038) x109/L. 77 pts were transfusions dependent for platelets and 66 for red cells. Bone marrow cellularity was low in 78 pts and normo/hypercellular in 8 pts. Cytogenetic analysis revealed a normal karyotype in 49 pts, an abnormal clone in 3 pts and no result in 39 pts. IST was started at a median of 67 (0-472) days after diagnosis. Fifty-four pts was given Thymoglobulin® (r-ATG group), 33 pts Lymphoglobulin® (h-ATG group), and 4 pts others. There were no statistical differences in patient characteristics listed above between r- ATG and h-ATG groups. Since 2007 h-ATG has been replaced by r-ATG. *Results.* 57 (63 %) pts responded to IST at 6 months (complete response =normal blood count (CR): n=6, partial response (PR): n=51), with no difference between r-ATG and h-ATG (57.6% vs. 68.5%, respectively, p=n.s.). There were also no differences in response rate according to age, days between diagnosis and IST, and ANC at IST. Clonal evolution occurred in 6 pts (5 pts in r-ATG-, 1 pt in h-ATG-groups; cumulative incidence at 5 years =10.2%), 3/6 pts developed -7 or 7q- aberrations Seven responders experienced recurrent disease (2 pts: r-ATG, 5 pt: h-ATG groups). One child (h-ATG) developed clinical PNH. Thirty-five pts received HSCT as the second line therapy. The overall survivals (OS) were 85.1% in r-ATG group (at 3 yrs) and 91.2% in h-ATG group (at 5 yrs), and the failure free survivals (FFS) were 41.2% in r-ATG group (at 3 yrs) and 43.6 in h-ATG group (at 5 yrs) (p=n.s.), respectively. The median follow-up after ATG was 510 days (105-1095) in r-ATG group and 1476 days (14-3672) in h-ATG group. *Summary.* In this selected patient population with RCC, about 60% of pts responded to IST. This finding suggests that the immune system plays the key role in the pathophysiology of bone marrow failure in some pts with RCC. There was no difference in response rate between r-ATG and h-ATG groups. Although the follow-up time is shorter in the r-ATG group, there were also no differences in OS and FFS between 2 groups. These results justify the further application of r-ATG in the treatment of RCC.

0264

THE QUANTITY OF CLONAL CELLS DETECTED BY CONVENTIONAL CYTOGENETIC ANALYSIS CORRELATES WITH BONE MARROW BLASTS AND LEUKEMIA FREE SURVIVAL IN MYELODYSPLASTIC SYNDROMES

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Backgrounds. Most researches studied clonal chromosomal abnormalities in MDS focusing on the relationship between the presence or absence of abnormality of certain chromosome and the prognosis of MDS. However, little has been known about the utility of the quantitative result of clonality studies in clinical practice. *Aims.* This study investigated the novel roles of CCA and FISH results in predicting outcome of MDS patients. The results of CCA and FISH were compared both in qualitative and in quantitative aspects. We analyzed the prognostic significance of the quantity of clonal cells in CCA or FISH in MDS. *Methods.* We performed the quantitative and qualitative analyses of conventional cytogenetic analysis (CCA) and interphase FISH (iFISH) results in 129 MDS patients, and investigated their unknown roles in predicting prognosis. *Results.* The abnormalities of -5/5q, -7/7q, +8, -20q and +1q were detected in 13.2%, 14.0%, 19.4%, 7.0% and 7.8%, respectively. iFISH detected occult abnormalities in 18.6% (24/129), changing IPSS grouping in 4.9% (6/129). The proportion of clonal cells for each chromosome of CCA did not correlate with the result of iFISH (Δ, from 0.580 to 0.778). The clonal cell percentage in CCA was higher in patients with >5% bone marrow blasts than those with <5% (37.4% vs. 24.4%, p=0.066). Multivariate analysis showed that high proportion of clonal cells in CCA analysis is an independent prognostic factor for progression free survival into AML in MDS (p=0.059). *Conclusions.* iFISH is advantageous in identifying cryptic cytogenetic abnormalities, and can change the IPSS risk grouping. We showed the quantity of clonal cells detected in CCA correlates with the bone marrow blast percentage, and the progression free survival into AML, suggesting the novel diagnostic utility of CCA in MDS.

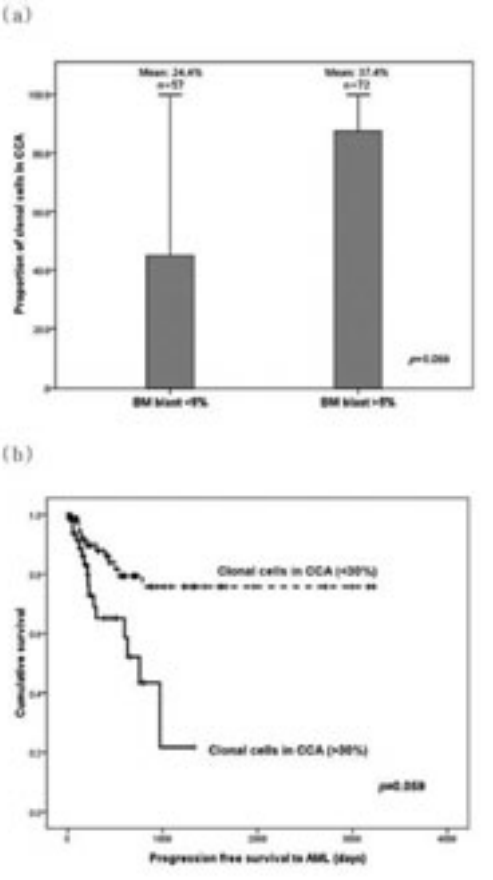

Figure 1. Prognostic impact of the proportion of clonal cell.

0265

ALLOGENEIC STEM-CELL TRANSPLANTATION IN PATIENTS WITH MYELODYSPLASTIC SYNROME; REVISITING THE NEED FOR PRE-TRANSPLANT INDUCTION THERAPY

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Background. Allogeneic stem-cell transplantation (SCT) is potentially curative therapy for myelodysplastic syndrome (MDS). SCT in MDS patients who are often elderly and heavily transfused may be associated with high incidence of non-relapse mortality (NRM). Reduced-intensity conditioning (RIC) may reduced NRM, but may be associated with increased relapse rate, such that the optimal conditioning regimen is unknown. Similarly, the need for induction chemotherapy prior to SCT is controversial. *Aims.* To determine the conditioning regimen with best outcome in previously untreated MDS. *Methods.* We retrospectively analyzed SCT outcomes in 82 patients with MDS given allogeneic SCT in a single institution over 11 years. Patients were not given any prior therapy for remission induction. Younger patients in good medical condition were given standard myeloablative conditioning (MAC) consisting of busulfan and cyclophospahmide. Older patients in good medical condition or patients with relative contraindications for MAC were given reduced-toxicity myeloablative conditioning (RTC) consisting of fludarabine and myeloablative doses of busulfan (total 12.8 mg/kg) or treosulfan (36 gr/m2). Patients considered to have absolute contraindication for MAC were given RIC consisting of fludarabine and reduced-dose busufan (6.4 mg/kg). *Results.* Median patient age was 58 years (21-73). The donor was matched sibling (n=42) or matched unrelated (n=40). The conditioning regimen was MAC (n=14), RTC (n=52) or RIC (n=16). IPSS score at SCT was intermediate-1 (n=29), intermediate-2 (n=33) and high (n=20). Thirty-nine patients had >10% marrow blasts at SCT and 23 had poor-risk cytogenetics. With median follow-up of 25 months (1 month-11 years), 37 patients are alive; 27 died of NRM and 18 of relapse. The estimated 5-year overall survival (OS) was 38% (95CI, 22-47). The cumulative incidence of NRM and relapse mortality was 35% and 27%, respectively. 5-yr OS was 29%, 49% and 18% after MAC, RTC and RIC,

respectively (p=0.04 for RTC vs. others). Multivariate analysis identified RTC as a favorable prognostic factor, HR 0.4 (0.2-0.9, p=0.04). Age, gender, donor type, IPSS, blast excess and cytogenetics were not predictive. MAC and unrelated donor were predicting factors for NRM with HR 2.3 (p=0.06) and 2.4 (p=0.05), respectively. NRM was 50% after MAC vs. 39% and 30 after RIC and RTC. RIC predicted for increased relapse risk, 44% Vs 22% in the other regimens, HR 2.8 (p=0.06). We compared these outcomes to a separate group of 39 patients with AML that was secondary to MDS who were given induction chemotherapy and transplanted after achieving CR1. 5-year OS after SCT was 51% (95CI, 33-68). *Conclusions.* RTC is associated with optimal results in untreated MDS. MAC may be associated with excessive NRM, while RIC may result in excessive relapse rate. Although not a direct comparison, patients with secondary AML transplanted in CR1 had similar outcome as MDS patients (including with excess blasts) transplanted upfront with RTC. Considering that only a fraction of MDS patients will achieve CR1 with induction chemotherapy and some will not be able to proceed to SCT due to treatment complications, this suggests that prior induction chemotherapy may not be needed when using upfront SCT with RTC.

0266

CLINICAL CHARACTERISTICS AND PROGNOSTIC FACTORS OF HYPOCELLULAR MYELODYSPLASTIC SYNDROME: ANALYSIS FROM THE WEB-BASED KOREAN MDS REGISTRY

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Background and Aims. Hypocellular myelodysplastic syndrome (MDS) comprised approximately 10-20% of total MDS cases. The difference in clinical features and prognosis between hypocellular MDS and other cellular MDS remains uncertain. Therefore, we conducted this study to define the characteristics and prognostic factors of Korean hypocellular MDS patients. *Methods.* We collected clinical and laboratory data from web-based Korean MDS registry (www.mdsregistry.or.kr). A total of 1274 evaluable MDS patients, who were diagnosed between the years 1993 and 2010, form 28 Korean hospitals were registered in Korean MDS Registry. Hypocellularity was defined as less than 30% of cellularity in patients <70 years, and <20% in patients 70 years or older in the bone marrow biopsy specimen. *Results.* We identified 146 (15.0%) patients with hypocellular MDS from 973 adult primary MDS patients according to the WHO classification criteria. Median age, gender, performance status, Hgb, transfusion dependency, bone marrow blast percentage, and karyotype did not differ between two groups. However, hypocellular MDS patients were more neutropenic (median ANC 0.9x109/L vs. 1.19x109/L, p=0.001) and thrombocytopenic (61x109/L vs. 72x109/L, p=0.013). There was no difference in estimated overall survival between the hypocellular and the normo/hypercellular MDS (40.7 months vs. 38.0 months, P=0.471) with the median follow-up length of 12. 2 months. 7.5% of hypocellular MDS patients and 10.2% of normo/hypercellular MDS patients transformed to AML (p=0.368). Time to AML transformation was 9.1 and 10.1 month, respectively (p=0.707). To define prognostic factors in the hypocellular MDS series, we performed univariate and multivariate analysis. At univariate analysis, the factors associated with a poor overall survival were age (≥60), thrombocytopenia (<50x109/L), neutropenia (<0.5x109/L), transfusion dependency and high LDH (≥600 IU/L). Using the Cox proportional hazard regression model, neutropenia, transfusion dependency and high LDH were independent, statistically significant, prognostic indicators. *Conclusions.* Hypocellular MDS had similar clinical features and survival compared to normo/hypercellular MDS. However, hypocellular MDS patients had a different pattern from those of normo/hypercellular MDS regarding prognostic factors.

0267

A 5-DAY OUTPATIENT REGIMEN OF 5-AZACITIDINE IS WELL-TOLERATED AND EFFECTIVE FOR HIGH-RISK MYELODYSPLASTIC SYNDROME AND ACUTE MYELOID LEUKEMIA PATIENTS UNSUITABLE FOR AGGRESSIVE CHEMOTHERAPY

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Background. Azacitidine has been established as an effective agent for patients with myelodysplastic syndrome (MDS), with prolongation of survival and delayed progression to Acute Myeloid Leukemia (AML). Patients with AML who are unsuitable for aggressive therapy or those with refractory disease may also benefit from therapy with this agent. Azacitidine is usually delivered according to a 7-day regimen, which may be difficult to deliver in the day ward setting. *Aims.* To examine the use of azacitidine delivered as a five-day regimen in patients with high risk MDS and AML patients not suitable for intensive chemotherapy (off-label use of azacitidine). *Methods.* We report the use of a 5-day regimen of azacitidine in 70 patients with MDS (n=34) or AML (n=36) in 4 centres in Ireland. Patients received azacitidine 75mg or 100mg/m^2/day subcutaneously or intravenously for 5 days in a day-ward setting. Cycles were given at 28-day intervals and continued until disease progression. *Results.* Of the 70 patients treated 48 were male and 22 female. Median age at time of treatment was 71 years (range 36-89 years). MDS patients included 14 with RAEB-2, 9 with RAEB-1, and 7 with CMML. Others included RCMD (n=2), RARS (n=1) and MDS/MPD (n=1). Most patients had an IPSS of Int-1, Int-2 or High. Patients received a median of 7 cycles of therapy (range 2-29). Twelve patients (35%) had partial remission with a 50% reduction in bone marrow blasts or change to a lower grade of MDS, 19 (56%) had haematological improvement (as assessed by IWG criteria, Cheson B *et al.*, Blood 2006), and 5 had stable disease. Nine red cell transfusion-dependent patients became transfusion-independent. Five patients requiring platelet transfusion prior to therapy became platelet transfusion-independent. 17 patients (50%) remain alive and 17 have died. Median survival was 10 months (range 1-35 months). Of 7 patients with CMML, 4 patients had prolonged survival >20 months. AML patients (n=36) included 18 with de novo AML and 15 with AML secondary to MDS (data not available for 3 patients). Most patients had received prior intensive AML induction chemotherapy but were unsuitable for further intensive therapy due to age, infection or comorbidities, had primary refractory AML or relapsed AML. Eight patients received azacitidine as primary therapy for AML. Median survival in this very poor prognosis AML group was 5 months (range 1-14 months). 50% had stable disease during treatment. Of 22 patients who had > 4 cycles of therapy, median survival was 10 months (range 5-14 months). *Conclusions.* Azacitidine was well-tolerated and effective in both MDS and AML patients, with many remaining transfusion independent. In the poor-prognosis AML group, there was significant reduction in in-patient admissions, compared to the use of intensive chemotherapy. A 5-day regimen of azacitidine, conveniently delivered in the day ward or outpatient setting, is effective in high-risk MDS and offers a well-tolerated palliative out-patient therapy for patients with very poor prognosis AML.

0268

CLINICAL AND BIOLOGICAL ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND ITS RECEPTORS IN MYELODYSPLASTIC SYNDROMES

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Background. The angiogenesis mediators are altered in myelodysplastic syndromes (MDS) and abnormal angiogenesis is implicated in

the pathogenesis of these disorders. VEGF is the most important pro-angiogenetic factor and exerts its biologic effects by interaction with its specific receptors i.e. VEGF-R1 and VEGF-R2. The VEGF/receptor signaling system is involved in the regulation of angiogenesis and hematopoiesis, the former through a paracrine loop and the latter through an autocrin loop. *Aims.* We analyzed bone marrow (BM) immunohistochemical expression of VEGF-Rs and VEGF in MDS patients. Furthermore, we investigated if these parameters had an impact on patient clinical outcome, in order to define their potential prognostic value. *Methods.* Study population included 79 MDS patients, categorized according to WHO 2008 classification and stratified in prognostic categories by means of IPSS and the WPSS systems, and 20 age-matched normal controls (NC). Bone marrow VEGF (and VEGF-Rs) expression was correlated to BM cellularity through an index (i): [(% of BM cellularity x %VEGF-positive cells)/10^4]. *Results.* VEGF had a weak cytoplasmic expression in BM pro-erythroblasts, whereas normoblasts were not immunoreactive. In the myeloid lineage, VEGF expression was more intense in immature cells. VEGFi was higher in MDS in comparison to NCs (Mann-Whitney U-test, p=.006) and was evenly distributed among the different IPSS and WPSS categories. VEGF-R1 had a moderate cytoplasmic expression in myeloid precursors, at all stages of differentiation. VEGF-R1i was higher in MDSs than in NCs (Mann-Whitney U-test, p=.05). VEGF-R1i significantly differed among IPSS and WPSS prognostic classes (Kruskal-Wallis analysis, p=.04 and p=.003). VEGF-R2i was expressed in myeloid precursors with a moderate cytoplasmic positivity. Myeloid mature cells were not immunoreactive. There were no difference in VEGF-R2i in MDSs as compared to NCs. In MDSs, both VEGF-R1i and VEGF-R2i directly correlated with VEGFi (Spearman test, r=.64; p<.0001 and r=.47, p<.0001, respectively). Considering the 75° percentiles of VEGFi and VEGF-R1i values, Low and High VEGFi and VEGF-R1i classes could be determined. Low-VEGFi patients had a longer LFS (Kaplan-Meyer, p=.006) and a significant better OS (Kaplan-Meyer, p=.03) compared with High-VEGFi. Similarly, Low-VEGF-R1i had a longer LFS (Kaplan-Meyer, p<.001) and a better OS (Kaplan-Meyer, p<.001), compared to High-VEGF-R1i patients. Nevertheless, in a multivariable analysis stratified by IPSS, VEGFi and VEGF-R1i did not retain a significant effect on both OS and LFS. No significant correlation between VEGF-R2i and clinical variables was found. *Summary/Conclusions.* The enhanced cytoplasmic expression of both VEGF-R1 and VEGF in BM of MDS patients and their prognostic impact on LFS and OS, although in univariate analysis, may suggest the hypothesis of an intracrine loop that may provide a growth advantage to neoplastic cells. Further studies, integrated with molecular approaches, will be needed to verify the role and the alterations of the VEGF/ VEGF-receptors pathway in these neoplasms.

0269

FACTORS ASSOCIATED WITH HAEMATOLOGIC RESPONSES IN MYELODYSPLASTIC SYNDROMES (MDS) PATIENTS TREATED WITH DEFERASIROX: AN EPIC POST-HOC ANALYSIS USING INTERNATIONAL WORKING GROUP (IWG) 2006 CRITERIA

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[8]*Mater Hospital, Brisbane, Australia*
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Background. Reports of haematologic responses associated with iron chelation therapy in transfusion-dependent patients with MDS are emerging, although the mechanism by which these occur has yet to be elucidated. Not all patients have shown such haematologic improvements; hence it is important to determine factors that may be associated with this response. *Aims.* To evaluate change in serum ferritin and labile plasma iron (LPI) as potential predictors of haematologic responses to deferasirox in a *post-hoc* analysis of transfusion-dependent MDS patients enrolled in the 1-year prospective EPIC study. *Methods.*

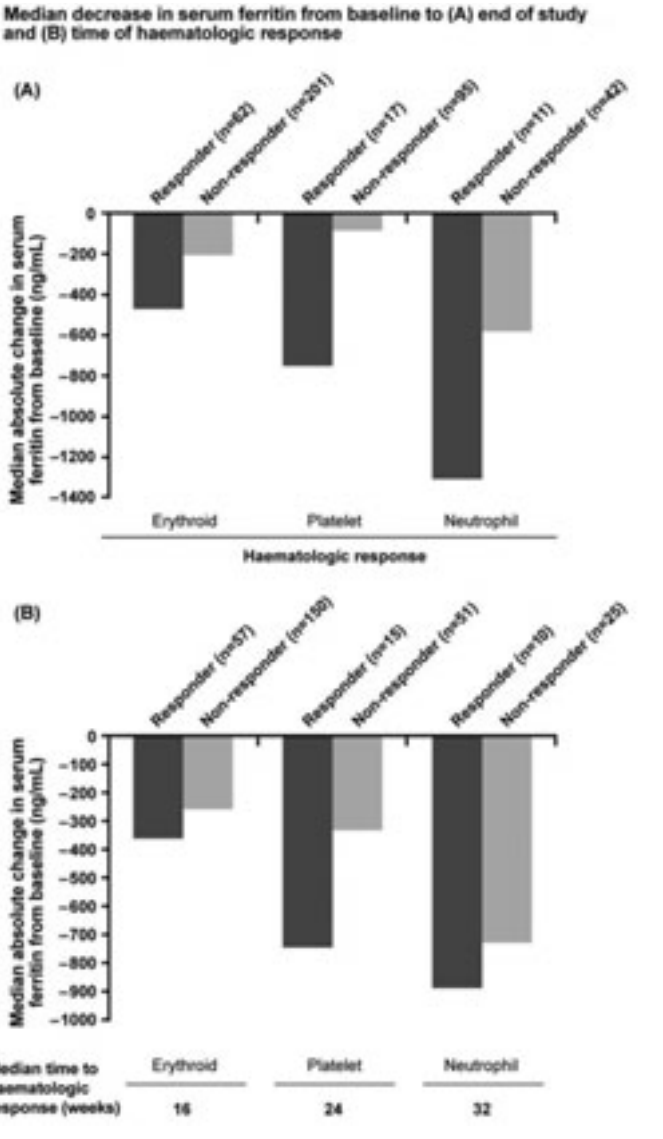

Figure 1.

Details of study design and inclusion/exclusion criteria for EPIC have previously been described (Gattermann *et al. Leuk Res* 2010). Recommended initial deferasirox dose was 20 mg/kg/day with dose adjustments of 5-10 mg/kg/day up to 40 mg/kg/day. MDS patients with haemoglobin (Hb) <11 g/dL or red blood cell (RBC) transfusion requirements >4 units/8 weeks and not receiving erythropoietin were eligible for erythroid response analysis. Patients with platelet counts <100 x 10^9/L or platelet-transfusion dependence, and absolute neutrophil counts <1.0 x 10^9/L and not receiving granulocyte colony-stimulating factor or granulocyte macrophage colony-stimulating factor were selected for the assessment of platelet and neutrophil responses, respectively. Erythroid response: Hb increase ≥1.5 g/dL or reduction in transfusion requirements of ≥4 RBC transfusions/8 weeks. Platelet response: increase ≥30 x 10^9/L for patients with >20 x 10^9/L platelets or increase from <20 x 10^9/L to >20 x 10^9/L and by at least 100%. Neutrophil response: ≥100% increase and absolute increase >0.5 x 10^9/L. All responses to last ≥8 weeks (IWG 2006 criteria [Cheson *et al. Blood* 2006]). Changes in serum ferritin and LPI over time were assessed for haematologic responders and non-responders. *Results.* 279, 121 and 56 patients were included in erythroid, platelet and neutrophil response analyses, respectively. Erythroid responses were observed in 22.6% (63/279) of patients. Median time to response was 109 days. Platelet and neutrophil responses were observed in 14.0% (17/121) of patients after a median of 169 days and 19.6% (11/56) of patients after a median of 226 days, respectively. Median baseline serum ferritin levels were comparable among responders and non-responders (erythroid analysis: 3129 vs 2679 ng/mL; platelet analysis: 3228 vs 3383 ng/mL; neutrophil analysis: 2946 vs 3043 ng/mL). Median absolute change in

serum ferritin from baseline was greater in the haematologic responders compared with non-responders at end of study [Figure (A)] and at the time of haematologic response [Figure (B)], but the differences were not statistically significant. Mean pre-administration LPI levels were high at baseline (>0.4 µmol/L), but were reduced to below this threshold with deferasirox from week 12 onwards; the extent of LPI change did not differ between haematologic responders and non-responders. *Summary/Conclusions.* Deferasirox is associated with improvements in haematologic parameters in some MDS patients. Change in serum ferritin was more pronounced in haematologic responders, suggesting that the serum ferritin decrease could play a role. However LPI did not appear to be related to the haematologic response seen with deferasirox. Additional factors influencing haematologic responses and the mechanisms behind these responses need further investigation.

0270

PRELIMINARY RESULTS OF A MULTICENTER RETROSPECTIVE STUDY ON EFFICACY AND SAFETY IN ERYTHROID STIMULATING AGENTS (ESAS) TREATMENT IN MYELODISPLASTIC SYNDROME (MDS)

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Background. The Erythropoiesis-Stimulating Agents (ESAs) remain the gold standard treatment of anemia in Low Risk MDS. This study was performed in order to evaluate the efficacy, safety and response prognostic factors of ESAs treatment and to correlate response to IPSS and WPSS. *Patients and methods.* From January 2002 to December 2009 a total of 582 MDS patients from 11 Hematologic Centers of the Roman Group of Myelodysplasies (GROM) were retrospectively evaluated. Diagnosis was made according to FAB and WHO Criteria; response was evaluated using IWG Criteria (2006). Patients were divided in Low Risk group (LR) including Low and Int-1 IPSS, and High Risk group (HR) including Int-2 and High IPSS. As for WPSS, patients were stratified as LR (WPSS very low and Low), Intermediate (IR, WPSS Intermediate), and HR (WPSS High and very High). *Results.* Among 311/582 (53,4%) patients who received ESAs only 117 (37.7%) were fully evaluatble. Of these 117, 3 patients (2,5%) who received ESAs 80.000 UI weekly, presented adverse events (1 deep venous thrombosis, 1 phlebitis and 1 hypertensive crisis); all of them were Low Risk. Therapy was discontinued by1 patient. Overall response rate was 63,2% (n=74), a Major and minor Haematological response was observed in 49 patients (41,8%) and 25 patients (21,4%) respectively. No significant statistical differences were found between ESA dosage 40.000 UI vs 80.000 UI. Of 74 responders 70 were LR (95%) and 4 were HR (5%) according to IPSS, whereas 58 were LR (78%), 10 IR (13,5%) and 6 HR (8%) according to WPSS. After a median time of 9,4 months (range 0,7-42,4 mo) 15 patients (20,2%) lost the response (12 pts LR and 3 HR). In our study predicting factors of response were: Haemoglobin and serum EPO dosage at diagnosis and ESAs initiation, serum ferritin at diagnosis and number of RBC transfusions within 2 months before starting ESA treatment. The analysis of prognostic score by IPSS revealed that 109/117 (93%) were LR and 70/109 (64.2%) were responsive to ESAs treatment. A total of 12/70 (17%) responding patients relapsed after a median time of 10 months. Among 8 HR patients 4 (50%) were responsive to treatment, of these and 3 (70%) lacked response after a median time of 4.6 months (range 2,4-7,2 mo). Using WPSS, 84 patients (71,8%) resulted LR, 20 (17,1%) INT and 13 (11.1%) patients HR. Response was obtained respectively in 58/84 LR (69%), 10/20 IR (50%) and 6/13 (49%) HR patient. *Conclusions.* our study demonstrate the safety of ESAs therapy, corroborate (uphold) predicting factors of response already reported and confirm the efficacy of ESAs in LR MDS moreover in HR MDS ESAs are able to obtain a response in about 50% of patients. Prospective studies are needed to validate these retrospective results.

0271

RENAL IMPAIRMENT IS A RISK FACTOR FOR EARLY MORTALTIY IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

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Introduction. PNH is a chronic and progressive disease characterized by chronic complement mediated hemolysis leading to significant ischemic morbidities, end organ damage and shortened lifespan. It has been reported that renal failure accounts for 8-18% of PNH related deaths. The proposed complement mediated mechanism is multifactorial that includes development of renal-mircothombi, ischemia, hemosiderin deposition due to high levels of free hemoglobin, and vasoconstriction of renal arteries reducing eGFR. *Aims.* To understand the impact of late stage renal impairment in patients with PNH, defined as history of acute renal failure or reported eGFR<60 ml/min/1.73m2, we retrospectively analyzed medical charts of 301 PNH patients from national data registry in South Korea over the last 41 years. *Results.* Patient ages ranged from 8 to 88 years (median 37 years), median PNH duration was 7.6 years (1 month to 41 years), and median PNH granulocyte clone size was 49% and median LDH was 4 fold above normal. Approximately 16% (50/301) of patients had a history or presence of late stage renal impairment, similar to the reported 20.5% of PNH patients with CKD 3-5 in the eculizumab clinical study (N=195). Median age of patients with late stage renal impairment was 38 years, and median granulocyte clone was 34%. Patients with renal impairment accounted for 35% of patient deaths. Using a multivariate regression analysis renal impairment was a strong predictor of mortality ($p<0.0001$; odds ratio 3.1; 95% CI 1.15 - 8.18). Patients with renal impairment had a significantly worse overall survival with a hazard ratio of 2.53 (95% CI (1.35, 4.74); P=0.003) compared to PNH patient with no late stage renal impairment. Renal impairment was reported equally in PNH patients with (48%) or without (52%) a bone marrow disorder. *Conclusion.* Late stage renal impairment in patients with PNH is underappreciated. These data establish renal impairment as a risk factor of early mortality in PNH patients.

0272

TREATMENT OF INTERMEDIATE AND HIGH RISK MYELODYSPLASTIC SYNDROMES(MDS) WITH AZACYTIDINE. THE HELLENIC EXPERIENCE

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Background. Myelodysplastic syndromes constitute an heterogenous group of clonal hemopoetic disorders characterized by ineffective hemopoiesis and frequent evolution to acute leukemia. Azacytidine is a hypomethylating agent with significant efficacy and already approved for the treatment of MDS. *Aim.* The aim of this retrospective analysis was the investigation of the efficacy of azacytidine in a group of patients with intermediate and high risk MDS *Methods.* 114 patients (37 female, 77 male) with a median age of 70 years (47-85) were included, Azacytidine was administered at the dose of 75mg/m2 SC for 7 days every 28 days. Response to treatment was evaluated according to the International Working Group (IWG) criteria for MDS. Patients were

classified accoding to FAB as follows RA n=15, RAEB n=67, RAEB-t n=16, AML n=6, RARS n=5, CMML n=5 and according to WHO as:RA n=3, RCMD n=8, RCMD-RS n=3, RARS n=2, RAEB-I n=32, RAEB-II n=44, AML n=17. The IPSS was int-1 n=38, int-2 n=48, high n=26 and the WPSS, low n=5, intermediate n=14, high n=63, very high n=24. 65% of the patients were previously treated. 68% of the patients were RBC and 19% PLT transfusion dependent. *Results.* The median time to therapy initiation since diagnosis was 8 months (0-114), while the median number of cycles administered was 5(1-34). The median time to best response was 3 months (0.5-17) and 4 cycles (1-18). 17.5% of patients achieved CR, 14.6% PR,13.6% HI with an overall response rate of 45.7% while 46.6% had SD and 7.8% PD. The median duration of response was 4 months (4-23). Improvement in WPSS was observed in 17.5%, no change in 32.5% and deterioration in 8.8%.The improvement in WPSS observed after treatment was statistically significant (p=0.011)Toxicity was acceptable with localized skin reaction in all cases, hematologic toxicity grade II-IV in 72,respiratory tract infections in 22, and neutropenic fever in 6 cases. Loss of response was observed in 38% whereas in 26% of cases a stable response was observed. 28.3% and 27.3% of patients who were RBC and PLT transfusion dependent respectively, became transfusion independent . Overall 26% of patients experienced transformation to AML within a median time of 9 months post treatment (1-90). 60.5% (69/114) patients remain alive, with an overall mortality rate of 37.7%. No significant association was observed between response to treatment and baseline clinical characteristics, prior therapy, transfusion dependencey, and time to treatment onset since diagnosis.On the opposite patients who responded to treatment or had a stable disease had a significantly lower percentage of transformation to AML compared to patients with stable disease (p=0.002). Moreover transformation to AML positively correlated with lower neutrophil (p=0.004) and platelet counts (p=0.011), higher bone marrow blasts at baseline (p<0.0001), and with FAB, WHO and IPSS high risk categories. *Conclusions.* Azacytidine is a safe and effective treatment for intermediate and high risk MDS with an overall response rate of 45.7%. A significant improvement of WPSS score was observed following treatment while the risk of transformation to AML was significantly reduced in responding as well as patients with stable disease.

0273

CLINICAL HETEROGENEITY AND OUTCOMES OF UNRELATED BONE MARROW TRANSPLANTATION IN 3 JAPANESE CHILDREN WITH FAMILIAL PLATELET DISORDER WITH PREDISPOSITION TO ACUTE MYELOGENOUS LEUKEMIA

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Background. Familial platelet disorder with predisposition to acute myelogenous leukemia (FPD/AML) is an autosomal dominant disorder characterized by thrombocytopenia and high risk for developing leukemia. Heterozygous germ-line mutations in *RUNX1* have been identified in at least 32 FPD/AML families. As a contribution to the literature on this rare disorder, we present here the clinical courses and genetic studies in 2 Japanese families with FPD/AML. Case report: The proband of family I, who presented with isolated thrombocytopenia at the age of 1 year and was suspected of having moderate immune thrombocytopenic purpura (ITP), developed severe thrombocytopenia at the age of 7 years. Bone marrow findings showed features of myelodysplastic syndrome (MDS): presence of micromegakaryocytes and up to 14% of myeloblasts. Eight months later, he developed pancytopenia and was diagnosed with overt leukemia. Cytogenetic analysis showed normal karyotype and the immunophenotype of the blasts was CD7+, 13+, 33+ and 34+ and HLA-DR+. He received 3 courses of intensive chemotherapy. Because he did not achieve complete remission, he underwent unrelated donor marrow transplantation (UR-BMT), but relapsed 18 months later. At the age of 10 years, he underwent a second UR-BMT on relapse. The patient is still in remission 3 years later. The 3-year older sister of the proband also presented with moderate isolated thrombocytopenia at the age of 1 year and developed severe thrombocytopenia at the age of 11 years. Bone marrow findings showed features of ITP; increased numbers of immature megakaryocytes without blasts. Two years later, platelet number decreased below $2.0x10^4/\mu l$, and megakaryocytes disappeared in the bone marrow. This finding was quite different from the previous one. Cytogenetic analysis showed normal karyotype. Finally, she was diagnosed with MDS and received a successful UR-BMT at age 14 years. The patient is alive and well 1.5 years later. In contrast, their father has presented with only mild isolated thrombocytopenia ($13.5x10^4/\mu l$ at age 36 years and $12.5x10^4/\mu l$ at 48 years). The proband of family II, who presented with moderate isolated thrombocytopenia at the age of 2 years, developed chronic myelomonocytic leukemia at age 9 years. She received a successful UR-BMT at age 12 years. The patient is alive and well 2 years later. The 2-year older sister of the proband also has a 10-year history of moderate isolated thrombocytopenia from the age of 1 year ($8.5x10^4/\mu l$ at age 16 years) without bleeding tendency. Their father died of MDS at age 43 years. Sequencing analyses of *RUNX1* revealed a heterozygous mutation in each family. Discussion: Clinical courses vary among FPD/AML patients within the same family. Our findings support the clinical heterogeneity of the disease as reported by other investigators. Because bone marrow findings in the early phase of FPD/AML mimic that in ITP, genetic analysis of *RUNX1* is necessary to screen for FPD/AML. Finally, we consider that early indication of stem cell transplantation (SCT) leads to better outcome although the optimal conditioning regimen remains to be determined.

Myeloma and other monoclonal gammopathies - Biology 1

0274

CONSTITUTIVE ACTIVE FIBROBLAST GROWTH FACTOR RECEPTOR 3 (FGFR3) WITH LYS650GLU MUTATION ENHANCES THE BORTEZOMIB SENSITIVITY IN PLASMA CELL MALIGNANCY

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Background. The t(4;14)(p16.3;q32.3) translocation occurs in 15-25 % of multiple myeloma (MM) patients and leads to the deregulation of fibroblast growth factor receptor 3 (FGFR3) gene. This results in ectopic expression of FGFR3, which promotes the proliferation and survival in myeloma cells. Clinical data indicate that patients with this translocation demonstrate resistance to conventional chemotherapy and early progression after a response occurs, leading to poor prognosis. Activating mutations of FGFR3, such as Lys650Glu (K650E) and Tyr373Cys (Y373C) have been identified in MM patients with t(4;14), who show more aggressive clinical course. Recently, it is reported that bortezomib is able to overcome the poor prognosis associated with t(4;14) or FGFR3 expression. Therefore, we are interested in the bortezomib-sensitivity of malignant plasma cell possessing FGFR3 and its mutation *Aims.* This study investigated whether FGFR3 and its mutations are associated with the cytotoxic effect of bortezomib in malignant plasma cells. *Methods.* Human plasmacytoma cell line, FR4 and human myeloma cell line, RPMI8226 were transfected with the plasmid encoding a full length of FGFR3 cDNA of wild type (WT), K650E and Y373C mutations. Cell apoptosis assays were performed in these cells after exposure to bortezomib. The localization of the FGFR3 receptors in the cells was observed by immunocytochemical analysis, and the induction of endoplasmic reticulum (ER) stress protein was compared between each type of cells after bortezomib treatment. Furthermore, we observed the alteration of bortezomib sensitivity by co-treatment with tunicamycin or cycloheximide. *Results.* FR4 cells with FGFR3 K650E showed enhanced sensitivity to bortezomib, as compared to FR4 cells with mock, FGFR3 WT or FGFR3 Y373C. Similar results were obtained when RPMI8226 cells were used in place of FR4 cells. According to the immunocytochemical analysis of FR4 cells, FGFR3 K650E mutant was preferentially localized in the ER and constitutively activated, whereas FGFR3 WT or FGFR3 Y373C receptors existed on the cell surface and the former was activated only after the stimulation by the FGF ligand. These results indicated that the FGFR3 K650E receptor accumulated and activated in the ER, aberrantly. Transcriptional up-regulation of ER stress proteins, such as BiP, Edem1 and CHOP were observed by real-time PCR after treatment with bortezomib. As a result, BiP, Edem1 and CHOP were strongly induced by bortezomib in FR4 cells, the magnitude of which were significantly increased in FR4 K650E transfected cells. The combination of bortezomib and ER stressor, tunicamycin, enhanced the cytotoxicity of bortezomib, resulting in the comparable apoptosis in each cell. In contrast, ameliorating ER stress with cycloheximide reversed the cytotoxicity activity of bortezomib, leading to similar survival of each cell type. These results suggested that increased ER stress is associated with the enhanced sensitivity of FGFR3 K650E expressing cells to bortezomib. *Summary/Conclusions.* This study indicated that FGFR3 with K650E mutation showed aberrant ER localization and enhanced the bortezomib-sensitivity in malignant plasma cells via ER stress pathways.

0275

IGH TRANSLOCATION AND DEL(13Q14) ARE FREQUENTLY ABSENT IN THE ANCESTRAL PLASMA CELL CLONE IN MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background. Previous cytogenetic studies in multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS) have been mainly done in whole bone marrow or CD138+ microbead-sorted plasma cells (PC) and suggested that both entities typically share karyotypic patterns, where IGH translocations and to a lesser extent del(13q14) frequently are primary oncogenetic events. However, also the technique of PC sorting by CD138+ microbeads is hampered in several aspects such as CD138 expression by normal and reactive PC as well as coexistence of CD138+ and CD138- clonal PC in monoclonal gammopathies. In addition, rapid loss of CD138 by apoptotic PC and insufficient purity are among the principal limitations of this technique. *Aims.* We analyzed 208 patients with MM (n=148) and MGUS (n=60) for the presence of different cytogenetic abnormalities - e.g. t(14q32), del(13q14) and del(17p13) - using highly purified flow cytometry-sorted clonal PC to get more insights in patterns of cytogenetic abnormalities and intratumoral clonal evolution profiles. *Methods.* Immunophenotyping of normal and clonal PC was carried out using multiparameter flow cytometry and clonal PC were sorted according to their specific immunophenotype with a ≥98% median purity using a FACSAria II cell sorter. Cytogenetic abnormalities were assessed by multicolor interphase fluorescence in situ hybridization using purified clonal PC. *Results.* Del(13q14), del(17p13) and t(14q32) were significant more frequent among MM than MGUS patients (55%, 11%, 42% vs. 22%, 0%, 22%), whereas del(13q14) in conjunction with t(14q32) was a common finding in MM but not in MGUS (26% vs. 3%, p<0.001). The presence of 2 or more cytogenetically different clones was more frequently observed in MM than in MGUS patients (64% vs. 37%, p<0.001). A primary clonal PC clone (ancestral tumor cell clone) without any cytogenetic abnormality for all probes tested was detected in 49% and 73% of MM and MGUS cases, respectively (p=0.002). Even more interestingly, t(14q32) and del(13q14) arose as a non-primary clone in 24% and 43% of MM vs. 62% and 38% of MGUS patients carrying the respective cytogenetic alteration (p=0.02 and p>0.05). Acquisition of cytogenetic abnormalities occurred stepwise in MM and MGUS, both groups frequently showing different cytogenetic evolution profiles when other cytogenetic variables such as DNA ploidy status, deletions/gains of IGH, FGFR3, CCND1 and MAF, were additionally considered in their respective chronological sequences of acquisition. *Summary/Conclusions.* Overall, our results indicate that acquisition of cytogenetic abnormalities may occur stepwise in different types of monoclonal gammopathy. In contrast to the general belief, IGH translocations and del(13q14) are present in only part of all clonal PC in a significant proportion of MGUS and MM cases once evaluated at the intratumoral cell level.

0276

EARLY RELAPSE AFTER AUTOLOGOUS TRANSPLANTATION FOR MYELOMA IS CHARACTERIZED BY GENES MAPPING TO CHROMOSOME X

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Background. High dose therapy (HDT) followed by autologous stem cell transplantation (ASCT) is currently the most widely accepted approach in newly diagnosed myeloma patients who are considered fit enough to undergo the procedure; however, almost all patients will eventually relapse with a median progression free survival (PFS) of 2 to 3 years. As PFS is the most robust predictor for overall survival (OS) in myeloma patients, it is important to identify those who are likely to relapse early from ASCT, so that additional treatment could be given to modify the outcome. *Aims.* This study aims to examine the molecular basis of HDT resistance and to develop a predictive signature for patients at high risk of early relapse following ASCT. *Methods.* Gene expression profiling (GEP) data is available in 80 newly diagnosed myeloma patients who underwent front-line ASCT following induction therapy after informed consent. PFS was calculated from the date of HDT to the date of progression, with those dying without evidence of relapse censored at the time of death. OS was defined as the time from HDT to the date of death from any cause. Univariate Cox analyses were conducted on PFS to identify significant genes associated with early relapse with multiple testing adjustment. The independence of the genes from other prognostic factors was tested using multivariate Cox regression. *Results.* Nine genes were identified as being associated with early relapse after ASCT, among which five were on chromosome X. Three of the five chromosome X genes belong to the cancer/testis gene family which has been shown to be prognostic in myeloma patients. NUDT11 has been reported to be involved in vesicle trafficking, DNA repair and apoptosis, and has been linked to drug resistance in some cancer types. The identified chromosome X genes were also significantly associated with shorter OS in our dataset,

which is independent from any other known prognostic factors such as β2m, alb, delp53, gain1q and adverse chr14 translocations identified by FISH ($p < 0.05$). The prognostic value of the genes identified was also validated in an external cohort of patients treated with ASCT. *Conclusion.* By analyzing GEP data we identified genes significantly associated with high risk of early relapse following ASCT, most of which mapped to chromosome X. Upon further investigation, theses genes could give insight into the biology underlying HDT resistance. The development of a predictive signature based on the identified genes is currently undergoing and will be presented at the conference.

0277

This abstract has been withdrawn.

0278

CANONICAL AND NON CANONICAL HEDGEHOG PATHWAY IN THE PATHOGENESIS OF MULTIPLE MYELOMA

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Background. The Hedgehog (Hh)-pathway is required for cell-fate determination during the embryos life, cell growth and differentiation in the adult organism. In mature tissues it regulates tissue homeostasis and repair and, in those tissues undergoing constant renewal as skin, colon, liver and blood it is also implicated in maintaining a stem/progenitor cell compartment. In this context, it is easy to understand how Hh-pathway deregulation may cause development defects during the embryos life, while leads to tumorigenesis during the adult life for a stem cell pool expansion or for onset, within more differentiated cells, of mutations affecting the normal growth-regulatory mechanisms. We already showed that an aspect of plasma cells (PCs) malignant transformation is the aberrant expression of developmental genes as those of Wnt- and Hh-pathways. Several evidences support a role of Hh-signaling in regulating a stem cell niche also in Multiple Myeloma (MM) and in modulating clinical response to conventional and novel therapeutic agents. Indeed, Hh-ligands produced by mice-derived Bone Marrow Stromal Cells (BMSCs) have been recently identified as new soluble factors supporting growth and survival of human primary CD19+ lymphoma and CD138+ MM cells demonstrating a role of Hh-pathway also in lymphoma and in MM terminally differentiated cells. Finally, we recently showed ciliary proteins over-expression as a possible cause of constitutive and non canonical Hh-pathway activation suggesting a cilia-dependent mode of Hh-signaling in MM. *Aims.* Here our aim was to demonstrate that Hh pathway plays a role in cancer formation and survival also in MM. *Methods and Results*. First we evaluated the Hh-genes expression in CD138+ PCs isolated from BM aspirate of 4 healthy persons, 12 MGUS, 132 MM and 9 PCL patients using oligonucleotide microarrays analysis. As compared with CD138+ cells isolated from healthy persons, we found that PCs from MGUS and MM patients express significantly higher levels of Hh-genes suggesting that Hh-pathway activation might play a role in MM disease initiation and pathogenesis. Conversely the down-regulated Hh-genes expression observed in PCL, a more advanced and BM-independent disease, suggests a role for stroma-derived Hh-signals in triggering a paracrine Hh-activity in MM. We also demonstrated a Smoothened (Smo)-dependent Hh-signaling showing that NVP-LDE225, a novel synthetic Smo-inhibitor (Novartis), affected MM cells viability, as assessed by MTT, inducing specific down-regulation of Gli1 and Ptch1, hallmarks of Hh-activity, as demonstrated by western blotting analysis. Additionally we detected, by immunofluorescence, an unexpected nuclear localization of Gli1 which is completely abrogated by Forskolin, a Gli1 modulating compound, demonstrating Smo-independent mechanisms leading to Hh-activation in MM. Finally we found that MM patient-derived bone-marrow stromal cells (BMSCs) were source of Shh-ligand although they showed resistance to Hh-inhibitor probably due to defective Smo expression as well as Ptch1 up-regulation. *In vivo* studies showed little antitumor efficacy of NVP-LDE225 as single agent that significantly increased when combined with proteasome inhibitor Bortezomib. *Conclusions*. All together our data demonstrate canonical as well as non canonical Hh-pathway activation in MM and provide the rationale for further investigations and for using Hh-inhibitors to improve MM patient outcome.

0279

THE ANALYSIS OF TRANSCRIPTIONAL NETWORK IN MULTIPLE MYELOMA REVEALS CRITICAL GENES WITH BIOLOGICAL AND CLINICAL IMPLICATIONS

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Background. The combined use of microarray technologies and bioinformatics analysis has improved our understanding of biological complexity of multiple myeloma (MM). In contrast, the application of the same technology in attempt to predict clinical outcome has been less successful with the identification of heterogeneous molecular signatures. *Aim.* This approach was aimed at the identification of robust and reproducible signatures associated with prognosis across independent datasets. *Methods.* We have reconstructed gene regulatory networks in a panel of 1883 samples from MM patients profiled on Affymetrix platform, derived from seven publicly available gene expression sets. The transcriptional networks were reconstructed using ARACNe (Algorithm for the Reconstruction of Accurate Cellular Networks). Critical analysis of network components was applied to identify genes playing an essential role in transcriptional networks, which are conserved between datasets, and proportional hazard models were used to evaluate the association of each gene with outcome. The correlation with overall survival was tested in three of the seven datasets for which clinical data were available. *Results.* The network critical analysis revealed that i) *CCND1* and *CCND2* were the most critical genes; ii) among the top critical genes *CCND2*, *AIF1* and *BLNK* had the largest number of connections shared among the datasets; and iii) robust gene signatures with prognostic power were derived from the most critical transcripts and from shared primary neighbors of the most connected nodes. In particular, a "critical-gene"model, comprising *FAM53B*, *KIF21B*, *WHSC1* and *TMPO*, and a "neighbor-gene"model, comprising *BLNK* shared neighbors *CSGALNACT1* and *SLC7A7*, predicted survival in all datasets with follow-up information. *Conclusions.* The reconstruction of gene regulatory networks in a large panel of primary tumors suggested novel molecular mechanisms central to MM biology and identified specific genes with prognostic importance.

0280

INHIBITION OF PROTEIN KINASE CK2 AFFECTS THE HOMEOSTASIS OF THE UNFOLDED PROTEIN RESPONSE PATHWAYS IN MULTIPLE MYELOMA CELLS AND EMPOWERS THE CYTOTOXIC EFFECT OF HSP90 INHIBITORS

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Background. Hsp90, a central chaperone molecule involved in the maturation and folding of several cellular client proteins, is essential for malignant plasma cell survival. Hsp90 inactivation in multiple myeloma (MM) cells has been shown to cause perturbation of the ER stress/unfolded protein response (UPR), eventually triggering the apoptotic cascades. Protein kinase CK2 is an important regulator of Hsp90 activity phosphorylating the Hsp90 cochaperone Cdc37 stabilizing a macromolecular complex containing Hsp90, Cdc37 and client proteins. We previously described that CK2 is over-expressed in a fraction of MM patients and is an essential MM pro-survival molecule. Currently, phase I clinical trials are ongoing with Hsp90 inhibitors and orally available ATP-competitive CK2 specific inhibitors. *Aims.* We have here investigated the role of CK2 in the ER stress/UPR pathways and in Hsp90

inhibition-induced apoptosis in MM cells. We analyzed CK2 activity upon ER stress and the consequences of the effects of CK2 inhibition/silencing on ER stress induced-apoptosis triggered by chemicals and Hsp90 inhibitors. *Methods.* MM cell lines, human bone marrow stromal cells and plasma cells from patients were exposed to geldanamycin or 17-AAG (17-(demethoxy)-17-allylamino geldanamycin) (Hsp90 inhiibtors) and CK2 inhibitors K27 and CX4945. RNA interference was used to silence the CK2 catalytic α subunit. Thapsigargin and Tunicamycin were used to trigger ER stress. Annexin V and propidium iodide staining and analysis of PARP cleavage were employed to assess cell growth and viability. UPR related signaling pathways were studied with western blot and real time-polymerase chain reaction analysis. *Results.* Down-regulation of the catalytic CK2α subunit with chemical inhibitors or RNA interference resulted in modifications of the main UPR regulating signaling cascades: a reduction of IRE1α protein levels; a reduction of BiP/GRP78 chaperone protein levels; an increase of PERK activity and phospho eIF2α levels. CK2 partly localized to the ER and the ER-stressor Thapsigargin triggered its kinase activity. CK2 inactivation enhanced Thapsigargin-induced apoptosis and opposed CHOP/GADD153 and IRE1α rise. Treatment of CK2-inhibited/silenced MM cells geldanamycin or 17-AAG resulted in a much more pronounced reduction of IRE1α protein levels; a marked inhibition of GA or 17-AAG-triggered BiP/GRP78 protein level raise; a more evident increase of eIF2α phosphorylation. Of note, CK2 plus Hsp90 inhibition was followed by apoptotic cell death to a much greater extent than that obtained with the single inhibition of the two molecules. Noteworthy, these effects were also reproduced upon modelling the MM bone marrow microenvironment by co-culturing MM cells with BM stromal cells and on plasma cells isolated from MM patients. Mechanistically, we demonstrated that CK2 inhibition leads to a reduction of IRE1α/HSP90/CDC37 complexes in MM cells, a phenomenon that could justify the reduced IRE1α half-life in MM cells. *Summary/Conclusions.* These data highlight the importance of CK2 in tuning HSP90 function and the ER stress/UPR cascades in MM cells. In view of the very recent development of phase I clinical trials with both CK2 inhibitors and Hsp90 inhibitors as anti-MM agents, our results might provide useful insights to better set the groundwork in designing novel combination treatments for this disease.

0281

INITIATING ROLE OF RECURRENT IGH TRANSLOCATIONS DURING THE ONCOGENESIS OF PLASMA CELL MYELOMA IS QUESTIONABLE IN A NOT NEGLIGIBLE SUBSET OF CASES

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Background. Only indirect evidence is available regarding the exact temporal sequence of cytogenetic aberrations during the oncogenesis of plasma cell myeloma (PCM). *Aims.* In this study, the correlation of cytogenetic changes to each other and the incidence of abnormalities within the purified plasma cell population were analyzed by interphase fluorescence in situ hybridization (FISH). Combined FISH analysis was applied in order to get direct evidence regarding cytogenetic evolution of PCM. *Methods.* We investigated diagnostic bone marrow specimens from 185 patients with PCM. Ethical Committee approval and written informed consent from the patients has been obtained. Aspirated plasma cells were purified with immunomagnetic cell separation using CD56 and CD138 antibodies. FISH probes were used for the detection of monosomy or deletion of chromosome 13 (Δ13), deletion of *p53* gene and the most frequent recurrent immunoglobulin heavy-chain gene (*IGH*) translocations: *IGH/FGFR3*, *IGH/CCND1* and *IGH/c-MAF*. After initial screening, patients' samples with more than one specific aberrations were chosen for the combined FISH analysis which was performed using motorized microscopy. *Results.* The Δ13, *p53* deletion and *IGH* disruption were found in 47.2, 7.5 and 58.9% of cases, respectively. Incidences of *IGH/FGFR3*, *IGH/CCND1* and *IGH/c-MAF* aberrations within the *IGH* positive group were 22.6, 21.7 and 6.6%, respectively. 25 cases harboring at least two specifically identified aberrations were found. Performing combined FISH analysis on these samples, more than one abnormal cell clones suggesting clonal evolution were observed in 16% of cases. The Δ13 was preceded by the *IGH/FGFR3* and *IGH/c-MAF* translocations. Deletion of *p53* gene was a secondary aberration compared to the Δ13 and the *IGH/CCND1* translocation. Interestingly, in 25% of *IGH* positive cases examined by combined FISH analysis the recurrent *IGH* translocations were presented only in a subset of purified plasma cells. Subsequently, we extended our observation to all 185 patients screened in this study. In 21.8% of cases harboring specifically identified recurrent *IGH* translocation (*IGH/FGFR3* 20.0%, *IGH/CCND1* 16.7% and *IGH/c-MAF* 28.6%) the aberration was presented in less than two-thirds of purified plasma cell population. The presence of more than one *IGH* translocations was excludable in these cases. *Conclusions.* Combined FISH analysis addressing the issue of clonal evolution of structural cytogenetic aberrations in patients with PCM has not been published yet; therefore our results not only support the current model of the oncogenesis of PCM, but provide the first direct evidence at single cell level. Other workgroups investigating patients with MGUS and smoldering myeloma have also found that sometimes recurrent *IGH* translocations are present in a subset of plasma cells. In these early lesions, the presence of normal plasma cells may confound results, since the count of abnormal plasma cells is relatively low. However, it is widely recognized that this diluting effect of normal plasma cells is negligible at the time of diagnosis of PCM. Consequently, our results suggest that recurrent *IGH* translocations investigated in this study are not primary events at least in approximately one-fifth of cases. Contact: donat.alpar@kk.pte.hu

0282

AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA: IMPACT OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNP) IN GENES INVOLVED IN INFLAMASSOME AND MIRNA NETWORK IN SURVIVAL AND PROGRESSION

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Background. Polymorphisms (SNPs) in proteins involved in cytosolic macrocomplex with regulatory functions in the immune system have previously shown to have prognostic impact after stem cell transplantation. Otherwise, SNPs in miRNA proteins pathway and in the target genes binding sites (miR-SNPs) have been observed with different prognostic implications in some tumors. However, data in multiple myeloma (MM) has ever been reported. *Patients and Methods.* One hundred and thirty seven patients with chemosensitive MM (73M/64F, median age 55 years) intensified with autologous stem cell transplantation (ASCT) have been studied in one institution. The patients achieved at least a minimal response after one (117) or two (20) induction regimens prior to ASCT. The genes (SNPs) evaluated in genomic DNA by allelic discrimination (TaqMan assays) were NLRP2 (rs1043684), NLRP3 (rs10925027), ATBF1 (rs7193297) and EP300 (rs20551) for innate immune system, and KRT81 (rs3660), AFF1 (rs17703261), FAM179b (rs1053667) and XPO5 (rs11077) for miR-SNPs. *Results.* Overall survival (OS) was significantly longer in patients with SNPs in KRT81 (rs3660; p=0.029), NLRP2 (rs1043684; p=0.053) and XPO5 (rs11077; p=0.012)(Figure). A correlation of this latter polymorphism in XPO5 with progression-free survival (PFS) was also observed (p=0.013). This miR-SNP retained its prognostic impact on PFS and OS when a Cox

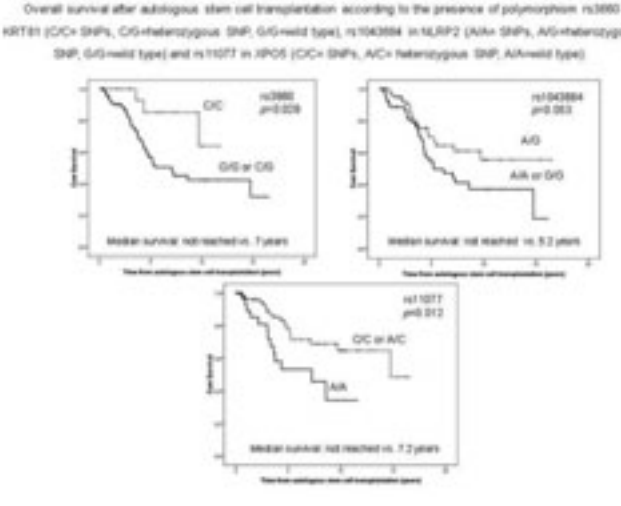

Figure 1.

multivariate regression analysis, including age, international staging system and immunoglobulin isotype, was performed (p=0.028 and p=0.014, respectively) There was a trend toward a longer PFS for miR-SNP in KRT81 (p=0.17), but not for NLRP2. EP300 (rs20551) SNP was associated with higher incidence of implant syndrome (42% vs. 12%, p=0.015). No other associations with prognosis or toxicities were observed in the remaining SNPs. *Conclusion.* Among the immune system genes, only NLRP2 was associated with longer OS in this ASCT series, as previously reported in the allogeneic SCT setting. EP300 is the first genomic risk factor associated with the development of implant syndrome. Concerning to the miRNA network, this is the first report in haematological malignancies that a miR-SNP in a keratin gene (KRT81), target of diverse myeloma-miRNA clusters and relevant in the structural cytoplasm framework, has been associated with prognosis. Moreover, a miR-SNP in XPO5 was significantly associated with longer PFS and OS in our study. This gene, exportin 5, mediates pre-miRNA nuclear export. This SNP could modify the miRNome of the cell, with a miRNA-target disturbance due to a global impairment of mature miRNAs. Further studies on proliferation and miRNA interaction are encouraged.

0283

THE EFFECT OF LENALIDOMIDE AND DEXAMETHASONE COMBINATION ON BONE REMODELING OF RELAPSED/REFRACTORY MYELOMA: FINAL RESULTS OF TWO STUDIES OF THE GREEK MYELOMA STUDY GROUP WITH 205 PATIENTS

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Background. Lenalidomide plus dexamethasone (RD) is very effective for the management of refractory/relapsed multiple myeloma (MM). However, there is limited information for the effect of RD on bone remodeling of MM patients. *Aims.* We performed both a retrospective analysis and a prospective study to evaluate the effect of RD regimen on bone remodeling in relapsed/refractory MM. *Methods.* Firstly, we evaluated 106 consecutive patients (54M/52F; median age 68 years) who received lenalidomide at the standard dose of 25mg PO daily (or adjusted to creatinine clearance) on days 1-21 of a 28-day cycle in combination with dexamethasone at a dose of 40mg PO on days 1- 4 and 15-18 for the first four cycles and only on days 1-4 thereafter. All patients were under zoledronic acid both pre- and during treatment period. The following serum indices of bone metabolism were measured on day 1/cycle 1, and then on day 28 of cycles 3 and 6: (i) osteoblast inhibitor dickkopf-1 (Dkk-1); (ii) osteoclast regulators: sRANKL and osteoprotegerin (OPG); (iii) bone resorption markers: CTX and TRACP-5b; and (iv) bone formation markers: bone-specific alkaline phosphatase (bALP) and osteocalcin (OC). These markers were also evaluated in 44 healthy controls of similar gender and age. Then, to confirm the results of the retrospective analysis, we scheduled a prospective study in which 99 patients received either RD (n=50) or VRD (bortezomib+RD, n=49), based on previous peripheral neuropathy status. The above bone markers were measured on day 1/cycle 1 and on day 28 (for RD) and 21 (for VRD) of cycles 3 and 6. Radiological skeletal survey was performed at baseline, after 6 cycles of therapy and then as needed, while patients were assessed for skeletal-related events (SREs) throughout the period of the study. *Results.* In the retrospective analysis, before RD, myeloma patients had increased serum Dkk-1, sRANKL, and bone resorption markers and reduced OC and bALP compared to controls. The objective response rate was 55%. RD produced a reduction of CTX and sRANKL/OPG only in responders, with no effect on bone formation. In the prospective study, VRD reduced Dkk-1 and increased OC after 3 cycles, while it reduced sRANKL/OPG and increased bALP after 6 cycles. These changes were irrespective of treatment response which was similar among treatment arms (63%). In VRD, % Dkk-1 reduction strongly correlated with % increase of bALP. RD reduced CTX only in responders, while it increased Dkk-1; however, responders had a median increase of 9% while non-responders of 91%. No SREs were observed in the VRD arm while two patients treated with RD who had not responded to therapy developed a vertebral pathological fracture. *Summary/Conclusions.* RD regimen reduces bone resorption only in responding patients with relapsed/refractory myeloma but has no effect on bone formation, possibly due to the presence of high dose dexamethasone and to the enhancement of Dkk-1 expression by lenalidomide. On the contrary, VRD enhances bone formation, at least partially due to a significant reduction of Dkk-1, reflecting the strong anabolic effect of bortezomib in MM patients.

0284

INHIBITION OF PROTEIN KINASE CK2 ENHANCES THE CYTOTOXIC EFFECTS OF BORTEZOMIB ON MULTIPLE MYELOMA CELLS

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Background. Multiple Myeloma (MM) cells are exquisitely sensitive to the cytotoxic effects of proteasome inhibitors (PI). Bortezomib (BZ) is a first-in class PI currently widely used in the therapy of MM patients. BZ causes MM cell apoptosis through different mechanisms which are only partially known. The pro-survival protein kinase CK2 has been implicated in human cancer and phase I clinical trials are ongoing utilizing oral ATP-competitive CK2 inhibitors in MM and other tumors. *Aims.* In this study we have investigated the role of CK2 in the regulation of BZ-induced MM cell death. We aimed to assess apoptosis and proliferation of MM cells exposed to BZ and CK2 inhibitors. We investigated pro-survival signaling pathways associated with MM cell resistance to BZ and chemotherapy. *Methods.* MM cell lines, human bone marrow stromal cells and freshly isolated plasma cells from patients were cultured and exposed to BZ and the CK2 inhibitors K27 and CX4945. Cell growth and viability was assessed upon the different treatments by annexin V and propidium iodide staining, MTT-assays, evaluation of mitochondrial potential depolarization and FACS analysis of cell cycle. Survival signaling pathways were studied with WB analysis and RT-PCR. *Results.* BZ-induced apoptosis and cell cycle arrest were significantly increased by the simultaneous inhibition of CK2. This effect was observed both in MM cell lines grown in suspension, in a model of bone marrow microenvironment as well as in malignant plasma cells isolated from MM patients. Mitochondrial membrane potential measurements revealed that CK2 inhibition enhanced BZ-triggered intrinsic apoptotic cell death. Also, CK2 inhibition together with BZ treatment was associated with reduced MM cell proliferation, as shown by cell cycle analysis with propidium iodide staining. We observed that unwanted side effects of BZ treatment were the activation of the MM growth-promoting NF-κB and STAT3 signaling pathways and the rise in the levels of the unfolded protein response-associated kinase IRE1α. These changes could lend MM cells the ability to escape the cytotoxic effects of BZ. Oppositely, CK2 inhibition was associated with a strong reduction of phopspho-p65 NF-κB, phospho-STAT3 and IRE1α levels in MM cells. Remarkably, the simultaneous treatment of BZ with CK2 inhibitors was accompanied with a significant reduction of BZ-triggered p65 NF-κB and STAT3 activation and we found that CK2 inhibition was also able to hamper the BZ-induced rise in IRE1α levels. *Summary/Conclusions.* These results indicate that protein kinase CK2 can antagonize BZ-induced apoptosis and regulates critical signaling pathways in MM cells, such as the NF-κB and STAT3 cascades. Our findings indicate that CK2 inhibition could represent a rational therapeutic strategy to be tested in designing novel BZ-based anti-MM combination therapies.

0285

USE OF SPECIFIC IMMUNOGLOBULIN HEAVY/LIGHT CHAINS PAIRS FOR THE DIAGNOSTIC AND FOLLOW-UP OF MULTIPLE MYELOMA PATIENTS: NORMAL RANGES AND ASSAY UTILITY

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Background. Multiple myeloma (MM) is a B cell disorder and is characterized in most of the cases by the production a monoclonal protein (MP). The detection and quantification of the MP by serum protein electrophoresis is the most used technique for the screening of monoclonal gammopathies. However, this can often be difficult, especially in cases where the paraprotein is of low amount and in cases where the band is masked by other proteins. Immunofixation (IFE) improves sensitivity to the detection protocol but it is not a quantitative method, therefore not suitable for the follow-up. More accurate methods could benefit the patient in several ways: by avoiding problems related to a delayed diagnostic; and by allowing a closer monitoring of the applied therapy, and by doing so helping in treatment decisions such as when

Table 1.

IgA

	IgA k (g/L)	IgA λ (g/L)	ratio IgA k/λ
N	83	83	83
Mean	1,37	0,92	1,47
Median (95% Range)	1,34(0,38-3,12)	0,92(0,32-2,01)	1,44(0,66-2,47)
Median (95% Range)[1]	1,27(0,43-2,36)	0,87(0,4-1,73)	1,40(0,58-2,52)
Median (95% Range)[2]	1,19(0,48-2,82)	1,00(0,36-1,98)	1,27(0,80-2,04)

IgG

	IgG k (g/l)	IgG λ (g/l)	Ratio IgG k/λ
N	75	75	75
Mean	7,10	4,10	1,81
Median (95% Range)	7,00(4,08-9,56)	4,02(1,76-6,24)	1,75(0,98-3,69)
Median (95% Range)[1]	7,76(4,23-12,08)	4,00(2,37-5,91)	1,96(1,26-3,2)
Median (95% Range)[2]	6,85(4,03-9,78)	4,81(1,97-5,71)	1,87(0,98-2,75)

1 - Clinical Chemistry 55:8, 1846-1853(2009)
2 - Serum free light chains plus Hevylite (2010) 4th Edition

Normal HLC ranges from a Spanish population and comparison to previously described ranges.

an adjustment is required or when avoiding undesired secondary effects that therapies can cause. A new assay (HevyliteTM - The Binding Site) is now available that allows the quantification of specific heavy chain/light chain pairs (HLC) (IgAk, IgAλ, IgGk, IgGλ, IgMk, IgMλ) and it is our aim to determine normal ranges in healthy individuals considering that the use of HLC ratios (HLCr) will help us improve the diagnostic and follow-up of monoclonal gammopathies. *Methods.* We measured HLC and immunoglobulin's (Igs) G and A concentrations in blood donor sera by turbidimetry (SPA+ - The Binding Site). 37 myeloma samples of treated and untreated patients (16 IgA MM + 19 IgG MM + 2 MM IgM), together with 4 MGUS, 1 Hodgking Lymphoma and 1 Mielodisplasic syndrome were analyzed. In addition, SPE and IFE were performed for all the samples *Results.* Normal HLC ranges were calculated (Table 1) and were found to be in agreement with the ranges previously published. There was a good correlation between the summation of HLC K + λ and the values of total Igs (tIgs) (tIgA vs HLC IgA, r2 =0,91; tIgG vs HLC, IgG r2 =0,91). Regarding the sensitivity of the assay for the identification of pathological cases, 13/16 IgA and 15/19 IgG patients presented altered HLCr. Interestingly, patients who presented normal HLCr for both IgA and IgG were in complete response with negative IFE. The 2/2 IgM, 3/4 MGUS patients, 1/1 Hodgking Lymphoma and 1/1 Mielodisplasic syndrome presented normal HLCr. *Conclusion.* HLC assays allowed the determination (typing and quantification) of specific individual immunoglobulin heavy/light chains concentrations and the respective ratios. Due to the higher sensitivity, HLCr could be a more sensible tool for quantitatively monitoring treatment response since altered ratios were observed in patients during complete response. It seems to present an enormous potential for the identification and the follow-up of patients with very low monoclonal components or in cases where it is difficult to identify a monoclonal protein hidden by other proteins.

0286

ANKHD1 IS HIGHLY EXPRESSED IN MULTIPLE MYELOMA AND PLAYS A ROLE IN PROLIFERATION AND IN THE ACCUMULATION OF CELLS IN THE S PHASE OF CELL CYCLE

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Background. Ankyrin-repeat-containing proteins regulate multiple cellular functions including transcription, cell cycle and cell survival. Ankyrin repeat and KH domain-containing protein 1 (ANKHD1) is one such protein ubiquitously expressed in normal human tissues and has a varied and high expression in cancer, including acute leukemias. Multiple myeloma (MM) is a plasma cell malignancy preliminary localized in the bone marrow and characterized by its capacity to disseminate. Previous study by our group showed that ANKHD1 is highly expressed in plasma cells of MM patients compared with normal individuals; however its role in the development of MM is still undetermined. *Aim.* The present study was aimed to study the expression of ANKHD1 mRNA and protein in a panel of multiple myeloma cell lines. In addition we used lentiviral mediated RNA interference technique to down regulate the expression of ANKHD1 gene in human myeloma cell line U266 and studied its effect on apoptosis, proliferation and cell cycle. *Methods.* MM cell lines U266, MM1S, MM1R and RPMI 8266 were used. ANKHD1 mRNA and protein expression were evaluated using real time RT-PCR and Western Blot. Localization of ANKHD1 in cells were analysed by laser confocal microscopy. Specific shRNA-expressing lentiviral vector to ANKHD1 or LacZ gene (control) was designed and used for transduction in U266 cell line. Quantitative PCR (q-PCR) and Western blot analysis were performed to determine the inhibition of ANKHD1 expression. After 48 hours of culture, proliferation was analyzed by MTT assays, apoptosis by Anexin-V and propidium iodide (PI), cell cycle by incubation with PI and RNase A buffer and flow cytometry. *Results.* ANKHD1 mRNA and protein was found to be highly expressed in all MM cell lines as evident by q-PCR and Western blot when compared to K652, a leukemia cell line (positive control). Confocal microscopy showed ANKHD1 to be predominantly localized in cytoplasm of MM cell lines. Lentiviral mediated ANKHD1 shRNA downregulated ANKHD1 mRNA expression and protein level significantly, with a downregulation of 88% and 92%, respectively when compared with control cells (P<0.0001). MTT assays showed that the proliferation was significantly reduced by 70% in ANKHD1 knockdown cells when compared with control cells (P<0.0001). Cell cycle analysis showed an increase of cells in S phase (59.3±1.5 versus 49.4±2.4; P<0.0001) in ANKHD1 knockdown cells as compared to control cells. However, annexin-V analysis showed no significant increase in apoptosis of cells on silencing ANKHD1 expression. *Conclusion.* ANKHD1 is highly expressed in all MM cell lines studied and is predominantly localized in the cytoplasm. Downregulation of ANKHD1 in MM cell line U266, effectively caused decrease in cell proliferation and increase in S phase cells suggesting that ANKHD1 plays a role in cell proliferation and in the accumulation of S phase cells. Further studies are being carried out to elucidate the underlying mechanisms.

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0287

99MTC-TRICARBONYL-TOCILIZUMAB: A NEW MOLECULAR IMAGING AGENT IN MULTIPLE MYELOMA

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Background. Interleukin-6 (IL-6) is a key molecule in the pathogenesis of multiple myeloma (MM), resulting in myeloma cell proliferation.[1] Overexpression of IL-6 receptor (IL6R) in MM cells is being studied as a molecular target for imaging as well as for treatment. Tocilizumab, a humanized anti IL6R monoclonal antibody has been conjugated with ^{99m}Tc through HYNIC showing good stability *in vitro* and *in vivo*.[2] Conjugation of ^{99m}Tc-Tocilizumab by means of tricarbonyl could be a suitable option as imaging agent in MM, with the advantage of not requiring derivatization previous to its labeling. *Aims.* Development of 99mTc(CO)3-Tocilizumab. Evaluation as imaging agent in MM. *Methods.* 1ml of 99mTc-pertechnetate solution was added to the IsoLink carbonyl labeling agent (Mallinckrodt). 1 mg Tocilizumab (Roche) was incubated with 1mL $[^{99m}Tc(CO)_3(OH2)_3]^+$ at pH 7, during 45 min at 37°C. Radiochemical purity was controlled by chromatographic systems: ITLC/NaCl 0.9%, Whatman 1MM/MEK, ITLC in BSA/EtOH-NH3-H2O (2:1:5) as stationary and mobile phase and by HPLC using size exclusion column and a phosphate buffer 0,01 M, pH7 isocratic gradient as mobile phase at 1ml/min. Stability of $^{99m}Tc(CO)_3$-Tocilizumab was evaluated in PBS and challenged with hystidine for 24 h. Binding studies to U266 MM cells were performed incubating 200000 cpm of the conjugate with 1000000 cells in 1ml culture medium at 30, 60 and 120 min. Specificity of binding was supported by competition experiments using unlabeled antibody. Tocilizumab was derivatized with FITC and purified by PD10 column. Laser scanning confocal microscopy was done with an excitation/emission wavelength of 488/530 nm. Fluorescent images were obtained. Atomic force microscopy imaging of U266 cells were done. Biodistribution studies were performed at 24 h in CD1 normal mice (n=3). Each mice was in-

jected with 37 MBq of $^{99m}Tc(CO)_3$-Tocilizumab and sacrificed 24 h after injection. Organs of interest were collected. *Results.* Radiochemical purity was 91.0 ± 1.1% and 93,5 ± 0.5% at 1 and 2 h respectively, remaining stable at 24 h, showing no significant transchelation. Competition experiments using cold antibody showed a reduction in binding to U266 cells superior to 50%, confirming the specificity of binding. Confocal microscopy proved the ability of the fluorescent antibody to recognize the IL6R in U266 myeloma cells. Biodistribution at 24 h showed blood (4.2) %act/g, liver (3.4) %act/g, kidney (1.2)%act/g and spleen (1.1) %act/g uptake with hepatic and renal elimination. *Conclusion.* Tocilizumab was easily labeled to 99mTc through tricarbonyl with good stability, radiochemical purity and specificity. These results were similar results to those obtained in its conjugation through HYNIC (2). $^{99m}Tc(CO)_3$-Tocilizumab may be a useful imaging agent in MM.

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0288

EXTRACELLULAR MATRIX REMODELING AND STROMAL CELL-DERIVED TUMOR PROMOTION IN THE BONE MARROW REFLECT THE PROGRESSION OF MGUS TO MULTIPLE MYELOMA

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Background. The pathogenesis of multiple myeloma (MM) is regarded as a multistep process, in which an asymptomatic stage of MGUS precedes virtually all cases of MM. Molecular events leading to transition from MGUS to MM are still poorly defined. Genetically, MGUS plasma cells resemble MM plasma cells in many features, and the clinically apparent step-wise progression of MGUS to MM is poorly reflected by genetic aberrations. *Aims.* We hypothesized that the bone marrow microenvironment is critically involved in the pathogenesis of monoclonal gammopathies. Therefore, we performed a comparative proteome profiling study and investigated the contribution of bone marrow fibroblast precursor cells to disease progression in MM. *Methods.* Primary bone marrow fibroblasts from patients with MGUS and MM were compared to control fibroblasts obtained from hip replacement surgery. Primary cells were cultured for 3 to 5 passages, characterized by immunophenotyping (FACS analysis), fractionated into cytoplasmic, nucleic and secreted protein fractions and then analyzed using shotgun proteomics. Confirmatory experiments were performed using Western blotting. *Results.* Strikingly, a group of extracellular matrix (ECM) proteins, ECM receptors and ECM-modulating enzymes was found to be progressively up-regulated from controls to MGUS and to MM. These proteins include laminin 8, lysyl hydroxylase 2, nidogen-2, integrin alpha-5, macrophage mannose receptor 2, PAI-1 and MMP-2. Additionally, the growth factors periostin and stem cell growth factor as well as PDGF-receptor beta showed a similar progression-related pattern. *Conclusion.* Our results indicate that ECM remodeling and stromal cell-derived tumor promotion in the bone marrow takes place already at the level of MGUS and becomes even more pronounced in MM. Thus, for the first time, marker proteins could be identified indicating a step-wise progression from MGUS to MM.

0289

TUMOR-PRIMED NATURAL KILLER CELLS FROM PATIENTS WITH MULTIPLE MYELOMA LYSE AUTOLOGOUS, NK-RESISTANT, BONE MARROW-DERIVED MALIGNANT PLASMA CELLS

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Background. Natural killer (NK) cells are cytotoxic lymphocytes able to kill tumor cells and virus-infected cells. Human resting NK cells can be activated by co-culture with NK-resistant CTV-1 cells. These tumor-activated cells (TaNK) are cytotoxic to a range of NK-resistant tumor cells *in vitro*. This potential, has not been explored in multiple myeloma (MM). *Aim.* The current study was design to assess the relative function *in vitro* of NK and TaNK cells from MM patients compared to normal controls in the lysis of tumor cell- lines and freshly isolated autologous and allogeneic MM cells. In addition we explored whether the ability to generate TaNK cells *in vitro* correlates with patients' characteristics, including the disease status and treatment, including novel agents. *Methods.* Freshly isolated CD56+ NK cells from normal donors and 21 MM patients, separated with CD56 immunomagnetic Microbeads were co-incubated with CTV-1 cells or lysates there from, overnight at 37oC, 5% CO2, to generate TaNK cells. For the cytotoxicity assay, the erythroleukemia cell-line K562 with known sensitivity to NK lysis was used as a positive control, the Burkitt's lymphoma cell-line Raji, known to be refractory to NK lysis was used as a negative control of NK killing. The Myeloma cell-line U266 as well as freshly isolated bone marrow (BM) autologous and allogeneic CD138+ plasma cells from MM patients were used to evaluate the sensitivity of plasma cells to MM patients' NK and TaNK lysis. Cytotoxicity was measured in a 4-h assay. Loss of membrane integrity was measured by ingress of To-Pro-3 iodide as determined by flow cytometry. Bone marrow plasma cells, were acquired after electronic gating on the CD138(+) cells, and the mean proportion of CD138 positive/To-Pro 3 iodide- positive cells from the samples was determined. *Background.* Target-cell death was determined from cells incubated in the absence of effector cells and the "percent lysis - CD138+"was calculated by subtraction of the background cell death. To determine the specificity of malignant plasma cell lysis, CD138-ve cells were gated in the same analysis and the "percent lysis - control cells"determined as above. This was subtracted from the "percent lysis - CD138+"to give a "percent specific lysis". *Results.* We have demonstrated that TaNK cells from MM patients lyse several myeloma targets, including autologous and allogeneic CD138+ myeloma cells whilst sparing CD138-ve BM cells. Myeloma patients' TaNK-induced lysis of the U266 cell-line was significantly higher compared to normal controls (median specific lysis 79.1% vs 69.5%) (p=0.003). In addition, TaNKs induced substantial lysis of autologous and allogeneic CD138+ myeloma cells (median specific lysis 52.5% and 37.4%, respectively). The percentage of specific lysis did not correlate with important disease characteristics, (ISS, age, high-risk cytogenetics), nor with the disease status and anti-myeloma treatment, including novel agents and dexamethasone. *Summary/Conclusions.* Tumor-primed NK cells are able to induce substantial lysis of myeloma targets including autologous and allogeneic CD138+ myeloma plasma cells and could be an additional therapeutic approach in MM, particularly in the era of novel agents.

0290

ANALYSIS OF SHELTERIN COMPONENTS MRNA EXPRESSION IN MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE. CORRELATION WITH CLINICAL CHARACTERISTICS

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Background. Shelterin is the mayor protein complex (TRF1, TRF2, TIN2, POT1, TPP1 and RAP1) bound to mammalian telomeres, involved in telomere length (TL) regulation and chromosome ends protection. Previous data from our group have provided the first evidence of modifications in the mRNA expression of TRF1 and TRF2 in plasma cell disorders (Panero *et al.,* Mol Med 2010). *Aim.* The aim of the present study was to evaluate the participation of the other four members of the shelterin complex: TIN2, POT1, TPP1 and RAP1, in patients with multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS). Results were correlated with TRF1, TRF2 and hTERT (telomerase catalytic subunit) expression, and TL. Clinicopathological characteristics of patients were also evaluated. *Methods.* Bone marrow samples from 145 patients: 77 with MM (35 males; mean age: 68 years; range: 30-87 years; 35.5% ISS stage 3) and 68 with MGUS (29 males; mean age: 69.1 years; range: 39-88 years) and, 15 normal controls were studied. All patients gave informed consent and the study was approved by the local Ethics Committee. Real-Time Quantitative PCR was used to quantify gene expression and TL measurements were performed by Terminal Restriction Fragments. For statistical analysis, Mann-Whitney test, Kendal coefficient and receiver operating characteristic (ROC) were used. *Results.* Differences in the

Table 1.

Analysis of telomere-associated genes expression and mean TL according to hTERT groups in patients with multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS).

Groups (n)	Telomeric genes (ΔΔCt)							TRF (kb)
	hTERT	TRF1	TRF2	TIN2	POT1	TPP1	RAP1	(ΔΔCt)
MM								
GA (48)	0.32±0.04	0.29±0.10	0.07±0.01	2.05±0.2*	0.05±0.01*	0.54±0.05	0.24±0.02*	7.02±0.38
GB (30)	6.08±1.04*	0.20±0.03	0.12±0.02*	2.54±0.31	0.06±0.01	0.84±0.09*	0.37±0.07	5.36±0.35*
MGUS								
GA (53)	0.28±0.05	0.41±0.08	0.04±0.003	1.36±0.12	0.016±0.001	0.45±0.05	0.19±0.04	7.33±0.42
GB (15)	4.47±0.8*	0.23±0.04	0.07±0.02	2.10±0.32	0.019±0.006	0.41±0.08**	0.24±0.06	6.83±0.81

Significant difference in MM of group B (GB) with respect to group A (GA): *p<0.01.
Significant difference in GB between MGUS and MM: **p<0.001.
Significant difference in GA between MGUS and MM: p<0.01

mean mRNA levels of telomere-associated genes in patients with respect to normal controls were observed (p<0.04). The comparison between both entities showed higher POT1 (p=0.0007), RAP1 (p=0.02), TPP1 (p=0.03) and TIN2 (p=0.003) mRNA levels in MM with respect to MGUS patients. In both pathologies an up-regulation of hTERT and a positive association with RAP1 and TPP1 (p<0.0002) were found. For a better analysis, patients were divided into two groups according to hTERT levels using ROC curves: hTERT mRNA levels <1.08 (Group A; GA) and >1.08 (Group B; GB). Table 1 shows the global analysis of all shelterin genes, hTERT and TL. In both entities, an increased gene expression in GB with respect to GA, with significant differences in MM for TPP1 and TRF2 as well as shorter TL (p<0.01) was observed. TPP1 also showed significant differences between GBs from MM and MGUS (p=0.003). Higher levels of RAP1 (p=0.009), POT1 (p=0.002) and TIN2 (p=0.01) in GAs from MM and MGUS were also found. In MM, the analysis of clinical characteristics showed a negative association between hemoglobin and POT1 and RAP1 expression (p<0.03), while the percentage of bone marrow infiltration was positively correlated with POT1 and TPP1 mRNA levels (p=0.03). RAP1 expression was also positively associated with calcium and creatinine levels (p<0.001). Although non-significant, a shorter overall survival in MM patients of GB compared to GA was observed. *Conclusion.* Our findings show a global modification in the expression of telomere-associated genes in MM and MGUS, suggesting that the up-regulation of most of the shelterin components may contribute to the stabilisation of short telomeres by delaying/repressing the telomere damage signals, contributing to the development and/or progression of the disease.

0291

CLONALITY ASSESSMENT USING 8-COLOR FLOW CYTOMETRY

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Background. Monoclonal gammopathies (MG) are characterised by presence of different numbers of clonal plasma cells (PC), lower in monoclonal gammopathy of undetermined significance (MGUS) and higher in multiple myeloma (MM). Discrimination between normal polyclonal PC (N-PC) and abnormal clonal PC (A-PC) is important in time of diagnosis and especially after treatment (transplantation etc.). Expression of surface markers CD19 and CD56 on PCs is widely used for this discrimination, but unfortunately in some cases is not enough sufficient. *Aims.* Verification and validation of 8-color flow cytometry protocol for clonality assessment of PC and B cell subsets in MGs. *Methods.* Analyses of 22 MG patients in various stages of diagnosis and treatment were done (14 newly diagnosed MGUS/MM, 6 treated MM and 3 relapsed/progressed MM patients). Combination of CD38/CD138/CD19/CD56/CD45/CD27/ck-appa/clambda was used to detect clonality of PCs subpopulations, mature B cells and memory B cells by flow cytometry. *Results.* There was found 0.4% (0.04-13.40) [median (min-max)] of $CD38^+CD138^+$ PCs in bone marrow. Only N-PCs ($CD19^+CD56^-$ PCs) were found in 4 patients (2 new diagnoses, 1 progression and 1 post-treatment) and only clonal A-PCs ($CD19^-CD56^{+/-}$ PCs) were found in 3 patients (1 new diagnosis, 1 progression and 1 post-treatment). Remaining cases were characterized by mixture of N-PCs and A-PCs, but detailed analysis of PC subpopulations using cytoplasmic kappa/lambda expression shown that not all $CD19^-$ PCs and/or $CD56^+$ PCs are clonal as was expected. This detailed analysis could be very useful in minimal residual disease (MRD) monitoring. Simultaneous analysis showed presence of clonal mature B cells and clonal memory B cells as well in 5 cases (4 new diagnoses and 1 post-treatment), so presence of lymphoproliferation should be verified in these patients. *Summary/Conclusions.* Analysis using combination of surface and intracellular markers in 8-color setting can improve detection even small clone of pathological PCs and together with B cell assessment can be very important for determination of diagnosis and monitoring after treatment.

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0292

CENTROSOME ASSOCIATED GENES IN MULTIPLE MYELOMA

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Background. Multiple myeloma (MM) is a lymphoproliferative disease characterized by clonal expansion of neoplastic plasma cells within the bone marrow. The genome of malignant plasma cells is extremely unstable characterized by a complex combination of structural and numerical abnormalities. It is suggested that centrosome abnormalities, of which centrosome amplification is the most prominent, occur early in MM pathogenesis and increase with disease progression. Centrosome amplification is therefore associated with deregulation of cell cycle, mitosis, DNA repair and proliferation. *Aims.* The objective of our study was to evaluate changes in expression profile of genes involved in centrosome structure/function in MM with known role in oncogenesis. *Methods.* 57 patients were evaluated by gene expression profiling of PCs. CD138+ cells were separated by MACS. Total RNA was transcribed into cDNA (Ambion WT Sense Target assay), labeled and hybridized to the Affymetrix GeneChip Human Gene ST 1.0 array according to a manufacture protocol. Acquisition of Affymetrix array images, RMA normalization algorithm and hierarchical clustering algo-

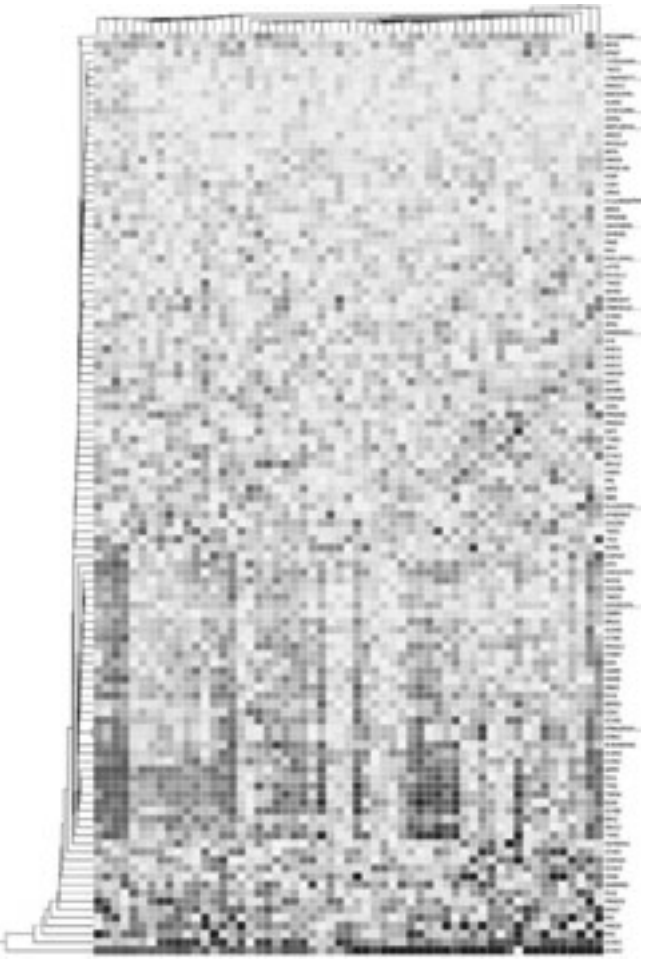

Figure 1.

rithm with Euclidean similarity measure were performed using appropriate software. 100 genes were selected for clustering. All of these genes are known to be involved in regulation of centrosome duplication and function with known role in oncogenesis. *Results.* We have found one distinct cluster of 36 genes. Based on ontology, the following gene groups can be distinguished: genes associated with cell-cycle, including CDK1 and CDK2 families, E2F and Plk families AURKA and NEK2; kinetochore and microtubule attachment genes (AURKB, BIRC5, CENP family); mitotic checkpoint genes presented by BUB1 and MUD2 families; DNA damage checkpoint genes presented with centrosome amplification supressors, integrity and reduplicate regulators (RAD51, XRCC2, MSH2, CHEK1) and structural genes presented with TUBG1. The rest of genes from revealed cluster (HMMR, TACC3, TOP2A, BRCA1 and BARD1) are also closely functionally connected with mentioned gene groups. *Conclusions.* In our study, we showed distinct cluster of 36 mitotic genes involved in centrosome abnormality in MM. Similarity of expression profile in revealed cluster gives us the possibility to suspect that these genes are involved in MM cell oncogenesis as a whole complex. This fact needs more detailed investigation and in our futher work we anticipate new insights into pathogenesis and possible crucial role of centrosome abnormalities in myeloma malignancy.

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0293

CANCER-TESTIS ANTIGENS OF THE MAGE FAMILY INDUCE SPONTANEOUS HUMORAL RESPONSES IN PATIENTS WITH MULTIPLE MYELOMA

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Background. We have previously shown that cancer-testis (CT) antigens are specifically expressed in the bone marrow (BM)-infiltrating plasma cells of patients with multiple myeloma (MM) and that certain members of the MAGE family are the CT genes most commonly detected in MM. In addition MAGE genes, such as MAGE-C1/CT7, MAGE-C2/CT10 and MAGE-A3, seem to independently promote the progression of MM. *Aims.* In this study, we investigated for the first time the occurrence of spontaneous humoral responses against these promising targets for the antigen-specific therapy of MM. *Methods.* Peripheral blood (PB; N=1347) plasma samples from 225 MM patients and PB samples from 97 healthy donors were screened for antibody responses against MAGE-A1, MAGE-A3, MAGE-A8, MAGE-A11, and MAGE-C2/CT10 by ELISA and western blot. A B cell ELISPOT assay was applied to determine the number of CT antigen-specific memory B cells in the PB of MM patients. *Results.* MAGE-A11-, MAGE-A1-, MAGE-A8-, and MAGE-A3-specific antibody responses occurred in 17 (7.6%), 5 (2.2%), 4 (1.7%), and 3 (1.3%) MM patients respectively, at least once throughout the course of their disease. The most commonly detectable humoral responses were directed against MAGE-C2/CT10 being present in 33 (15%) of MM patients. In agreement with this finding, we were also able to demonstrate for the first time the presence of MAGE-C2/CT10-specific memory B cells in the PB of MM patients by ELISPOT. In a western blot analysis, spontaneous MAGE-C2/CT10-specific immune responses in the patients were found to be highly specific for both natural and recombinant protein. Epitope mapping in an ELISA using overlapping MAGEC2/CT10 20mer peptides further showed that antibody responses were restricted to regions of the full-length protein spanning amino acids 40-60, 160-180, 180-200, and 270-290. MAGEC2/CT10-specific antibodies consisted mainly of IgG2 and to a lesser extent of IgG1, IgG3 and IgG4 subtypes. *Conclusions.* Cancer-testis antigens of the MAGE family, especially MAGE-C2/CT10, are capable of inducing spontaneous humoral response in MM patients. These antigens represent promising targets for the antigen-specific immunotherapy of MM but might also be of use as diagnostic and/or prognostic parameters for myeloma represent potential targets for immunotherapy in patients with multiple myeloma, vaccination targeting this kind of antigen could remarkably enhance the corresponding response.

Red blood cells - Clinical and transfusion

0294

A MULTICENTER, OPEN LABEL STUDY OF LENALIDOMIDE AND PREDNISONE (RP) FOLLOWED BY LENALIDOMIDE, MELPHALAN AND PREDNISONE (MPR) IN NEWLY DIAGNOSED ELDERLY MULTIPLE MYELOMA PATIENTS

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Background. The combination of Melphalan-Prednisone-Lenalidomide (MPR) has shown promising results in elderly newly diagnosed myeloma patients. In young myeloma patients, low-dose chemotherapy (induction) precedes high-dose chemotherapy (autologous transplantation consolidation). This approach reduces tumor mass, with few side effects, before achieving the maximum cyto-reduction with transplantation consolidation. The same approach has been designed for the elderly patients: lenalidomide plus corticosteroids precedes consolidation with MPR. *Aims.* To evaluate the safety and efficacy of Lenalidomide-Prednisone (RP) as induction, followed by Melphalan-Prednisone-Lenalidomide (MPR) as consolidation and RP as maintenance in elderly myeloma patients. *Methods.* Unfit patients with newly diagnosed symptomatic myeloma older than 65 years were enrolled in a two-stage phase II clinical trial designed according to Bryant and Day method. No exclusion criteria were included in the protocol, to avoid the selection of fit elderly subjects only. Patients with low blood count, abnormal performance status, hepatic, renal, cardiac or pulmonary functions were enrolled. Patients received 4 RP courses (Lenalidomide 25 mg/day for 21 days for 4 cycles, plus Prednisone 50 mg three times/week for 4 months) followed by 6 MPR cycles (Melphalan 2 mg and Prednisone 50 mg three times/week for 6 months plus Lenalidomide 10-15 mg/day for 21 days for 6 cycles) and maintenance with RP (Lenalidomide 10 mg/day for 21 days and Prednisone 25 mg three times a week until PD). Two different dose-levels of Lenalidomide were tested in combination with MP: 15 mg (dose-level 1) and 10 mg (dose-level 2). Each cohort included 12 patients, with additional 22 patients enrolled at dose-level 2. *Results.* Forty-six patients (median age 75, range 65-88) were enrolled. Forty-four patients were evaluable after a median follow-up of 13.3 months. During RP induction, the most frequent grade 4 hematological adverse events were neutropenia (7%) and anemia (2 %), no grade 4 thrombocytopenia was observed. During MPR consolidation no increase in grade 4 adverse events was registered, incidence of neutropenia was 11%, while no grade 4 anemia and thrombocytopenia were observed. Non-hematological toxicities were more frequent during RP cycles and reduced during MPR cycles (cutaneous rash and infections). Discontinuation rate was higher during induction (14% vs 5%). After RP induction, at least partial response (PR) rate was 73%, at least very good partial response (VGPR) was 16%. During MPR consolidation, PR rate increase to 78%, including 24% of patients who achieved at least a VGPR. CONCLUSION: Induction with RP followed by consolidation with MPR showed a manageable safety profile with a reduced the risk of severe haematological toxicity in unfit elderly myeloma patients.

0295

AN UPDATE ON THE PHASE 1B/2 DOSE-ESCALATION STUDY OF CARFILZOMIB WITH LENALIDOMIDE AND LOW-DOSE DEXAMETHASONE (CRD) IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background. Carfilzomib, a novel epoxyketone that specifically and irreversibly inhibits the proteasome, has shown promising single-agent activity in relapsed/refractory multiple myeloma (R/R MM) with a favorable side-effect profile and minimal myelosuppression. Lenalidomide with low-dose dexamethasone (Rd) is a standard of care for patients with relapsed MM. In preclinical studies, lenalidomide sensitized MM to bortezomib, suggesting that combination therapy with these complementary agents may enhance clinical activity. Although the clinical utility of bortezomib/Rd is limited by toxicity, the combination of carfilzomib with lenalidomide and dexamethasone (CRd) may be similarly efficacious and prove to be more tolerable. *Aims.* This phase 1b/2 study evaluated the maximum tolerated dose (MTD) of CRd in patients with relapsed or refractory MM. The safety and antimyeloma activity of the highest dose was further assessed in an expanded cohort. *Methods.* Eligible patients included those with relapsed or refractory MM following 1-3 prior therapies (including prior lenalidomide and/or bortezomib). CRd was given on 28-day cycles to 6 cohorts in a 3+3 dose-escalation design. The dosing cycle included carfilzomib on days 1, 2, 8, 9, 15, and 16; lenalidomide on days 1-21, and dexamethasone on days 1, 8, 15, and 22 (Table). An additional ~40 patients enrolled in an expansion cohort at the highest dose level. Primary endpoints included safety and establishment of the MTD. Grading of adverse events (AEs) was performed according to NCI CTC v3.0. Additional endpoints included overall response rate (ORR, including partial response or better) as assessed by IMWG criteria, with secondary assessment of clinical benefit response (CBR, minimal response or better) using EBMT criteria. *Results.* Approximately 75% of the patients enrolled were previously treated with bortezomib. MTD was not reached. Eighty-one patients (50 at the highest dose) were response-evaluable. Initial responses generally occurred within the first 2 cycles and improved with continuing therapy. Responses were observed at all dose levels (Table). As of December 2010, median duration of response has not been reached (>14 mo). Patients have continued on therapy for up to 28 months, and prolonged administration has not resulted in any unexpected toxicities. Most AEs were reversible and manageable. AEs ≥ Grade 3 were primarily hematologic, including neutropenia, thrombocytopenia, and anemia. Twelve patients discontinued treatment due to AEs. *Conclusions.* The combination of full-dose carfilzomib (20 or 27 mg/m^2) with Rd was well-tolerated in MM patients who had received 1-3 prior treatment regimens, including bortezomib or immunomodulators. At the highest dose tested ORR was 78%, and prolonged administration led to no new or overlapping toxicities. CRd is being directly compared to Rd in patients with relapsed MM in ASPIRE, an ongoing phase 3 open-label, international, multicenter trial.

Table 1.

	CFZ, mg/m^2	LEN, mg	Dex, mg	nCR/CR	VGPR	PR	MR	SD
Cohort 1 (n=6)	15	10	40	-	2	1	-	2
Cohort 2 (n=6)	15	15	40	-	1	-	2	2
Cohort 3 (n=8)	15	20	40	1	2	2	1	1
Cohort 4 (n=5)	20	20	40	1	2	1	1	-
Cohort 5 (n=6)	20	25	40	-	2	2	1	-
Cohort 6+Exp (n=50)	27*	25	40	9	11	19	1	4
			Total	11	20	25	6	9
			ORR	69.1%				
			CBR	76.5%				

0296

AN ABNORMAL NON-HYPERDIPLOID KARYOTYPE IN MULTIPLE MYELOMA PREDICTS FOR AN ADVERSE OUTCOME AFTER HIGH DOSE THERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANATATION (HDT/ASCT)

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Background. Despite routine HDT/ASCT in younger patients (pts), a huge heterogeneity in overall survival (OS) outcomes is still observed. This relates to the underlying biological heterogeneity of MM. We evaluate the impact of pre-transplant characteristics and the eventual post-transplant response on the OS of pts who underwent HDT/ASCT in 2 major transplant centers in Singapore. We sought to identify important prognostic factors that may predict clinical outcomes in the era of novel therapy. *Methods.* 122 of 483 newly diagnosed MM pts were enrolled to our HDT/ASCT programme from 1999 to 2009. Pt and disease characteristics including age, gender, cytogenetics, ISS staging, induction therapy, bortezomib exposure and the eventual post-transplant response were evaluated. For induction therapy, pts received VAD chemotherapy and thalidomide/dexamethasone (TD) before and from 2004 respectively. Bortezomib was available for treatment of relapsed MM and induction treatment of high-risk MM from 2005. All pts received cyclophosphamide 4g/m2 as mobilization followed by peripheral blood stem-cell collection prompted by G-CSF. HDT/ASCT entailed conditioning with melphalan 200mg/m2. Response was defined according to IMWG uniform criteria. *Results.* The median age of all pts was 55 years. 22%, 43% and 35% of patients presented with ISS stage I, II and III disease respectively. Metaphase cytogenetics detected abnormalities in 44% of pts (hypodiploidy [16%], hyperdiploidy [22%], pseudodiploidy [4%] and near tetradiploidy [3%]). For MM subtypes: IgA 13%, IgG 34%, IgD 1%, and light chain MM 7%. Interphase FISH for high-risk markers [deletion17p, t(4;14), t(14;16)] was positive in 12/52 pts (23%) diagnosed after 2005. For induction, 40% received VAD, while 40% and 20% received TD and bortezomib-based combination respectively. At a median follow-up of 3 years, 65 pts (53%) have been exposed to bortezomib (31% in frontline and 69% at relapse). Overall, the median OS is 8.3 years. Median OS for pts with ISS stage I, II, and III were not reached, 8.1 and 5.1 years respectively (p=0.09). The median OS for pts with diploid, hyperdiploid and non-hyperdiploid karyotype were not reached, 5.1 and 2.8 respectively (p<0.001). When this analysis was further stratified by bortezomib exposure (non-exposed, frontline or relapse), the impact of cytogenetics was no longer apparent only among the group of pts who received frontline bortezomib induction. There was a non-significant trend for longer OS among pts attaining at least a very good partial response (≥VGPR) (9.5

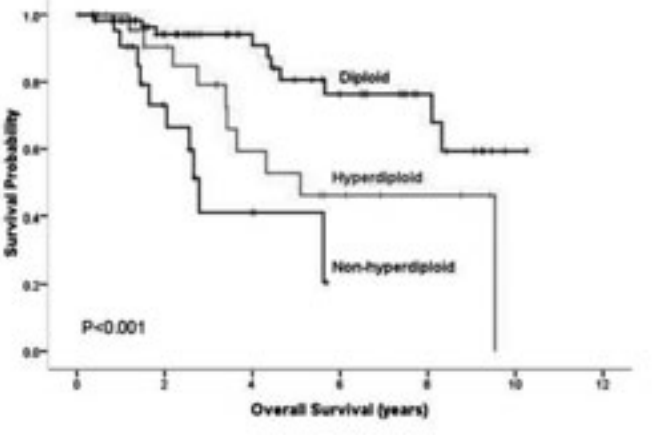

Figure 1. Overall survival by metaphase cytogenetics (N=122).

years vs 8.1 years, p=0.15). On Cox regression multivariate analysis, the presence of a non-hyperdiploid karyotype emerged as the single most adverse prognostic indicator after HDT/ASCT (hazard ratio 4.1, 95% CI 1.2, 14.0, p=0.03). *Conclusion.* Our study suggests that while HDT/ASCT may prolong the OS of transplant-eligible pts, it is still unable to overcome adverse cytogenetics detected on conventional metaphase karyotyping. Upfront bortezomib combination prior to HDT/ASCT rather than sequential use at relapse should be considered in this group of pts. Although the attainment of ≥VGPR post-HDT/ASCT has been reported as an important surrogate marker of better prognosis, it does not appear to confer any benefit for pts with a non-hyperdiploid karyotype.

0297

A NOVEL ESTIMATED GFR FORMULA, BASED ON CYSTATIN-C, INDEPENDENTLY PREDICTS FOR SURVIVAL IN PATIENTS WITH NEWLY-DIAGNOSED, SYMPTOMATIC, MULTIPLE MYELOMA: RESULTS IN 157 PATIENTS

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Background. Renal impairment (RI) is a common complication of multiple myeloma (MM). Cystatin-C (Cys-C) is considered as a sensitive marker of glomerular filtration rate (GFR). A recent study in >3,400 patients with chronic kidney disease (CKD) showed that estimating GFR (eGFR) based on serum creatinine(sCr), Cys-C, age, gender and race (eGFR/Cys/Cr) provides the most accurate GFR estimates: eGFR=177.6 x Scr-0.65 x CysC-0.57 x age-0.20 x (0.82 if female) x (1.11 if black). However, this new formula has not been evaluated in MM. *Aim.* The aim of this study was to evaluate eGFR/Cys/Cr in newly-diagnosed MM patients, compare it with eGFR assessed by MDRD equation and explore possible correlations with clinical data, including survival. *Methods.* We studied 157 newly-diagnosed, symptomatic, MM patients (87M/70F, median age 68 years) before any kind of therapy and evaluated both eGFR/Cys/Cr and eGFR/MDRD. Serum Cys-C was determined by particle enhanced immunonephelometry (Dade Behring, Liederbach, Germany). *Results.* Serum Cys-C was increased in MM patients compared to 52 controls [median: 1.01 mg/L vs. 0.72 mg/L, p<0.0001] and was significantly correlated to sCr (R2=0.497, p<0.001) and beta2-microglobulin (R2=0.42, p<0.001). Median values of eGFR/Cys/Cr (68.7 ml/min/1.73m2) were not different compared with those of eGFR/MDRD (66.1 ml/min/1.73m2). There was also no difference in terms of number of patients with RI of different stages between the two estimates (39% of patients had CKD 3-5 by the MDRD equation and 41% by the eGFR/Cys/Cr equation). However, in 15% of patients, there was a difference in CKD staging according to the two methods: 13 (8%) patients were CKD 3-5 by eGFR/Cys/Cr but CKD 1-2 by eGFR/MDRD, while 10 (6%) were CKD 1-2 by eGFR/Cys/Cr but CKD 3-5 by eGFR/MDRD. eGFR/Cys/Cr was correlated with ISS (p<0.001). The median survival was 48 months (median follow-up: 20 months). In the univariate analysis, CKD 3-5 assessed by eGFR/Cys/Cr (HR:2.93, p<0.001) was a stronger prognostic factor than CKD 3-5 assessed by eGFR/MDRD (HR:1.85, p=0.018). Elevated LDH, ISS stage, bone disease and myeloma subtype (light chain only MM vs. others) were also associated with survival in the univariate analysis. In ISS-2, eGFR/Cys/Cr could identify a subgroup of patients with poor survival: 19 patients with eGFR/Cys/Cr <68.7 ml/min/1.73m2 had a median survival of 23.9 months, while the median survival of all other (n=32) has not been reached (p=0.003). However, eGFR/MDRD, either by using the median as a cutoff or by using the CKD stage, could not discriminate prognostic groups within ISS-2. In a multivariate analysis eGFR/Cys/Cr was the strongest prognostic factor for survival, either as continuous variable (HR 0.986; p=0.004) or as a dichotomous at median (HR:0.380, p=0.01); other factors included bone disease (HR 2.220; p=0.018) and LDH (HR 2.782; p=0.025). *Summary/Conclusions.* These results suggest that eGFR/Cys/Cr estimates RI comparable to eGFR/MDRD. However, only eGFR/Cys/Cr strongly correlates with survival, while it could identify a subgroup of patients with ISS-2 disease with poor outcome. This may be due to the better reflection of both renal function and tumor burden by eGFR/Cys/Cr, as Cys-C is also overproduced by myeloma cells.

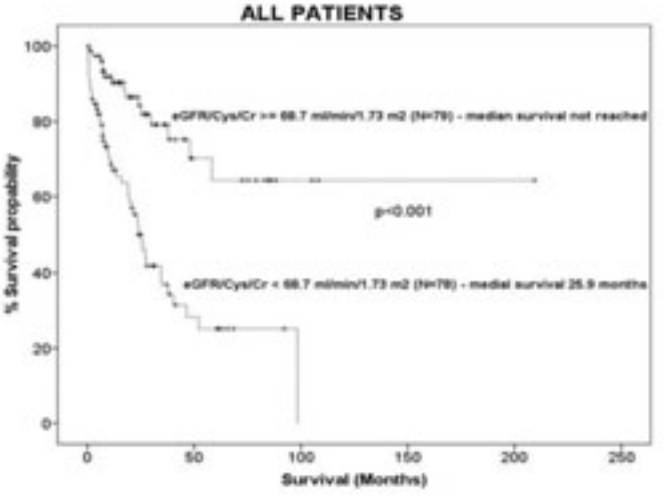

Figure 1.

0298

PATIENT-REPORTED QUALITY OF LIFE (QOL) IN PREVIOUSLY UNTREATED, ELDERLY MULTIPLE MYELOMA (MM) PATIENTS TREATED WITH BORTEZOMIB-BASED REGIMENS: RESULTS FROM THE PHASE 3B UPFRONT STUDY

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Background. The ongoing community-based phase 3b UPFRONT study compares the safety and efficacy of three bortezomib (Vc)-based regimens, Vc-dexamethasone (VcD), Vc-thalidomide-dexamethasone (VcTD), and Vc-melphalan-prednisone (VcMP), followed by Vc maintenance therapy, in previously untreated, transplant-ineligible MM patients. *Aims.* To measure changes in patient-reported QoL during Vc-based induction and maintenance. *Methods.* Patients with symptomatic, measurable MM were randomized (1:1:1) to receive 49 weeks of treatment; eight 3-week induction cycles with VcD, VcTD, or VcMP followed by five 5-week maintenance cycles with single-agent Vc. All patients provided written consent. Adverse events (AEs) were graded by NCI-CTCAE v3.0. Responses were assessed according to IMWG criteria. Patient QoL was recorded using the EORTC QLQ-C30 questionnaire, which assesses global health status, multiple function scores and symptom scores including fatigue, nausea and vomiting, pain, dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties. Patients completed the questionnaire at baseline, on day 1 of every odd-numbered cycle, at the end-of-treatment visit, and every 12 weeks thereafter. Data were analyzed after 300 patients, 100 per arm, had the opportunity to undergo all 13 treatment cycles. Data imputation was used for patients who died within a year of randomization with missing QoL assessments assigned the worst possible score of zero. Changes in mean global health status scores from baseline, within and between treatment arms, were calculated. Observed data were further analyzed using a linear mixed effect model; a sensitivity analysis using last observation carried forward was also performed. *Results.* Patient baseline characteristics were well balanced across the treatment arms, as reported previously (Niesvizky, ASH 2010). Patients received a median of 9 (VcD), 6 (VcTD), and 7 (VcMP) treatment cycles; 56%, 33%, and 43% of patients, respectively, received Vc maintenance. After 13 cycles, re-

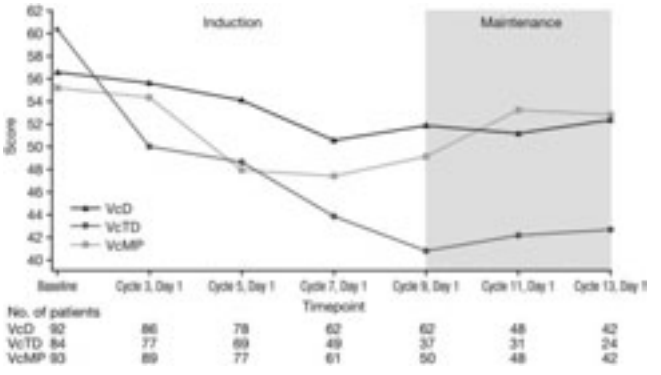

Figure 1. Mean observed global health status score by arm.

sponse rates were 71% (VcD), 79% (VcTD), and 73% (VcMP); ≥VGPR rates were 39%, 47% and 44%, respectively. Rates of grade ≥3 AEs after 13 cycles were 74% (VcD), 86% (VcTD), and 80% (VcMP); serious AEs were highest for VcTD (61% vs 57% VcD and 51% VcMP), as was the rate of discontinuation due to AEs (41% vs 29% VcD and 35% VcMP). QoL assessments were available at baseline and ≥1 post-baseline time point for 80% (VcD), 67% (VcTD), and 80% (VcMP) of patients. The observed data indicate that all treatment groups experienced a downward trend in mean global health status score until cycle 7 or 8, followed by an increase or stabilization by the end of treatment (Figure); there were no differences between treatment arms. Symptom scores changed very little during induction with all Vc-based regimens, with moderate improvements seen during maintenance, except for nausea/vomiting and diarrhea. *Conclusions.* The trends to decreased QoL score seen during Vc-based induction may reflect the onset of treatment-associated AEs. Post-induction increases in QoL may reflect the positive impact of achieving a response and the limited toxicity profile associated with Vc maintenance. Patients continue to be followed for QoL assessment and long-term outcomes.

0299

ADVERSE CYTOGENETICS DO NOT AFFECT RESPONSE RATE OR DURATION IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (R/R MM) TREATED WITH SINGLE-AGENT CARFILZOMIB

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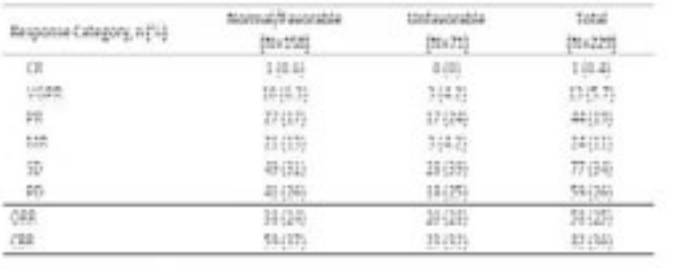
Background. The impact of cytogenetic abnormalities on the response to therapies and survival in patients with multiple myeloma (MM) is well-established in the literature. Bortezomib, alone or in combination with other agents, may overcome the adverse impact of several common unfavorable cytogenetic features. Additionally, responses with lenalidomide (LEN)/dexamethasone and pomalidomide have been reported in patients with unfavorable cytogenetics. Carfilzomib is a novel, highly selective epoxyketone proteasome inhibitor that produces durable single-agent activity in patients with R/R MM. *Aims.* The objective of this analysis was to evaluate the influence of cytogenetics in a large phase 2b study (PX-171-003-A1) of single-agent carfilzomib in patients with R/R MM. *Methods.* 229 of 266 patients enrolled (86%) were response-evaluable and had available metaphase cytogenetics and/or fluorescence in situ hybridization (FISH) analyses for adverse cytogenetics defined per mSMART criteria (hypodiploidy, chromosome 13 deletions, del 17p13, t(4:14), and t(14;16) chromosomal abnormalities). Metaphase data were available for 200 patients (75%), FISH data for 205 patients (77%). All patients received carfilzomib at 20 mg/m^2 IV on days 1, 2, 8, 9, 15, and 16 in a 28-day cycle (C) in C1 followed by 27 mg/m^2 in each C thereafter for up to 12 C. Patients who completed 12 C of therapy were eligible to continue carfilzomib on extension study PX-171-010. Responses were assessed and confirmed by an Independent Review Committee. Overall response rate (ORR) was defined as ≥PR by International Myeloma Working Group criteria, and clinical benefit response (CBR) was defined as ≥MR by European Group for Blood and Marrow Transplantation criteria. *Results.* Patients had disease that was relapsed after ≥2 treatment regimens including bortezomib and either thalidomide or lenalidomide, and were refractory to their most recent regimen. Patients in this heavily pre-treated population had received a median of 13 prior anti-myeloma drugs and a median of 5 prior treatment regimens. Patients had previously received treatment with bortezomib (99.6%), thalidomide (74%), lenalidomide (93%), and stem cell transplantation (72%). 71 of 229 patients (31%) had ≥1 cytogenetic abnormality. Of these, 47/71 (66%) had abnormalities detected via metaphase cytogenetics, 44 (62%) by FISH, and 20 (28%) by both methods. The presence of deletion 13 or hypodiploidy by cytogenetics or del17p13, t(4;14), or t(14;16) by FISH did not significantly impact the ORR. Specifically, 28% of patients with ≥1 abnormality responded compared to 24% with none. The CBR was also unaffected at 32% versus 37% for patients with ≥1 and no abnormalities, respectively. For patients with ≥1 abnormality, the median duration of response (DOR) was 6 months (95% CI, 4-10) compared with a DOR of 8 months (95% CI, 6-11) in patients with no abnormalities. *Conclusions.* Carfilzomib demonstrated durable and comparable activity in patients with R/R MM in both the absence and presence of cytogenetic abnormalities including hypodiploidy, chromosome 13 deletions, del 17p13, t(4:14), or t(14;16). The results of the present study suggest that durable responses to carfilzomib can be achieved in heavily pretreated patients and these responses are not influenced by poor prognostic cytogenetic features.

Table 1.



0300

SINGLE-AGENT CARFILZOMIB ACHIEVES HIGH RESPONSE RATES IN PATIENTS WITH BORTEZOMIB-NA VE RELAPSED MULTIPLE MYELOMA (MM): UPDATED RESULTS FROM STUDY PX-171-004

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Background. The selective, epoxyketone proteasome inhibitor carfilzomib produces potent, sustained proteasome inhibition while lacking many of the off-target activities associated with bortezomib. Carfilzomib produces durable single-agent activity in patients with relapsed/refractory multiple myeloma (R/R MM) who have received multiple prior therapies, as well as in patients with advanced-stage disease or significant comorbidities. PX-171-004 is an ongoing phase 2 study of single-agent carfilzomib in patients with relapsed or refractory MM following 1-3 prior therapies. *Aims.* Here we present updated data on the bortezomib-naïve patients treated on study. *Methods.* Patients received either 20 mg/m^2 for all treatment cycles (Cohort 1) or a stepped-up dose-escalating regimen of 20 mg/m^2 for Cycle 1 and 27 mg/m^2 for all treatment cycles thereafter (Cohort 2). Carfilzomib was administered on days 1, 2, 8, 9, 15, and 16 of every 28-day cycle, for a maximum of 12 cycles. The primary endpoint was the overall response rate (ORR; CR + VGPR + PR) determined according to the International Myeloma Working Group Uniform Response Criteria. Secondary endpoints included the clinical benefit response (CBR; ORR + MR) rate, time to progression (TTP), duration of response (DOR), and safety. *Results.* 123 of 125 enrolled bortezomib-naïve patients were evaluable for response. Prior therapies included thalidomide (58%), lenalidomide (59%), alkylating agents (82%), and stem cell transplant (73%). 44 patients had disease refractory to the most recent therapy. A median TTP of 8.3 months and a median DOR of 13.1 months were observed for Cohort 1. The median TTP and DOR for Cohort 2 have not been reached. The most common treatment-emergent adverse events (AEs), regardless of relationship to carfilzomib, were fatigue (60%), nausea (45%), anemia (40%), and dyspnea (36%). These were primarily ≤ Grade 2 in severity. The most common Grade 3/4 AEs were anemia (13%), lymphopenia (13%), pneumonia (13%), neutropenia (12%), and thrombocytopenia (11%). Treatment-emergent peripheral neuropathy (PN) was infrequent (18%) and mild. Only 1 case of Grade 3 PN (0.8%) was observed. Overall, 49 patients (~40%) completed 12 cycles (~1 yr) and 38 patients (29%) continued to receive carfilzomib therapy on extension protocol PX-171-010, including 11 patients from bortezomib-naïve Cohort 1 and 27 patients from bortezomib-naïve Cohort 2. Significantly, no cumulative toxicities have been noted. *Conclusions.* Single-agent carfilzomib achieves high response rates in bortezomib-naïve patients with relapsed myeloma, with minimal neuropathy. At the recommended phase 3 dose of carfilzomib, the 53% ORR is noteworthy for a single-agent regimen for patients with myeloma who had received 1-3 prior regimens for MM. Moreover, prolonged carfilzomib treatment is well-tolerated, with ~40% of patients completing 12 cycles and 29% continuing treatment beyond 1 year.

Table 1.

Best response	Cohort 1 N=59 20 mg/m^2 n (%)	Cohort 2 N=64 20/27 mg/m^2 n (%)
CR	2 (3)	1 (2)
VGPR	8 (14)	17 (27)
PR	15 (25)	16 (25)
MR	10 (17)	6 (9)
SD	13 (22)	12 (19)
ORR (CR + VGPR + PR)	25 (42)	34 (53)
CBR (ORR + MR)	35 (59)	40 (63)

0301

EVALUATION OF TWICE-WEEKLY AND WEEKLY DOSING OF THE INVESTIGATIONAL AGENT MLN9708, AN ORAL PROTEASOME INHIBITOR, IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: PHASE 1 DOSE-ESCALATION STUDIES

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Background. MLN9708 is an investigational, orally bioavailable, potent, reversible, and specific 20S proteasome inhibitor. MLN9708 is active in solid tumor and hematologic xenograft models. IV and oral formulations are in phase 1 trials. *Aim.* Two studies of oral MLN9708 are ongoing to assess its safety, maximum tolerated dose (MTD), pharmacokinetics (PK), and anti-tumor activity in patients with relapsed/refractory multiple myeloma (MM). *Methods.* Adults with MM after ≥2 prior therapies, which must have included bortezomib, thalidomide/lenalidomide, and corticosteroids, were eligible. Patients provided informed consent. Patients received MLN9708 on days 1, 4, 8, 11 of 21-day cycles (twice-weekly [TW]) or days 1, 8, 15 of 28-day cycles (weekly [W]). Dose-escalation proceeded from 0.24 mg/m^2 using a standard 3+3 schema based on the occurrence of dose-limiting toxicities (DLTs) in cycle 1. Adverse events (AEs) were graded by NCI-CTCAE v3. PK samples were collected after single and multiple doses for both schedules. Response was assessed by modified EBMT criteria. *Results.* To date, 26 patients (16 male, median age 64.8 years [range 50-83]) have received MLN9708 TW at 0.24, 0.48, 0.8, 1.2 (each n=3), 1.68 (n=4), 2.0 (n=7), and 2.23 mg/m^2 (n=4), and 22 patients (12 male, median age 63.5 years [range 40-76]) have received MLN9708 W at 0.24, 0.48, 0.8, 1.2 (each n=3), 1.68 (n=4), 2.23, and 2.97 mg/m^2 (each n=3). Patients received medians of 4 cycles (range 1-12+) on the TW and 2 cycles (range 1-8+) on the W schedules; 9 and 8 patients remain on treatment, respectively. Safety data are shown in the Table. The MTD for MLN9708 TW was established as 2.0 mg/m^2 and MTD on the W schedule has not yet been reached. All-cause grade 3/4 AEs on the TW schedule included thrombocytopenia (n=6), neutropenia (n=2), and non-cardiac chest pain (n=2) and on the W schedule included

Table 1.

	MLN9708 TW (n=26)	MLN9708 W (n=22)
DLTs	Grade 3 rash, Grade 4 thrombocytopenia (both at 2.23 mg/m²)	0
MTD	2.0 mg/m²	Not reached (escalated to 2.97 mg/m²)
All-cause common AEs, % Fatigue Diarrhea Nausea	 42 42 31	 41 27 27
All-cause grade ≥3 AEs, %	50	14
Drug-related grade ≥3 AEs, %	35	0
Serious AEs, % (all unrelated)	35	9
Discontinuations due to AEs, n	0	1
On-study deaths, n	1 (unrelated)	0

syncope, pathologic fracture, and musculoskeletal pain (each n=1). Baseline grade 1 neuropathy was present in all patients reporting treatment-emergent neuropathy (peripheral [PN] or sensory [PSN]) or paresthesia (P). With TW dosing, 6 patients had grade 1 PN/P and 1 had a grade 2 event (unrelated). With W dosing, one grade 2 and three grade 1 PSN/P events were seen. For both TW and W schedules, MLN9708 was absorbed rapidly with T_{max} of ~0.5-2.0 hr and terminal half-life of ~5-7 days. Steady-state was not achieved following Day 11 dose for TW and Day 15 dose for W dosing. Exposures appeared to increase proportionally with increasing dose from 0.8 to 1.68 mg/m^2. With TW dosing, 1 patient receiving 1.2 mg/m^2 achieved a partial response in cycle 5, which was maintained through cycle 11+; 16 had stable disease, of whom 5 have received ≥8 cycles. With W dosing, 3 patients have reported stable disease to date. Response evaluation is ongoing in both studies. *Conclusions.* These data suggest oral MLN9708 is generally well tolerated and has early signs of activity. W dosing appears to enable delivery of higher MLN9708 doses. Combination trials guided by these data are ongoing.

0302

A SUMMARY OF SAFETY AND EFFICACY DATA ACHIEVED WITH LONG-TERM CARFILZOMIB (CFZ) TREATMENT IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA (R/R MM)

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Background. Carfilzomib (CFZ) is a novel, highly selective proteasome inhibitor that differs from BTZ both structurally and mechanistically. CFZ does not cause cumulative toxicity (including neurotoxicity) in long-term (6-9 month) chronic animal toxicology studies. Phase 2 clinical trials of single-agent CFZ in MM demonstrated durable responses in patients with relapsed and relapsed/refractory (R/R) multiple myeloma (MM), including patients with pre-existing peripheral neuropathy and substantial renal dysfunction. *Aims.* Here we report on the updated clinical experience with long-term treatment (>12 cycles) with carfilzomib in patients with MM. *Methods.* Patients treated in phase 1 or 2 MM trials were eligible to enroll in an extension study after 12 cycles. Patients initially received carfilzomib on Days 1, 2, 8, 9, 15, and 16 of every 28-day cycle. The dosing frequency could be reduced to alternate weeks (ie, Days 1, 2, 15, and 16). Dose increases up to a maximum of 56 mg/m^2 were also permitted. Depending upon the time of administration from the patients' original study, IV carfilzomib was administered over a period of either 10 minutes or 30 minutes. In selected patients, a second anti-myeloma agent was added. Patients continued treatment until evidence of progressive disease or unacceptable toxicity, or individual withdrawal of consent. *Results.* As of 31 January 2011, 78 patients with MM had enrolled. The relative proportion of patients rolling over was highest from the 2 phase 2 trials: 29/266 patients (11%) from 003-A1 (R/R MM pts) and 38/129 patients (29%) from 004 (bortezomib-naïve, 1-3 prior regimens). Sixty-one MM patients (78%) remain on study and are receiving a median dose of 27 mg/m^2 (range: 15-56 mg/m^2). The median total duration of treatment (original study + extension study) is 18 months. 29 patients remain on their original dosing schedule, and 21 patients are receiving the intermittent dosing schedule. The longest total duration of treatment is >30 months. 13 patients are off-study due to progressive disease, 1 patient was removed at the investigator's discretion, and 1 patient withdrew consent. 2 patients had dose reductions due to toxicity, and there have been no withdrawals due to toxicity. 15 patients had their doses of carfilzomib increased, 2 patients added lenalidomide, and 4 patients added 40 mg/wk dexamethasone. Clinically significant cumulative toxicities were not observed. *Conclusions.* CFZ can be safely administered to patients with MM for extended therapy using the either the original dosing schedule or an intermittent schedule. The maintenance carfilzomib treatment regimen sustains disease control and provides excellent long-term tolerability.

Table 1.

Baseline Parameters	(n=50)
Median time since initial diagnosis	6 years
Unfavorable cytogenetics/FISH	16%
Prior regimens, median (range)	3 (1–12)
Prior bortezomib	60%

0303

EFFICACY AND SAFETY OF LENALIDOMIDE IN COMBINATION WITH LOW DOSE DEXAMETHASONE (LD) AS FIRST LINE TREATMENT OF PRIMARY PLASMA CELL LEUKEMIA (PPCL)

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Background. PPCL is an aggressive, rare variant of multiple myeloma, whose prognosis is usually poor. Lenalidomide is an IMiD®-immunomodulatory agent with proven efficacy in multiple myeloma. There are some sporadic reports of PPCL treated with lenalidomide as salvage therapy, but no data are currently available on the use of this drug as first line therapy. *Aims.* On March, 2009, we started the first multicenter pilot study aiming to evaluate safety and antitumor activity of LD in previously untreated PPCL. *Methods.* Newly diagnosed patients received lenalidomide at a dose of 25 mg/d for 21 days and oral dexamethasone at a dose of 40 mg on days 1, 8, 15, and 22 for each 28-day cycle. After 4 cycles, responding patients not eligible for stem cell transplantation (SCT) continued until 8 cycles of full-dose LD, if tolerated, followed by a maintenance dose of single agent lenalidomide equal to 10 mg/d on days 1-21 of each 28-day cycle. Patients responding after 4 cycles and eligible for SCT proceeded according to single Centre transplant policy. Patients not responding after 4 cycles or progressing during treatment were considered off-study. The primary endpoint was early response rate according to International Uniform Criteria. The secondary endpoints were PFS, OS, safety, and percentage of eligible patients able to undergo autologous or allogeneic SCT. Biological markers (cytoge-

netics, SNP and GEP studies) were also evaluated (results reported in a different abstract). Appropriate dose reductions, contraception methods and anti-thrombotic prophylaxis were applied. *Results.* According to the Simon Optimal Two-Stage Adaptive Design, twenty out of 22 planned patients have been so far enrolled (M/F 11/9; median age 66 years, range 45-81). Circulating plasma cells ranged from 655 to 70.000 x 10e9/l. Moderate renal failure, increased LDH and extramedullary disease occurred in 35%, 55% and 30% of patients, respectively. Hb was < 10 g/dl in 14 patients (70%), while platelet count was < 50 x 10e9/l in 5 patients (25%). Cytogenetic abnormalities were frequently combined in complex karyotypes and detected in 15 out of 17 (88.2%) analyzed patients: there were 12 del13, eight del17, four t(11;14), seven t(14;16) and one t(4;14). With a median follow-up of 14 months, eleven out of 18 evaluable patients achieved at least PR (61.1%), with 39% of patients achieving at least VGPR on intention-to-treat analysis. Causes of early interruption were progressive disease (four patients), adverse events (one acute renal insufficiency, one Stevens-Johnson's syndrome), one death in PR due to causes unrelated to treatment or disease. Grade 3-4 hematological and non hematological toxicities occurred in 29.4% and 35.2% of patients, respectively. Dose adjustment was required in 11/18 patients (61%), including those with initial renal failure. One-year OS and PFS were 77% and 50%, respectively. Four out of 6 eligible patients underwent autologous SCT (one patient refused, one patient failed to collect PBSC): to-date, all transplanted patients are alive in remission phase. *Conclusions.* LD is a promising initial therapy for PPCL, which can rapidly control the disease in the majority of cases, allowing following single patient-adapted therapeutic strategies.

0304

REAL-LIFE DATA ON THE CURRENT APPROACH TO OLDER PATIENTS WITH MULTIPLE MYELOMA

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Background. The combination melphalan-prednisone (MP) has represented the standard of care for older patients with multiple myeloma (MM). The introduction of new agents has challenged the role of MP and led to new standards of care, even in aged individuals. All randomized studies comparing MP with MP plus thalidomide (MPT) showed advantage in progression free survival for MPT; in addition, in two of these studies a survival advantage was recorded. More recently, superiority versus MP was also demonstrated for the combination of MP plus bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the daily practice a proportion of aged patients still receive MP treatment, the real number of them and exact reasons accounting for suboptimal therapy are unknown. *Aims.* In this study, we retrospectively analyzed a consecutive series of 237 patients with MM aged over 65 years, with the aim of assessing the number of patients who did not received a 3-drug regimen and the causes of suboptimal treatment. *Methods.* From 2008 to 2010, 237 patients over 65 years were diagnosed at our institutions. The median age was 74 years and all of them had symptomatic disease requiring treatment. Twenty-five patients (12%), all aged 66-70 years, who received autologous stem cell transplantation were excluded from the analysis. Among the remaining 212, 47 patients received MP (22%), 102 MPT (48%) and 63 (30%) MPV. Among MPT and MPV patients, 66 % were accrued into prospective clinical trials; conversely, no patients within the MP subgroup were judged as eligible for any trial (p >0.001). The median age was 77 years for the MP patient subgroup, as opposed to 71 for MPV and 73 for MPT; the difference was statistically significant between MP and MPV (p=0.004) as well as between MP and MPT (p=0.007), while it was not significant between MPV and MPT (p=.14). The median number of comorbidities requiring specific treatment was 3 (range 1-5) in the MP subgroup as opposed to 1 in the MPT (range 0-1) and 0 (range 0-1) in the MPV subsets. Once again, differences between MP and MPT (p=0.03) and between MP and MPV (p=0.01) were statistically significant. The main criteria for the selection between MPT and MPV were distance from hospital, ability of the patients to travel and the period of observation (more patients received MPV after the registration as first line). *Conclusions.* We conclude that 22% of older MM patients receive suboptimal therapy, i.e. the old MP combination. More advanced median age and number of severe comorbidities requiring specific treatment account for the therapeutic selection. Among patients treated with current standard of care, the choice between MPV and MPT is strictly related to the ability of patients to travel as well as to distance from the hematological institution. Of note, most of data refer to specialized hematological institutions; it is conceivable that in non hematological wards, the percentage of exclusion from the current standard of care can be higher.

0305

BORTEZOMIB-BENDAMUSTINE-DEXAMETHASONE FOR TREATMENT OF RELAPSED/REFRACTORY MYELOMA

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Background. Bendamustine, an alkylating agent with purine analogue like activities and non-cross resistance with several other alkylators, exerts significant activity when combined with the proteasome inhibitor bortezomib. In addition, evidence has been created for a favorable risk/benefit ratio for the triple combination of bendamustine -bortezomib - dexamethasone. *Aims.* In this ongoing phase II trial we evaluate the efficacy of the triple combination in patients with relapsed/refractory myeloma. *Methods.* Up to now 21 patients with relapsed/refractory multiple myeloma have been enrolled. Informed consent was obtained from all patients. Median age at baseline was 62 years (range 46-86), male/female: 8/13. Data documentation is available for 20 patients: ISS stage I: 5, stage II: 7, stage III: 8. 13 patients presented ECOG performance status 2, 7 patients with ECOG 1. Twelve patients had 1-2, 6 patients 3-4, and 2 patients >4 previous treatment lines. 13 of 20 patients had previously been exposed to bortezomib. Treatment regimen: Bendamustine 70 mg/m2 day 1+4, Bortezomib 1.3 mg/m2 days 1, 4, 8 and 11, Dexamethasone 20 mg on days 1, 4, 8 and 11, repeated every 4 weeks. Treatment should be applied for a maximum of 8 cycles; in case of no response or stable disease (SD) after 4 cycles, patients should be switched to another therapy. *Results.* Responses are presently evaluable in 12 patients (≥ 2 cycles completed and fully documented). 2 patients completed 7, 6, 3 and 2 cycles, respectively; 3 patients received 4 cycles and in 1 patient 5 cycles were applied. ORR (CR + nCR + PR + MR) was 66.6% with 1 patient achieving CR (8.3%), 4 nCR (33.3%), 1 PR (8.3%), and 2 MR (16.6%), respectively. Median time to response was 87 days. Responses were observed in 5 (62.5%) of 8 patients previously exposed to bortezomib therapy. In 17 fully documented patients the following grade 3/4 toxicities were observed: thrombopenia in 8 (47.0%), anemia in 4 (23.5%), herpes zoster in 3 (17.6%), and acute renal impairment in 2 (11.8%) patients, respectively. Grade 3/4 diarrhea, constipation, and sepsis were seen in 1 (5.9%) patient each. Interestingly, grade 3/4 leukopenia was noted in 1 (5.9%) patient only, while up to now no grade 3/4 polyneuropathy has been observed. *Conclusions.* The BBD combination exerts significant activity (ORR: 66.6%) in pretreated patients with relapsed/refractory myeloma. Re-induction of response was noted in 5/8 patients previously exposed to bortezomib. The regimen was well tolerated with grade 3/4 thrombopenia being the most frequently (47%) observed side effect. Updated results will be presented at the meeting.

0306

LENALIDOMIDE-DEXAMETHASONE (LD) AS TREATMENT OF ACUTE CAST NEPHROPATHY-INDUCED RENAL FAILURE (ARF) IN MULTIPLE MYELOMA (MM). A PHASE II STUDY

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Background. Light chain-induced renal failure is a severe complication of MM associated with increased risk of infections, dependency on

chronic hemodialysis and shortened survival. Reversal of renal impairment requires immediate installment of effective anti-myeloma therapy and rapid reduction of the pathogenic light chains. *Aims.* To prospectively evaluate the efficacy of Lenalidomide (L)-Dexamethasone (D) in restoring renal function and tumor control in patients with light chain-induced acute renal failure (LC-ARF). *Patients and Methods.* 21 patients with LC-ARF as formerly defined (JCO 2010) have been identified for this trial and informed consent was obtained from all patients; 1 patient died before first study medication and for 2 patients data are not available as yet. Lenalidomide was given from d 1-21 with dose adaptation according to GFR. Dexamethasone 40 mg was administered on d 1-4, 9-12, 17-20 during cycle 1; thereafter 1x/week. Cycles were repeated q 4 weeks. *Results.* Baseline data are available for 18 pts at present: Median age: 68 years (range: 47-87 years), male/female: 8/10, 17 patients presented with ISS stage III (1 data missing). 17 (94.4%) presented with de novo MM and 1 (5.5%) with previously treated, but relapsing disease; median GFR 21.2 ml/min (range 6.1 - 27.6 ml/min). ECOG performance status was 0 in 6 patients, 1 in 4 pts, 2 in 5 pts, 3 in 1 pt and 4 in 2 pts. Presently, 12 patients are evaluable for response (completed ≥2 cycles and fully documented). 6 patients completed all 9 cycles, 2 patients 6 cycles and 1 patient each completed 7, 5, 3 and 2 cycles. nCR was achieved in 7 (58.3%), VGPR in 1 (8.3%), PR in 1 (8.3%), and MR in 3 (25.0%) patients, respectively. yielding a nCR-PR rate of 75%. Median time to tumor response was 129 days. Renal response was assessed as formerly defined (JCO 2010). 2 patients achieved CRrenal and 6 PR/MRrenal, respectively, yielding an ORRrenal in 8 (61.5%) of the evaluable pts. Median time to best renal response was 147 days. 3 of 8 dialysis dependent patients became dialysis independent. Median GFR of evaluable patients increased from 14.7 (range 6.1 - 27.6 ml/min) at baseline to a median best GFR of 28.9 ml/min (range 11.3 - 74.0 ml/min). In 7 patients with nCR median GFR improved from 9.4 to 30.6 (11.3 - 74 ml/min). In 5 patients with VGPR/PR/MR median GFR increased from 14.7 to 26.0 (16.8 - 40.7 ml/min). Full documentation of adverse events is presently available in 16 patients. 2 patients died due to infection (12.5%). Grade 3/4 anemia, thrombopenia and leucopenia, were seen in 9 (56.2%), 2 (12.5%), and 1 (6.25%) patients, respectively. Other common grade 3/4 toxicities were infection 5 (31.2%), and cardiac dysfunction 3 (18.8%). Exanthema, pulmonary embolism, macula edema, and fatigue were seen in 1 patient each (6.25%). *Conclusions.* LD showed significant anti-myeloma activity and improved renal function in 62% of this high risk population. The LD regimen with the dose of lenalidomide adjusted according to GFR was well tolerated. Updated results will be presented.

0307

BORTEZOMIB-BASED THERAPY OVERCOMES THE PROGNOSTIC ROLE OF ISS SCORE IN MULTIPLE MYELOMA PATIENTS NOT ELIGIBLE FOR TRANSPLANT

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Background. Novel agents had considerably changed the outcome in multiple myeloma (MM) patients not eligible for transplant. The achievement of a CR correlates with long-term progression free survival (PFS) even in this setting. PFS could be significantly variable in patients with the same type of response. Among baseline prognostic factors, ISS seems to maintain its role even in patients treated upfront with novel agents. *Aims.* We explore the role of ISS in predicting the length of progression free survival (PFS) in MM patients not eligible for transplant stratified according to response. *Methods.* We retrospectively reviewed the records of 511 patients enrolled in the controlled multicenter phase III GIMEMA trial VMPT-VT vs VMP (Palumbo *et al.*, JCO 28:5101-5109, 2010). Patients were randomly assigned to received 9 VMPT cycle (bortezomib +melphalan+prednisone+thalidomide) followed by VT maintenance (bortezomib+thalidomide) for 2 years (or until progression) (VMPT-VT arm, 254 patients) or 9 cycle of VMP (bortezomib+melphalan+prednisone) without maintenance (VMP arm, 257 patients). The study was performed according to the Declaration of Helsinki, and approved by the ethics committee of each participating institution. Response to therapy was defined according to EBMT criteria. Patients were considered responsive (ORR) when obtaining at least a PR. *Results.* ISS distribution was balanced in the two arms (p=0.40) with 19%/39%/23% and 22%/34%/22% of patients with ISS 3/2/1 in VMPT-VT and VMP arm respectively. ORR was 89% (CR 38%, VGPR 21%, PR30%) in VMPT-VT arm vs 81% (CR 24%, VGPR 26%, PR 31%) in VMP arm (p=0.01). Longer PFS was observed in patients obtaining a CR with no statistical difference between patients with VGPR and PR both in VMPT-VT and VMP arm (p=0.35 and p=0.33 respectively). The prognostic role of ISS on PFS was evaluated in the whole population and after stratification according to response and study arm. In univariate analysis ISS showed no significant impact on PFS (p=0.9). In patients reaching a CR, ISS showed no significant correlation with PFS in both treatment arm (p=0.8 and p=0.6 in VMPT-VT and VMP arm respectively). In patients with less than a CR (VGPR/PR) low ISS (1) was associated with better PFS, only in patients in VMP arm (p=0.04), without significant correlation for patients with similar response enrolled in VMPT-VT arm (p=0.18). *Conclusions.* In MM patients not eligible to transplant, the prognostic role of ISS is overcome by bortezomib-based treatment. In patients with less than a CR, ISS have a negative prognostic impact on PFS only in those who do not receive a maintenance.

0308

LONG-TERM FOLLOW-UP IN PX-171-003-A1, AN OPEN-LABEL, SINGLE-ARM PHASE 2 STUDY OF CARFILZOMIB IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (R/R MM): ANALYSIS BY SUBGROUPS OF INTEREST

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Background. Carfilzomib is a novel, highly selective epoxyketone proteasome inhibitor in development for treatment of MM. Single-agent carfilzomib has demonstrated durable activity in patients with R/R MM in phase 1 and 2 studies. *Aims.* Here we report on clinical experience with single-agent carfilzomib in the open-label, single-arm phase 2 PX-171-003-A1 trial in patients with multiply-relapsed and refractory MM, including those patients with Grade (G) 1/2 peripheral neuropathy (PN) at study entry. *Methods.* Patients must have received ≥2 prior therapies including bortezomib, either thalidomide or lenalidomide, and an alkylating agent. Patients received carfilzomib at 20 mg/m^2 on a QDx2 schedule (Days 1, 2, 8, 9, 15, and 16 every 28 days) in cycle (C) 1 and were dose escalated to 27 mg/m^2 on the same schedule thereafter for up to 12 C. Patients completing 12 C were eli-

Table 1.

Baseline characteristic	Total n	ORR (≥PR)	CBR (≥MR)
Overall	257	61 (24)	93 (36)
Number of prior therapies, n (%)			
<5	110	27 (25)	36 (33)
≥5	147	35 (24)	53 (36)
Baseline PN, n (%)			
None	55	14 (26)	20 (36)
G1/2	202	48 (24)	68 (34)
ISS disease stage, n (%)			
I	76	24 (32)	35 (46)
II	96	24 (25)	34 (35)
III	78	14 (18)	19 (24)

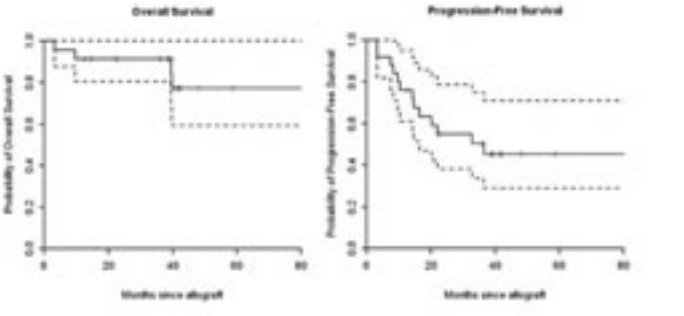

Figure 1.

gible to enter an extension study (PX-171-010). Responses and progression were determined according to the International Myeloma Working Group (IMWG) criteria and were assessed and confirmed by an Independent Review Committee (IRC). The primary endpoint was overall response rate (ORR) (≥ partial response [PR]). Secondary endpoints included: clinical benefit response (CBR) (ORR + Minimal response [MR]), duration of response for ≥PR (DOR), overall survival (OS), time to progression (TTP), progression-free survival (PFS), and safety. PN history, ISS/Durie-Salmon staging, and prior treatment history were collected for all patients for subset analyses. Newly incident PN or worsening PN were monitored by prospective neurologic exams every 2 C. *Results.* Of 266 patients enrolled, 257 were response-evaluable as detailed in the table below. The ORR was 24% with a median DOR of 7.4 months (range 6.2-10.3). 202 of 257 patients (79%) had G1/2 PN at baseline and achieved an ORR of 24%, with a median DOR of 7.4 months (95% CI 5.6-9.2). The OS for all patients was 15.5 months (95% CI 12.7-19.0). The most common treatment-emergent adverse events (AEs) ≥G3 regardless of relationship to study drug were predominantly hematologic and included thrombocytopenia (22%), anemia (20%), lymphopenia (10%), pneumonia (8%), neutropenia (8%), fatigue (7%), hyponatremia (5%), and hypercalcemia (5%). New-onset PN was infrequent and PN ≥G3 was reported in only 2 patients (0.97%). 27 patients completed 12C and continued on extension protocol PX-171-010. Additional subset analysis and updated long-term follow-up data will be presented. *Conclusions.* Single-agent carfilzomib achieved significant durable responses in patients with R/R MM whose disease had relapsed after all available therapies including bortezomib and immunomodulatory agents, including patients with active G1/2 PN at study entry. Carfilzomib was well-tolerated, and AEs were clinically manageable with no new, unexpected, or cumulative toxicities, allowing prolonged single-agent dosing for disease control. Notably, PN had no impact on depth or durability of responses, or on the tolerability of carfilzomib in these heavily-pretreated patients. Importantly, exacerbation of pre-existing PN was uncommon. The CBR and median DOR achieved with this steroid-sparing regimen establish the potential of carfilzomib to offer substantial clinical benefit to patients with relapsed or refractory disease.

0309

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN FIRST LINE MULTIPLE MYELOMA: DOES IT STILL EXIST? RESULTS FROM A MULTICENTRE STUDY

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Material and Methods. This study concerned MM patients (pts) prospectively allocated to receive a tandem auto-allo-HSCT for being of bad prognosis. They received RIC followed by allo-HSCT after achieving at least a PR to auto-HSCT. RIC regimen in majority combined Fluda. 30mg/m²/d (d-5[ARROWRIGHT]d-1), Busilvex IV 3.2mg/kg/d (d-4, d-3) and ATG 2.5mg/kg/d (d-2, d-1). This analysis included 25 pts, 18 males and 7 females, median age 51 years [28-67], there were 15 IgG, 6 IgA and 4 light chains MM, 14 pts had del13, 7 had del17 and 17 had high level of beta2µ; 7 pts had the 3 factors combined and 6 pts had 2 factors combined. For induction therapy, 16 pts received VAD and 9 patients received Vel.D. Pts received auto-HSCT after a median time of 5.5 months [3.6-15.3] from diagnosis. All pts were in PR after auto-HSCT and before allo-HSCT. Allografts came from 16 identical siblings, 8 matched (10/10) and 1 mismatched (9/10) unrelated donors. *Results.* At Day 90, 10 pts were in CR, 15<CR among them 9 received Velcade, 6 received other treatments including DLI. There were 8 acute GVHD (7 grade II and 1 grade III) and 11 chronic GVHD (3 lim. and 8 ext.). At the last follow-up, 10 pts were in durable CR1 post allo-HSCT. After a median follow-up of 40 months [3-125], the median OS was not reached with a 6 years probability of 70% [53-95], the median PFS was 36 months [16-125], with a 6 years probability of 45% [29-71], TRM was 8% at 2 years and reached 12% at 4 years. *Conclusion.* According to these very promising results, we should reconsider the allo-HSCT as a first line treatment for MM especially for pts with poor prognostic factors. The development of novel reduced-intensity preparative regimens, pre- and post-transplantation strategies enhancing the graft-versus-myeloma effect are the important key issues for the future. RIC Allo-HSCT should be optimized rather than replaced.

0310

EVALUATION OF 18F- FDG PET/ CT AND 99MTC- MIBI SCINTIGRAPHY IN PATIENTS WITH MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE: COMPARISON OF METHODS

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Newer imaging modalities, such as 18F- FDG PET/ CT and 99mTc-MIBI scintigraphy, have been recently introduced to assess the aktivity and extent of disease in patients with multiple myeloma (MM) and gammopathy of undetermined signifi cance (MGUS). *The aim* of our study was to compare the impact of these imaging modalities in the evaluation of MM and MGUS patients. *Materials and Methods.* A total of 101 patients with MM (81 patients) and MGUS (20 patients) were enrolled in the study (21 newly diagnosed and 44 relapsed patients with symptomatic MM, 16 with asymptomatic MM and 20 with MGUS). All patients were without therapy and underwent 18F- FDG PET/ CT and 99mTc- MIBI scintigraphy within a maximum interval of 14 days. The scans were classifi ed as normal (N), diff use (D), and focal or combined (F- FD) pattern. *Results.* There was no signifi cant diff erence in the detection of newly diagnosed MM and relapsed patients between the compared methods. 18F- FDG PET/ CT performed better than 99mTc-MIBI scintigraphy in the detection of focal lesions ($p < 0.039$), whereas 99mTc- MIBI scintigraphy was superior in the visualization of diff use disease ($p = 0.042$). 18F- FDG PET/ CT visualised signifi cantly more focal lesions than 99mTc- MIBI scintigraphy ($p = 0.002$), both generally in the cohort and when comparing the number of focal lesions per patient. Both the imaging modalities singly or in combination infl uenced the subsequent clinical management in 17% of patients. In our study, 18F- FDG PET/ CT predicted asymptomatic MM and MGUS transformatio ninto more aggressive forms with the necessity to start therapy more often than 99mTc- MIBI scintigraphy. *Conclusion.* 18F- FDG PET/ CT appeared to be a better imaging technique than 99mTc- MIBI scintigraphy in the detection of focal lesions in patients with symptomatic MM.99mTc- MIBI was superior in the visualization of diff use disease. On the other hand, despite its limited capacity in detecting focal lesions, 99mTc- MIBI scintigraphy still remains the most rapid and inexpensive technique for whole- body evaluation and may be an alternative option when a PET/ CT facility is not available.

0311

EFFECT OF TREATMENT WITH LENA / DEXA OF ASYMPTOMATIC MULTIPLE MYELOMA AT HIGH RISK OF PROGRESSION ON BONE REMODELING MARKERS AND CYTOKINES RELATED TO BONE DISEASE

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Background. We know the changes in bone remodeling markers and cytokines related to bone disease in Multiple Myeloma (MM) patients treated with different drugs (chemotherapy, Bortezomib, Lenalidomide,..), but so far this subject is not studied in smoldering MM (sMM) patients. *Aims.* To analyze sequential changes of serum bone parameters in sMM at high risk of progression treated with Lenalidomide and Dexamethasone. *Material and Methods.* A phase III trial has been conducted by the Spanish Myeloma Group (GEM), in which sMM patients at high-risk of progression were randomized to receive Len-dex (9 cycles) as induction followed by a maintenance phase with Len alone vs. no treatment in order to evaluate whether the early treatment prolongs the time to progression (TTP) to symptomatic disease. In the serum of this patients, we analyzed one bone resorption marker (C-Terminal telopeptide (Ctx)), one bone formation marker (bone alkaline phosphatase (bAP)) and some cytokines related to bone disease pathogenesis in MM (Macrophage inflammatory protein 1-alpha (MIP-1alpha), sRANKL, osteoprotegerin (OPG) and DKK1) before the start of treatment, after 3 and 9 cycles. *Results.* We analyzed these parameters in 33 patients in the treatment group who achieved at least partial remission, (20 of them underwent sequential analysis of the parameters above. The only two parameters that showed significant sequential changes in this subset of patients were DKK1 and OPG. In the case of DKK1, was a significant decrease of the same after 3 months of treatment (mean ± SD: 35.46 ± 26.9 (at baseline) to 20.80 ± 13.21 (after 3 cycles) ($p = 0.007$)), which stabilized after 9 cycles (22.17 ± 13.06). The OPG showed late decrease (6.28 ± 3.07 (at baseline) to 5.10 ± 2.37 (after 9 cycles) ($p = 0.013$)). *Summary.* The decrease in DKK1 has been described in patients with MM. It has been postulated to be due to the reduction of malignant plasma cells, which would also explain the decline in our patients after 3 cycles of treatment. The stabilization of DKK1 levels after 9 cycles could be explained because in patients with low tumor mass, tumor control could be done especially in the first cycles. The OPG is produced by osteoblasts and mesenchymal cells, so the late fall of OPG observed in our study would imply a decrease in the synthesis and osteoblastic activity, which could be due to the action of Dexamethasone.

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0312

NOVEL AGENTS ARE BENEFICIAL FOR REAL LIFE PATIENTS WITH MULTIPLE MYELOMA NOT ELIGIBLE FOR HIGH DOSE TREATMENT

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*Background.*High dose treatment (HDT) in Multiple Myeloma (MM) has been shown to be superior to standard chemotherapy. However, a large amount of patients are not eligible for HDT. Evaluating real life outcome in those patients is important to understand prognostic factors. *Aim.* Evaluate real life responses, time to progression (TTP), time to next therapy (TTNT) and overall survival (OS) and prognostic power of patient variables in real life non-HDT patients with MM. *Methods.* All non-HDT patients, n=269, diagnosed with MM between Jan 2000 and Jul 2010 at Karolinska Huddinge and Jan 2005 and Jul 2010 at Karolinska Solna were included. Near complete response (nCR) was defined as an immeasurable M-protein by standard electrophoresis. Very good partial response (VGPR), partial response (PR), no response (NR) and TTP were defined according to IMW criteria. Standard statistical methods were used. *Results.* The median age was 72 years and 51% male. Baseline median creatinine was 98 µmol/L, albumin 33 g/L, hemoglobin 110 g/L and β2-microglobulin 3.8 mg/l. The median number of treatment lines was 2. 58% of the patients were given at least 2 treatment lines and only 34% 3 or more. The response distribution nCR/VGPR/PR/NR was 15/16/29/40 in 1st line, 13/9/23/55 in 2nd line. nCR in the 1st line implied a 43% probability to receive a ≥VGPR and 26% probability to receive an nCR in 2nd line. NR in the 1st line implied a 58% probability to receive a NR in the 2nd line. Logistic regression analysis shows that the patients receiving novel agents (Bortezomib, Lenalidomide, Thalidomide) in 1st line had a higher probability of achieving nCR. Baseline creatinine values, β2-microglobulin and most likely also albumin and hemoglobin, seem to be of importance for the response. The median TTP/TTNT was 248/301 days in the 1st line 176/187 in 2nd line and 180/210 in 3rd line. There was a significant trend of increasing TTP/TTNT in 1st line depending on the increased depth of the response with TTP/TTNT for nCR of 423/481 days, VGPR 289/376 days, PR 269/376 days and NR 190/176. Kaplan-Mayer analysis shows that the use of novel agents in 1st line predicted a longer TTP. Median OS was 3.6 years 95% CI [2.9;4.2] with 44% censored. The median OS for patients with 1st line best response nCR was 4.9 years, VGPR 3.9 years, PR 4.0 years and NR 1.9 years. There is a correlation between TTP in 1st line and increased OS. Kaplan-Mayer analysis shows that use of novel agents in the 1st line predicted a longer OS, median 5.1 years vs. 2.8 years. Baseline patient variables that showed to have significant importance for OS were creatinine, albumin, hemoglobin and β2-microglobulin. *Summary.* Patients receiving 2nd and 3rd line treatment were declining rapidly. Receiving a nCR in 1st line seems to be very important. Receiving a VGPR or PR seems to give similar results, less good than nCR but better than NR. Variables of importance are creatinine, albumin and hemoglobin. The use of novel agents improves response, TTP and OS.

0313

A COMPARISON OF REDUCED-INTENSITY CONDITIONING FOR ALLOGRAFTING (RICT) FOLLOWING AUTOGRAFTING (ASCT) VERSUS DOUBLE AUTOGRAFTING FOR NEWLY MULTIPLE MYELOMA (MM) PATIENTS. TEN YEARS EXPERIENCE

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Background. Multiple Myeloma (MM) is the most frequent indication for autografting (ASCT). ASCT represents the most effective palliation for these patients. Even the double ASCT where there is demonstrated good long-term control in a minority of patients do not appear to result in cure of disease. Myeloablative AlloSCT is penalized by excessive transplant-related mortality (TRM) and toxicity. The introduction of reduced-intensity conditioning for allografting (RICT) has renewed interest in the use of AlloSCT for MM. *Aims.* Recent studies have reported encouraging results with tandem ASCT followed shortly thereafter by RICT in MM patients as compared to ASCT or myeloablative AlloSCT alone. Here we present an update at 10 years of the results

achieved in our Unit in patients receiving double ASCT compared to patients receiving tandem ASCT/RICT. *Methods.* We enrolled 132 consecutive patients 65 years of age or younger with stage II or III myeloma. One hundred seven patients had siblings and 93 patients and their siblings underwent HLA typing. Thirty-four of them had an HLA-identical sibling. All patients were initially treated with induction chemotherapy consisting of 3-4 courses of VAD regimen or modifications of VAD regimen. Soon after, peripheral blood stem cells (PBSC) were collected after 3-4 gr of cyclophosphamide per square meter of body surface are. G-CSF was given 4-5 days after chemotherapy. Daily aphereses was continued until at least >3x10^6 CD34 cells/kg were collected. After the first ASCT, patients who had an available HLA-identical sibling donor were offered RICT. Patients without an HLA-identical sibling donor underwent a second ASCT. The conditioning regimen of ASCT-1 and ASCT-2 consisted of melphalan 200 mg/m^2 infused over 30 minutes. The RICT consisted of fludarabine 30 mg/m^2 daily for 3 days and TBI (2 Gy or melphalan 70 mg/m^2). Graft-versus-host prophylaxis consisted of cyclosporine A and short-course methotrexate. *Results.* The rate of CR was significantly higher in RICT arm (RICT: 54,1%; ASCT: 28,5%). At December 2010 9 (37,5%) of 24 patients who received RICT and 4 (11,1%) of 35 patients who received double ASCT were in continuous CR after a median of 57 months (range, 14-88 months) and 56 months (range, 28-70 months), respectively. Thirteen (54,1%) patients in the ASCT/RICT group and 12 (34,2%) in the double ASCT were alive at a median of 104 months (range, 45-124 months) and 65 months, respectively. The cumulative incidence rate of acute and chronic GVHD after RICT was 41% and 54%. In the RICT arm, 12 patients died of: extensive chronic GVHD (4 patients), progressive disease, (5 patients) and cGVHD and infections (3 patients). At December 2010, 13 patients in RICT and 12 patients in double ASCT are alive after a median of 104 mo. (range, 45-124) and 62 mo. (range, 20-118), respectively. *Conclusions.* this update retrospective analyses suggests that ASCT/RICT may be able to reduce the incidence of disease progression.

0314

A PHASE IB STUDY OF ORAL PANOBINOSTAT PLUS INTRAVENOUS BORTEZOMIB IN RELAPSED OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA: UPDATED RESULTS AT THE EXPANSION PHASE

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Background. Panobinostat is an oral pan-deacetylase inhibitor (pan-DACi) that increases acetylation of proteins involved in multiple oncogenic pathways. In preclinical studies, panobinostat has demonstrated synergistic activity in combination with bortezomib which may occur through inhibition of the aggresome and proteasome pathways causing an accumulation of intracellular misfolded cytotoxic proteins leading to multiple myeloma (MM) cell apoptosis. *Aims.* This phase Ib study sought to identify the maximum tolerated dose (MTD) of the combination of panobinostat and bortezomib in patients with relapsed or relapsed and refractory MM. Safety and efficacy was further evaluated in an expansion phase using a modified dosing schedule of panobinostat at the MTD of the combination. *Methods.* This study completed enrollment (N = 62) in December 2010 with 47 patients in the dose escalation phase and 15 patients in the dose expansion phase. In the dose escalation phase, panobinostat was administered orally thrice weekly in combination with bortezomib (intravenous, days 1, 4, 8, 11) in 21-day cycles. Dexamethasone was to be added in case of suboptimal response from cycle 2 onwards (n = 16 patients). The MTD was determined to be 20 mg panobinostat plus 1.3 mg/m^2 bortezomib. In the expansion phase, 15 patients received the MTD of panobinostat plus bortezomib, with a modified panobinostat schedule (2 weeks on, 1 week off) and dexamethasone was introduced for all patients, from cycle 2 onwards allowing time for pharmacokinetic analysis of panobinostat and of bortezomib before as well as after introduction of dexamethasone. This dosing schedule was evaluated in an effort to increase tolerability and maximize therapy duration and is identical to the schedule in the ongoing phase II and III PANORAMA trials. *Results.* Patients in the dose-escalation phase received a median of 2 (1-10) prior therapies with 28 having received prior bortezomib and 15 refractory to last bortezomib based therapy (12 progressing under or within 60 days of this therapy). Responses of ≥ minor response (MR) were observed in 36/47 (76%) dose-escalation patients and 10/15 (66%) patients with bortezomib-refractory MM (Table 1). The ≥ partial response (PR) rate was 64% (30/47) and 40% (6/15) among patients with bortezomib-refractory MM. Response assessments are also available at this early cut-off date, for 12/15 patients in the dose-expansion phase with ≥ PR observed in 9/12 patients (75%) including 1/4 bortezomib-refractory patients. Safety data was available for all 62 patients and common grade 3/4 adverse events included thrombocytopenia (n = 47), neutropenia (n = 33), asthenia (n = 13), and anemia (n = 10), with no treatment-related mortality. *Summary/Conclusions.* The combination of panobinostat and bortezomib has a predictable and manageable safety profile with promising activity in advanced MM including in patients with bortezomib-refractory MM. Updated data from the dose-expansion phase will be presented.

Table 1.

Best Response by Cohort

Treatment Phase	Dose Escalation (n = 47, 15 bortezomib-refractory)												Dose Expansion (n = 15, 4 bortezomib-refractory)	
Cohort	1		2		3		4		5		6		7	
Panobinostat	10 mg		20 mg		20 mg		30 mg		25 mg		20 mg		20 mg (2 weeks on, 1 week off)	
Bortezomib	1.0 mg/m^2		1.0 mg/m^2		1.3 mg/m^2		1.3 mg/m^2		1.3 mg/m^2		1.3 mg/m^2		1.3 mg/m^2	
Dexamethasone	-		-		-		-		-		-		20 mg (from cycle 2)	
Patients, n Bortezomib - refractory, n	7	4	7	5	8	2	7	0	9	2	9	2	15	4
Complete Response			1	0	2	0	1	0			2	0		
Very Good Partial Response	1	0			1	0			2	0	1	0	3	0
Partial Response			3	3	2	1	4	0	6	2	4	0	6	1
Minor Response	1	1	1	1	2	1	1	0			1	1		
Stable Disease**	3	1	1	1	1	0	1	0			1	1	2	2
Progressive Disease	1	1											1	1
Not Evaluable	1	1	1	0					1	0				
No data													3	0

Data cutoff as of 14 January 2011
*Dexamethasone was optional
**SD and MR patients listed separately

Myeloproliferative disorders - Biology

0315

NF-E2 OVER-EXPRESSION ALTERS ERYTHROCYTE MEMBRANE STRUCTURE LEADING TO PREMATURE DESTRUCTION IN A TRANSGENIC MOUSE MODEL

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Background. We have previously demonstrated that the transcription factor nuclear factor erythroid-2 (NF-E2) is overexpressed in MPN patients, irrespective of the presence of the JAK2V617F mutation.[1,2] In order to investigate the role of NF-E2 overexpression in the pathophysiology of MPN, we have engineered a transgenic mouse model overexpressing NF-E2 specifically in haematopoietic cells. While this model recapitulates many features of MPN including thrombocytosis, leucocytosis, characteristic BM morphology, and transformation to acute leukaemia,[3,4] surprisingly the mice do not display polycythemia. However, spleens of NF-E2 tg mice show a large increase in iron containing histiocytes, indicating increased red cell destruction. NF-E2 has previously been implicated in the transcriptional regulation of several erythrocyte membrane structural genes, as well as in globin gene expression.[5,6] We therefore tested the hypothesis that NF-E2 overexpression leads to an alteration in erythrocyte membrane structure and physiology, promoting destruction of RBCs in our tg mouse model. *Aims.* To elucidate the effect of NF-E2 overexpression on the RBC phenotype in a novel mouse model of MPN. *Methods.* Two independent transgenic mouse lines were generated which overexpress human NF-E2 under the control of the hematopoietic specific vav-promoter.[3,7] Expression of RBC structural genes as well as of α and β globin was quantified by q-RT-PCR from mouse bone marrow of NF-E2 tg mice and wt littermates. Osmotic fragility assays were performed as previously described.[8] *Results.* NF-E2 tg mice showed a significantly increased expression of several structural erythroid membrane components: Erythroid Protein band 4.1 (Epb4.1, p=0.002 for wt vs tg, n≥15), Alpha Spectrin (p=0.009) and Band3 (Slc4a1, Solute carrier family 4 anion exchanger 1, p=0.03). In contrast, four other structural genes tested (Ankyrin-1, Beta-Adducin, Erythroid Protein band 4.2 and Dematin) were not significantly different between wt and tg animals. The expression of both α and β globin was elevated in the bone marrow of transgenic animals (p=0.03 and p=0.02, respectively). Because β globin expression was increased to a greater extent than α globin, the α/β globin ratio in tg animals fell to 0.69 compared to 0.91 in wt littermates (p=0.006). In addition, the osmotic fragility of NF-E2 tg RBCs was reduced (0.45% NaCl, p=0.01 and 0.5% NaCl, p=0.02), indicating increased rigidity. *Summary/Conclusions.* In NF-E2 tg mice abnormally increased expression of erythrocyte structural protein genes renders erythrocytes more rigid and, by inference, less flexible. We therefore propose that these abnormal RBCs are subject to premature phagocytosis in the spleen. In addition, the disbalance between a and b globin expression in erythrocytes from NF-E2 overexpressiong tg mice contributes to their early destruction. To substantiate this conclusion, *in vivo* RBC turnover measurements, which are currently ongoing, will be presented.

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0316

BONE MARROW STROMA-MEDIATED PARACINE INHIBITION OF RUXOLITINIB (INCB018424)-INDUCED APOPTOSIS OF JAK2V617F MUTATED CELLS: PROTECTIVE EFFECT OF MPN PATIENT-DRIVED BUT NOT HEALTHY DONNOR-DERIVED STROMA

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Myeloproliferative neoplasms (MPNs) are a class of stem cell-derived hematologic malignancies, characterized by an expansion of one or more myeloid lineage with resulting bone marrow (BM) hypercellularity. Because BM cells of most patients with MPN carry a gain-of-function mutation in the Janus kinase-2 gene (JAK2V617F) that contributes to the pathophysiology of the disease, several inhibitors of JAK2 have been developed and their activity has been tested in clinical trials. We have recently reported that Ruxolitinib (INCB018424), an oral inhibitor of JAK1 and JAK2, induced marked and durable clinical benefits in patients with primary myelofibrosis (Verstovesk S *et al*. N Engl J Med 12:1117, 2010). Ruxolitinib induced a dose-dependent suppression of phosphorylated signal transducer and activator of transcription 3 (STAT3), a marker of JAK signaling, however only a mean maximal suppression of 13% from baseline of the JAK2V617F allele burden was seen after 12 cycles of therapy. Therefore, we hypothesized that extracellular signals negate the effects of JAK2 inhibitors. To test our hypothesis, we designed co-culture experiments to test the interaction between the JAK2V617F mutated cells and their BM stromal microenvironment. Ruxolitinib inhibited the proliferation and induced marked apoptosis of the human JAK2V617F mutated SET2 (IC50 56nM) and UKE-1 (IC50 329nM), but not of the JAK2-wild type human mast MHC1.1 and MHC1.2 cells. However, the anti-proliferative effect of ruxolitinib was markedly attenuated when JAK2V617F mutated cells were co-cultured with either primary patient or immortalized patient derived human BM mesenchymal stromal cells (MSC). Specifically, co-culturing JAK2V617F-positive cells with TM-R1 MSC from the myelofibrosis patient hampered the anti-proliferative and pro-apoptotic effect of ruxolitinib and inhibited its ability to dephosphorylate STAT3 and STAT5. However when JAK2V617F-positive cells were co-cultured with MSC from healthy donors the protective effect was not observed. Subsequent experiment demonstrated that protective effect by TM-R1 MSC was due to a paracrine secretion of cytokines, since the effect was observed even when JAK2V617F-positive cells were cultured with MSC separated by micropore filters. Cytokine profile of supernatants from co-cultures of TM-R1 MSC with JAK2V617F-positive cells differed from those of supernatants from co-cultures of normal MSC with JAK2V617F -positive cells (with or without ruxolitinib). Interleukin (IL)-6 was elevated in both co-cultures whereas basic fibroblast growth factor (b-FGF-basic p= 1.05E-17) and CXCL10/IP10 (p= 2.19E-4) were significantly lower in normal MSC/JAK2V617F supernatants. Using neutralizing antibodies against IL6, b-FGF, and CXCL10/IP10, or using shRNA to down-regulate their mRNAs, we blocked the protective effect of TM-R1 MSC. Our results suggest that marrow stroma secreted cytokines play a significant role in protecting JAK2V617F-positive cells from JAK2 inhibitor therapy, thus highlighting the role of the microenvironment not only in the pathogenesis of MPNs but also in the resistance to JAK2-directed therapies.

0317

INCREASE IN CIRCULATING CD4+CD25+FOXP3+ T CELLS IN PATIENTS WITH PHILADELPHIA-NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS DURING TREATMENT WITH INTERFERON-ALPHA

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Background. Recent reports have shown complete or major molecular remission in patients with polycythemia vera after long-term treatment with the immunomodulating agent, interferon-alpha2 (IFN-alpha2). Accordingly, there are reasons to believe that the immune system is a key player in eradicating the JAK2-mutated clone in these patients. Foxp3+ regulatory T cells play a pivotal role in maintaining immune homeostasis and importantly preventing immune reactivity to self antigens. However, their suppressive activity can compromise an

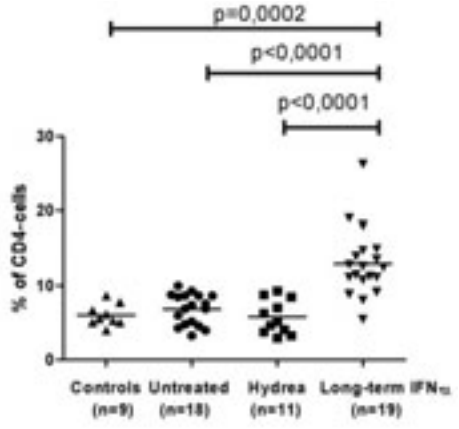

Figure 1. Frequency of CD25+Foxp3+-cells in CMPN patients.

effective anti-tumor immune response and high frequencies of regulatory T cells (Treg) in peripheral blood have been reported in both hematological and solid cancers. Foxp3 is the most specific marker of a regulatory phenotype, but cytokine-producing non-suppressive effector T cells (Teff) can transiently express Foxp3 upon activation. *Aims.* We have analyzed the frequency, phenotype and function of circulating CD4+CD25+Foxp3+ T cells in patients with chronic myeloproliferative neoplasms (CMPNs) treated with IFN-alpha2, hydroxyurea or untreated patients. *Methods.* 48 patients (19 women, 29 men; median-age 61, range 43-83 years) with a diagnosis of PV (n=31), ET(n=13) or PMF(n=4) according to the World Health Organisation (WHO) classification were included in this study. 18 patients were untreated, 11 patients treated with hydroxyurea and 19 patients were treated with pegylated IFN-alpha2 long term (>1 year; mean 40 months). Nine healthy subjects were used as controls (6 women, 3 men; median-age 63 years). Peripheral blood mononuclear cells were isolated and frozen using standard procedures. Flowcytometric analysis was performed after surface and intracellular staining with the following antibodies: APC-Cy7 anti-CD3, PerCP anti-CD4 FITC anti-CD127, APC anti-CD25, PE-Cy7 anti-CD45RA and PE anti-Foxp3. *Results.* We have analyzed the frequency of circulating CD25+Foxp3+ T cells in the CD4+lymphoid compartment of patients with CMPNs. Surprisingly, we found that patients on long-term IFN-alpha treatment had a marked increase in circulating CD4+CD25+Foxp3+ T cells (12,98%;CI 10,78-15,18%) when compared to healthy subjects (6,06%;CI 4,93-7,19%), untreated patients (6,87%;CI 5,84-7,37%) or patients treated with hydroxyurea (5,83%; CI 4,28-7,37%), P<0,0001. Results are given as mean with 95% confidence intervals. When distinguishing phenotypically between activated Tregs and Teff using CD45RA and Foxp3 expression, we found a significant expansion of both subpopulations in patients treated with IFN-alpha compared to other patient categories or healthy subjects (Tregs: P<0,0001; Teff: P=0,001). *Summary.* To date, IFN-alpha is the only therapy which is able to induce minimal residual disease with low-burden JAK2 V617F. We believe that immunological mechanisms are key factors in eradicating JAK2-mutated cells during IFN-alpha treatment. For the first time, we have shown a marked CD4+ T cell response in these patients, and further studies are ongoing to elucidate immunological responses triggered by IFN-alpha. In perspective, we hope to bring more focus on the potential benefit of immunotherapy as frontline treatment in JAK2-positive CMPNs.

0318

CLONALITY AND LOSS OF HETEROZYGOSITY IN MESENCHYMAL STEM CELLS OF PATIENTS WITH CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background. Little is known about Mesenchymal Stem Cells (MSC) derived from the bone marrow (BM) of patients with chronic myeloproliferative neoplasms (MPNs). It has been shown that MSCs from MPN patients with JAK2V617F positive hematopoiesis never harbor the mutation and their immunophenotype is similar to that of MSCs derived from healthy donors (HD). Previous data from our group, obtained by array-comparative genomic hybridization, have documented that recurrent cytogenetic abnormalities in *in vitro* expanded MSCs from MPN patients are present only at late passages (P). However, the possibility that MSCs from MPN patients can be clonal has been never thoroughly investigated. *Aims.* We have studied clonality and loss of heterozygosity (LOH) in *ex vivo* expanded BM-derived MSCs of MPN patients both at early and at late P. The study was approved by the institutional review board of the IRCCS Policlinico S. Matteo Foundation, and all patients gave written informed consent. *Methods.* Nine patients (3 males and 6 females) were studied: 6 patients were affected from Primary Myelofibrosis (PMF), 2 from Essential Thrombocythemia (ET), 1 from Polycthemia Vera (PV); 8 healthy, age matched individuals served as controls. MSCs were isolated and expanded *ex vivo*, according to standard protocols from BM biopsies. DNA was extracted by commercial kit from MSCs both at early (P2-P3), intermediate (P4-P8) and late passages (> P9). Clonality was assessed by the human androgen receptor assay (HUMARA) and LOH was investigated by PCR-based analysis of selected polymorphic microsatellite markers. Results. Four out of 6 female patients were heterozygous at the locus for HUMARA. Of them, 1 (a JAK2V617F positive ET) showed a skewed pattern of X-chromosome inactivation in MSCs at P2, indicating the presence of a clonal population of MSCs at the beginning of the culture. A second JAK2V617F positive ET patient had skewed X-chromosome inactivation at P12. Assessment of X-chromosome inactivation of MSCs at P3 of this patient is ongoing. The remaining 2 patients (both affected from PMF) showed a polyclonal pattern of X-chromosome inactivation. Microsatellite analysis was performed in 2 male patients (1 PV and 1 PMF, both with JAK2V617F positive hematopoiesis): 10% of MSC showed allelic loss at P3; the proportion of cells with LOH progressively increased at P7 and P13, reaching 90% and 100%, respectively. As expected, in all cases JAK2V617F mutation was not detectable and telomerase activity was found at low levels, as in HD-MSCs. Neither skewed X-chromosome inactivation nor LOH were observed in HD-MSCs both at early and at late P. Conclusion. Taken together, our data indicate that a clonal population of MSCs with genetic abnormalities is detectable in *in vitro* culture already at early passages, suggesting that small clones of mutated MSCs can expand in the bone marrow of MPN patients. Further studies are warranted to assess whether these MSCs can contribute to the pathogenesis of MPNs. (486/500).

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APOPTOMIRS EXPRESSION PROFILE IN POLYCYTHEMIA VERA PATIENTS

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Background. Polycythemia vera (PV) is a clonal disorder resulting in a multipotent hematopoietic progenitor cell that causes the accumulation of morphologically normal red cells, white cells, platelets, and their progenitors in the absence of a definable stimulus. Until now many aspects of physiopathology remains unclear and no effective treatment for curing or prevention of disease progress exists. Deregulation of apoptotic machinery might have a role in PV physiopathology. Entirely understanding of apoptotic machinery and its regulation by potential modulation of the microRNA in PV patients cells might unveil novel targets, which may be translated into novel therapies. *Aims.* To quantify the miR-26a, miR21, miR29c, miR-130b and let-7d, expression in Polycythemia Vera (PV) patients and to correlate the data with the expression of target genes. *Subjects and Methods.* 13 PV patients (6 males and 7 females with a mean age of 58.46y) and 13 healthy subjects (6 males and 7 females, m=57.6y). Peripheral leukocytes were obtained by Haes-Steril method, total RNA was extracted according to Trizol® method and High Capacity® Kit was used to synthesize cDNA. Quantification of apoptomirs and its target genes was performed by real time PCR and results were given as $2^{-\Delta\Delta Ct}$. Statistical

analyses were performed by Mann-Whitney tests. *Results.* miR26a, miR21, miR29c, miR130b and let7-d levels were increased in PV (median=4.01; 7.961; 2.135; 2.52 and 8.47; respectively) patients in comparison to controls (m=1.33, 1.19, 1.292; 1.05; 1.64) (p=0.0005; p<0.0001; p=0.0017; p=0.0177 and p<0,0001; respectively). In adition we detected the deregulated expression of *mcl*-1 (m= 2.80), bcl-2 (m= 0.10) anti-apoptotic and *bax* pro-apoptotic (m= 0.28) when compared to controls (m= 0,855; m=1.32 and m= 1.422, respectively) (p= 0.0076, p=0.002 and p=0.0052; respectively). These data suggest that *bcl*-2 expression may be regulated by the miR 21. *Conclusions.* The results indicate that peripheral blood cells patients with polycythemia vera have some microRNA signatures and genes expression distinct from those reported in literature and suggests the potential new targets and pathways that might be modulating apoptosis process and acting in physiopathology of PV.

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0320

CD133+/JAK2V617F+ CELLS DERIVED FROM PERIPHERAL BLOOD OF PRIMARY MYELOFIBROSIS PATIENTS DIFFERENTIATE INTO HEMATOPOIETIC AND ENDOTHELIAL PROGENITORS IN CULTURE

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Background. The JAK2V617F mutation, occuring in variable frequency in 50% of Primary Myelofibrosis (PMF) patients, has been used as a prognostic marker for several years. Even though JAK2V617F has been detected in terminally differentiated or CD34+ circulating cells in PMF patient peripheral blood, its contribution to the outgrowth of the malignant clone has not been elucidated yet. Characterization of JAK2V617F+ circulating primitive stem cells may shed light on our understanding of PMF pathophysiology. *Aim.* To identify the prime stem cell compartment responsible for PMF development, by investigating the clonogenic potential of CD133+ cell populations derived from peripheral blood of PMF patients and by assessing their JAK2V617 mutational burden variability. *Method.* CD133+ peripheral blood circulating cells were isolated from JAK2V617F+ PMF patients and cultured in liquid media supplemented to induce endothelial and hematopoietic differentiation. CD133+ expanded cells were also assessed for clonogenic potential in semisolid media enriched with various growth factors. Outgrown colonies were morphologically characterized, isolated and tested for JAK2V617F allele burden by real time PCR. *Results.* Purified CD133+/CD45+ stem cells derived from PMF patient peripheral blood were positive for the JAK2V617F mutation and expanded in suspension cultures supplemented with differentiation-inducing growth factors. Differentiation induced cultures of isolated CD133+ exhibited two different phenotypes. CD133+ cells expanded under presence of VEGF or G-CSF were found positive when immunostained for endothelial or hematopoietic lineage markers, respectively. RT-PCR analysis for JAK2V617F allele burden in isolated colonies outgrown from individual CD133+ cells indicated variability within progeny that is, differentiated into endothelial or diverge hematopoietic lineages. JAK2V617F allele burden analysis of various hematopoietic and endothelial colonies indicated heterozygotic mutational status to occur mostly in the endothelial and macrophage colonies, while homozygotic in more committed hematopoietic progenitors. *Conclusions.* Our data suggest that: (i) JAK2V617F mutation can occur early in the stem cell compartment of PMF patients contributing to extended genetic instability of one or more malignant clones. (ii) These JAK2V617F+ / CD133+ malignant clones are likely to derive from bipotent cells in the stem cell compartment that are able to differentiate into both hematopoietic and endothelial lineages. *In vitro* evidence indicates that PMF develops from acquired mutations of a stem cell population with hemangioblastic potential.

0321

This abstract has been withdrawn.

0322

IMPLICATION OF BIOLOGICAL FUNCTIONS OF RUNX1 MUTANTS ON OUTCOME OF PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA

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Background. RUNX1 is an essential transcription factor for normal hematopoiesis. We have observed a high mutation frequency (37%) of RUNX1 gene in chronic myelomonocytic leukemia (CMML) whereas the mutation status did not correlate with the clinicohematological features (Leukemia, 2009, 23: 1426-1431). The implication of biological functions of RUNX1 mutants and the disease progression remains to be determined. *Aims.* We aimed to study the biological effects of the RUNX1 mutants and to correlate the functions of mutants to sAML progression and survival. *Methods.* Bone marrow samples from 93 patients with CMML were analyzed for RUNX1 mutations. Mutational analysis of RUNX1 was performed by RT-PCR amplification of whole coding region of RUNX1 followed by sequencing of the PCR products. Each mutation was reconfirmed by PCR with alternative primers. Expression plasmids of wild-type RUNX1 and the mutants were constructed and applied for functional studies of the mutants. Immunoprecipitation followed by Western blotting was performed to confirm the interaction between RUNX1 mutants and CBFβ. Transactivation activities of RUNX1 mutants were examined by luciferase reporter assay. Mutation frequency of RUNX1 and transactivity of the RUNX1 mutants were correlated to sAML transformation of the patients. *Results.* Thirty-four mutations of RUNX1 were identified in 32 patients. Six RUNX1 mutants were homozygous. Seventeen of 32 RUNX1 mutation-positive patients (53%) progressed to sAML compared with 21 of 61 RUNX1 mutation-negative patients (34%) (P=0.081). There was no significant difference in the overall survival between RUNX1 mutation-positive and mutation-negative patients (median survival 11.5 and 13.4 months, respectively, P=0.180). Functional study revealed that RUNX1 mutants of C72W, S114L and P398L which retained more than 70% transactivities of wild-type RUNX1 were designated as high activity mutants; S195fsX209, G324fsX565, M341fsX569, S268fsX578, P332fsX573 and F369fsX572 which exhibited only 40% to 70% transactivities were designated as the medium activity mutants; H78Q, W79R, R80L, N109K, R139G, R177X, R293X, L71fsX94 and I150fsX185 which exhibited less than 40% transactivities were designated as low activity mutants. Patients with lower transactivation activity mutants had a higher risk of sAML transformation (P=0.003) and a shorter time to sAML transformation (P=0.044) whereas the transactivity did not influence the overall survival (P=0.722). *Conclusions.* Our results showed that RUNX1 activity was a determinant factor for the progression of CMML to sAML; lower transactivity mutants were associated with a higher risk and rapid progression to sAML.

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IMPLICATION OF MPL/JAK2 PROTEIN EXPRESSION DEREGULATION IN ABNORMAL CELLULAR PROLIFERATION OF MYELOPROLIFERATIVE NEOPLASMS

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Background. Megakaryopoiesis is a multiple stage differentiation process under the control of thrombopoietin (TPO). Megakaryocytic precursors proliferate, switch to polyploidization and stop DNA replication before terminal differentiation leading to platelet shedding. We reported that UT7 cells genetically modified to over-express the TPO receptor MPL (UT7-MPL) respond to TPO by inducing senescence and that a similar process occurs in normal megakaryocytes. In contrast, megakaryocytes from primary myelofibrosis (PMF) patients lack these senescence markers, suggesting that an escape from the senescence-like signaling pathways could participate in the abnormal megakaryocytic proliferation observed in myeloproliferative neoplasms (MPNs). *Aims.* The aim of our work was to study the involvement of MPL

and/or the associated protein kinase JAK2 expression deregulation in this escape. *Materials and Methods.* We selected 5 UT7-MPL cell clones for their ability to escape from TPO-induced cellular senescence (ie these clones proliferate in presence of TPO). To study megakaryopoiesis *in vitro*, CD34+ cells were cultured in serum-free medium supplemented with TPO. Platelets were isolated from normal, essential thrombocythemia (ET) and PMF patients. The mRNA and protein expression levels of MPL and JAK2 were examined in the different cell lines, primary CD34+ cells and platelets by TaqMan and Western Blot, respectively. Cell signaling was studied by Western Blot. *Results and Discussion.* TPO-induced signaling was stronger in UT7-MPL cells compared to the 5 derived clones, as determined by Western blotting for the phosphorylated forms of various key TPO/MPL downstream molecules. This correlated with MPL and/or JAK2 expression, decreased in the different clones. Accordingly, we over-expressed either MPL or JAK2 in these clones, and recovered the TPO-induced proliferation arrest and expression of the senescence markers. Thus, cellular response to TPO depends on the MPL/JAK2 protein expression levels. A weak signaling is proliferative and a strong signaling induces a growth arrest and cellular senescence. Moreover, we observed a progressive and continuous increase of MPL and JAK2 protein expressions during normal megakaryopoiesis. We then hypothesized that megakaryopoiesis could be regulated by MPL and JAK2 expression levels, allowing the transition from a weak TPO-induced proliferative signal in immature cells expressing few MPL/JAK2 to a strong signaling (due to a high MPL/JAK2 expression in more engaged cells) inducing a proliferation arrest and megakaryocytic maturation. Interestingly, MPL and JAK2 expressions were lower in platelets from ET and PMF patients compared to normal platelets confirming previous data on MPL level in MPN. Based on these results, we propose that the decrease in MPL and/or JAK2 protein expression may be involved in MPN abnormal megakaryocytic proliferation, by extending the proliferative signal in immature cells, resulting in the amplification of the progenitor cell compartment. This protein expression decrease could be a shared consequence between different events leading to a myeloproliferation. *Conclusion.* We show that MPL and JAK2 protein expressions are decreased in MPNs megakaryocytic cells and hypothesize that this down-regulation could be involved in the abnormal cellular proliferation characterizing MPNs.

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JAK2 46/1 HAPLOTYPE PREDISPOSES TO SPLANCHNIC VEIN THROMBOSIS-ASSOCIATED BCR-ABL NEGATIVE CLASSIC MYELOPROLIFERATIVE NEOPLASMS

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Background. The germline constitutive JAK2 haplotype, called GGCC or 46/1, is a susceptibility factor for BCR-ABL negative classic myeloproliferative neoplasms (MPNs). The mechanisms linking this haplotype and the acquired MPN remains unclear. Splanchnic vein thrombosis (SVT) is variably associated with MPNs, occurring either during the course of well defined MPNs, or as the event leading to the diagnosis of MPN. *Aim.* In this study we sought to clarify the potential role of 46/1 haplotype in the etiology of SVT. The study was approved by the institutional review board of the IRCCS Policlinico S. Matteo Foundation, and all patients gave written consent. *Methods.* Screening for the 46/1 haplotype was performed by assessing the tag SNP rs12343867 that is in complete linkage disequilibrium with this haplotype. This SNP consists in a T to C shift, with the C allele associated with the 46/1 haplotype. Analysis was performed both by a PCR reaction followed by restriction-enzyme digestion and using a commercially available RT-PCR SNP genotyping assay. The chi-square or Fisher's exact test were used in the statistical analysis; P values <0.05 were considered significant. Results. One-hundred-sixty-four subjects with SVT were studied. In 56 of them (11 with a Budd-Chiari syndrome and 45 with portal vein thrombosis) a diagnosis of MPN was excluded. One-hundred-eight patients (32 ET, 29 fibrotic and 26 pre-fibrotic PMF, 21 unclassified MPNs) received a diagnosis of MPN-associated SVT. Fifty-six healthy subjects served as controls (CTRLs). Patients with SVT but no MPN had a C allele frequency not different from that of CTRLs (0.241 vs 0.267, p=0.6453), and the genotypic frequencies reflected this similarity. SVT-associated MPN patients as a whole had a C allele frequency significantly increased compared to CTRLs (0.440 vs 0.267, p=0.0023). This difference was principally due to an excess in V617F positive cases (p=0.0001), whereas the 46/1 haplotype was not over-represented in V617F-negative cases vs CTRLs (p=0.847). Accordingly, the JAK2 mutated patients had the highest frequency of CC genotype. SVT-associated ET and PMF (prefibrotic and fibrotic type) showed a significant increase of C allele frequency compared to CTRLs, whereas SVT-associated unclassifiable MPNs did not (0.381, p=0.1720). Moreover, the 46/1 haplotype was overexpressed in JAK2V617F-negative patients with ET (frequency=0.363), and prefibrotic PMF (0.300) but not PMF-fibrotic type (0.187) or unclassifiable MPN (0.250). These differences, however, were not statistically significant, likely due to the small number of subjects considered. These latter results are in agreement with published data showing that patients with a proven JAK2V617F-negative MPN and Budd-Chiari syndrome showed increased frequency of the 46/1 haplotype. Conclusion. In summary, our observations exclude the hypothesis that the 46/1 haplotype confers susceptibility neither to SVT, nor to vein thrombosis. Rather, we suggest that the 46/1 haplotype confers susceptibility to JAK2 mutation positive MPNs presenting with SVT.

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DIFFERENTIAL GENE EXPRESSION PROFILE RELATED TO LEUKOCYTOSIS IN JAK2 V617F POSITIVE POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

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Background. Recognized risk factors to thrombosis in classic chronic BCR/ABL negative myeloproliferative neoplasms (MPN) are age at diagnosis (over sixty) and previous events of thrombosis. Recently, two additional risk factors seem to play a key role in pathogenesis of thrombosis. Leukocyte (white Blood cells, WBC) count -in addition to JAK2V617F point mutation status and allele burden- has been identified as a probable independent predictor of major thrombosis in both essential thrombocythemia (ET) and polycythemia vera (PV). However, whether leukocytosis should be simply considered a marker for vascular disease or whether elevated WBC levels actually contribute directly to causing such disorders is presently matter of many studies, to be corroborated by prospective studies as well as JAK2 V617F (De Stefano *et al.*, Am J Hematol. 2010). *Aims.* We aimed to analyze the differential gene expression profile of JAK2 V617F positive PV and ET, with and without leukocytosis (threshold, WBC>11 X109/L) before and after treatment with Hydroxyurea (HU). We hoped to identify underlying molecular alterations related with leukocytes, platelets activation and /or endothelium adhesion that may contribute to thrombosis. *Methods.* Twenty-one PV (10 with treatment, 11 at diagnosis), and 28 ET (16 with treatment, 12 at diagnosis) were included in the study. cDNA of granulocytes from venous peripheral blood was extracted. Low-density quantitative real time PCR array (LDA) was performed on selected genes from a previous microarray analysis (E Albizua *et al.*, Ann Hematol. 2011 Feb 18). Statistical analyses of data were performed using the non-parametric Wilcoxon analysis. Statistical significance was considered when P value was under 0.05. *Results.* Thirty genes were significantly over-expressed in ET and PV with leukocytosis at diagnosis compared to those with normal leukocytes. Among them are to be outlined genes involved in leukocytes activation, and endothelium adhesion - CD44 (P=0.016) and SELL (P=0.009)-,other genes playing key roles as transcription factors - LYN, (P=0.04) -, in MAP kinase (MAPK) signaling pathways - RAF1(P=0.01) MAP2K1 (P=0.031), MAPK14(P=0.009) -, in other proliferative pathways - JUN (P=0.013), IGF1R (P=0.004), - as tyrosine kinases - BTK (P=0.028) -, and in regulation of hematopoietic cell differentiation and development of lymphocytes - IKZF1(P=0.000). Interestingly, overexpression of these genes disappeared after treatment with HU. *Conclusions.* Neutrophil activation correlated with activation of both endothelial cells and the coagulation cascade is a well known phenomenon in ET and PV as well as that white cells contribute to the procoagulant response at sites of vascular injury (Bouchard *et al*. Curr Opin Hematol,2001; Falanga *et al.*, Blood 2000). Our results suggest an association between leukocytosis and a group of genes involved in activation of leucocytes, and endothelium adhesion, that could contribute to the underlying molecular mechanism to thrombosis in PV and ET. In addition, genes playing key role as transcription factors and in proliferative pathways are over-expressed in PV and ET with leukocytosis at diagnosis, that may participate to thrombosis as well.In addition, disappear-

ance of over-expression of these genes after treatment with HU could give additional molecular support to the antithrombotic effect of HU (Brun *et al.*, Pharmacogenomics J. 2003).

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ASXL1 MUTATIONS IN PRIMARY AND SECONDARY MYELOFIBROSIS

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Background. In patients with Myelofibrosis (MF), beyond the presence of the JAK2V617F mutation, additional genetic lesions have been recently identified, including ASXL1 truncating mutations, that might be relevant for the development of this disease. *Aims.* To elucidate the role of ASXL1 mutations in the molecular pathogenesis of primary MF (PMF), and their contribution to the disease progression from Polycythemia Vera (PV) and Essential Thrombocythemia (ET) to MF. *Methods.* We analyzed 43 PMF and 22 secondary MF patients (13 post-PV and 9 post-ET) by direct DNA sequencing of six amplicons encompassing ASXL1 exon 12 (~3 kb). For 10 PMF, paired DNA samples were screened at diagnosis and during follow-up. The same sequential study was performed in 6 PV and 5 ET before and after disease progression to MF. The DNA from 10 PV and 10 ET at diagnosis was also sequenced in parallel. *Results.* Overall, 19 distinct ASXL1 mutations (10 frameshift, 8 nonsense and 1 missense) were identified in 28/65 (43%) MF patients: 23/43 (53%) with PMF and 5/22 (23%) with secondary MF (3 post-PV and 2 post-ET). In contrast, ASXL1 mutations were rarely detectable at diagnosis in ET patients (0/10) and PV (only 1 out of 10). Notably, analysis of paired CD3+ T lymphocytes purified in parallel from the peripheral blood of 9 PMF and 5 secondary MF confirmed that all lesions were restricted to the neoplastic clone only. In PMF patients, ASXL1 mutations were identified at diagnosis in 18/36 (50%) cases and during the course of the disease in 11/16 (69%). In one patient two distinct ASXL1 mutations were detected at diagnosis, while in another case an additional aberration was acquired during the follow-up. Analysis of paired diagnosis/follow-up samples in PMF documented the presence of the same ASXL1 mutation at diagnosis and during follow-up in 6 out of 10 patients (60%); in one patient the mutation occurred during follow-up, while in three patients mutations were never detectable. No correlation was found between ASXL1 mutations and the presence of the JAK2V617F allele. In post-PV/ET MF, the analysis performed on paired samples collected during the course of the disease showed that ASXL1 mutations were subsequently acquired during the course of the disease in 3 out of 4 informative cases. *Conclusions.* ASXL1 mutations are frequently detectable in MF, albeit more commonly in primary compared to secondary cases, whereas they are rather uncommon in PV and ET at diagnosis. In secondary MF, ASXL1 mutations may possibly contribute to the molecular progression of the dis.

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PRV-1 OVEREXPRESSION IN ESSENTIAL THROMBOCYTHEMIA CORRELATES WITH TRAIL EXPRESSION

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Background. PRV-1, a molecular marker for Myeloproliferative Neoplasms (MPN), is a cell surface receptor expressed in blood cells, which is involved in cell signaling proliferation process. Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF) are MPN characterized by accumulation of myeloid cells in peripheral blood and bone marrow hyperplasia. Apoptosis process deregulation may be linked to the cell accumulation and, therefore, to the MPN physiopathology. *Aims.* 1) To determine *PRV-1* gene expression in CD34$^+$ cells and leukocytes from ET and PMF patients; 2) To correlate these results with extrinsic apoptosis pathway related gene *c-flip*, *fas*, *fasL*, *dr4*, *dr5* and *TRAIL* expression and with JAK2V617F mutation allele burden. *Subjects and Methods.* This study analyzed CD34$^+$ cells and leukocytes from 22 ET patients (5 males and 17 females; mean age(ma)=58.4 years), 12 MF patients (9 males and 3 females; ma=61.6y) and 44 controls (20 males and 24 females; ma of 43.5 y) as control group. The JAK2V617F allele burden was determined by real time allelic discrimination PCR assay. Ficoll-Hypaque protocol and Miltenyi CD34 isolation kit were used to obtain bone marrow CD34$^+$ HSC whereas leukocytes were obtained by Haes-Steril method. Total RNA was extracted and cDNA synthesized by reverse transcription using High Capacity® Kit. Gene expression was quantified by real time PCR, the results were given as fold change ($2^{-\Delta\Delta Ct}$) and statistical analyses were performed by Mann-Whitney and Spearman tests. *Results.* There was no significant difference in *PRV-1* expression in CD34$^+$ cells from control, ET and PMF groups (p>0.05). However, *PRV-1* expression was increased in ET and MF leukocytes (median= 2.33 and 4.40, respectively) compared to control (m=0.91) (p=0.014; p=0.002, respectively). Regarding JAK2V617F mutation, *PRV-1* expression in leukocytes from ET and PMF patients and JAK2V617F allele burden showed a positive correlation (r=0.420; p=0.026; r=0.537, p=0.047, respectively). Considering that *TRAIL* expression was decreased in ET and PMF leukocyte (m=0.30, m=0.49) in comparison to control (m=1.6) (p=0.0004; p=0.029), it was interesting to emphasize that we found a negative correlation between *PRV-1* and *TRAIL* expression in ET leukocytes (r=-0.6228, p=0.001). *Conclusions. PRV-1* overexpression is associated with apoptosis related gene expression *TRAIL*, a relevant molecule in death receptor pathway and a potential therapeutic target in neoplasms. These results indicate that *TRAIL* deregulated expression and *PRV-1* overexpression may contribute to the myeloaccumulation and to the physiopathology of the Myeloproliferative Neoplasms.

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EXPRESSION OF LEUKEMIA INHIBITORY FACTOR (LIF) IS INCREASED IN MYELOPROLIFERATIVE NEOPLASMS

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Background. The JAK2/STAT5 signalling pathway transduces signals from many cytokines and growth factors, and promotes essential hematopoietic events such as proliferation, cell migration and apoptosis. This pathway is frequently altered in chronic myeloproliferative neoplasms (MPNs) with activating mutations in *JAK2*. However, one *JAK2* mutation (V617F) leads to the development of MPNs with distinct phenotypic features. Some explanations have been proposed to account for this fact (such as V617F allele burden, other mutations in *JAK2* or mutations in *TET2* and other genes), but none of them fully explains the phenotypic differences observed in this group of diseases. This suggests that there might be other genes transcriptionally regulated by STAT5 that could contribute to the phenotypic variability of chronic MPNs. *Methods.* We performed a genome-wide search for the presence of STAT5-binding sites in human promoters. Affymetrix Exon 1.0 Arrays (Affymetrix Inc, Santa Clara, CA) were used to search for genes differentially expressed after activation of the JAK2/STAT5 pathway in cell lines. We validated expression changes by qRT-PCR. Cell lines used: - M07e, a myeloid cell line in which the JAK2/STAT5 pathway can be induced by IL-3 and inhibited by a specific inhibitor (STAT5 inhibitor, Cat. No. 573108, Calbiochem, San Diego, CA, USA). - HEL, a human erythroleukemia cell line that harbors the V617F activating mutation in *JAK2* gene. - SET-2, a cell line originally derived from a patient with essential thrombocytemia at megakaryoblastic transformation, heterozygous for the V617F mutation in *JAK2*. In HEL and SET-2 cells, JAK2/STAT5 pathway was inhibited by the JAK2 inhibitor AG490 (from the tyrphostin family of tyrosine kinase inhibitors, Cat. No. 658401 Calbiochem, San Diego, CA, USA). To confirm whether STAT5 binds to putative binding sites in the promoter of specific genes, we measured binding by chromatin immunoprecipitation (ChIP) with a specific STAT5 antibody and qPCR, so as to compare the amount of DNA bound by STAT5 before and after IL3-mediated induction of the pathway in M07e cells. We obtained peripheral blood samples from patients with chronic MPNs and measured the expression of *LIF* and *OSM* by qRT-PCR. *Results.* Bioinformatic analyses predicted STAT5-binding sites in the promoter of Leukemia Inhibitory Factor (*LIF*). ChIP experiments confirmed binding of STAT5 to one of these motifs in response to IL-3 mediated activation of the pathway.

Moreover, *LIF* expression was significantly upregulated by IL-3 in M07e cells and this was reversed by treatment with a specific STAT5 inhibitor. Treatment of HEL and SET-2 cell lines with a JAK2 inhibitor downregulated *LIF* expression. These results confirm that *LIF* behaves as a direct transcriptional target of STAT5. Finally, MPN patients showed significantly increased expression levels of *LIF* as compared with healthy donors. The finding of *LIF* as a novel STAT5-regulated gene might help to understand STAT5-mediated oncogenesis.

0329

LOW EXPRESSION OF GALECTIN-1 IS CORRELATED TO DOWN-REGULATION OF BAX PRO-APOPTOTIC GENE EXPRESSION IN CD34+ HEMATOPOIETIC STEM CELLS FROM POLYCYTHEMIA VERA PATIENTS

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Background. Polycythemia vera (PV) is a clonal hematopoietic stem cell malignance characterized by an accumulation of mature myeloid cells in bone marrow and peripheral blood. A specific point mutation in the Janus kinase 2 gene (JAK2V617F) has been identified in more than 90% of patients with PV however many aspects of pathogenesis and the description of diagnostic markers remains unclear. Apoptosis deregulation may play a role in PV physiopathology and we speculate that galectin-1 interferes in the apoptotic pathway of PV patients' myeloid cells. Galectin-1 may interact with proteins from Bcl-2 family, leading to apoptosis. The potential regulation of apoptotic machinery by this lectin in PV patients cells might emerge as novel target for therapy manipulation or as a possible diagnostic marker for PV. *Aims.* 1) To quantify *galectin-1* and genes from Bcl-2 family expression in $CD34^+$ hematopoietic stem cells in PV patients and controls; 2) To correlate the results of *galectin-1* expression and apoptosis-related genes and JAK2V617F allele burden. *Methods.* Bone marrow $CD34^+$ cells from 20 PV patients (9 males and 11 females with a mean age of 62,25y) and 15 healthy subjects (9 males and 6 females, m=29,73y). $CD34^+$ cells were enriched by using the MACS (magnetically activated cell sorting) CD34 isolation kit (Miltenyi Biotec). Total RNA was extracted according to Trizol® method and the High Capacity® Kit was used to synthesize cDNA. The expression of *galectin-1* and apoptosis-related genes was performed by real time PCR. The JAK2V617F mutation and the allele burden were conducted by real time allelic discrimination PCR. The gene expression results were given as $2^{-\Delta\Delta Ct}$. Statistical analyses were carried out by Mann-Whitney and Spearman tests. *Results. Galectin-1* mRNA levels in $CD34^+$ bone marrow cells were decreased in PV patients (median=0.6) in comparison to controls (m=1.0, p=0.017). Pro-apoptotic *bax* (m= 0.09) and the anti-apoptotic *mcl-1* (m=3.74) expression were different from controls (m= 1.049, m=1.415; respectively) (p= 0.027, p= 0.027; respectively). *Galectin-1* expression correlated with the expression of pro-apoptotic gene *bax* (r= 0.46; p= 0.032). The *bax* expression also correlated with JAK2V617F mutation allele burden (r=-0,46; p=0,036). *Conclusions.* Apoptosis impairment in myeloid cells from PV patients may be linked to low levels of *galectin-1* and *bax* pro-apoptotic mRNA and to the overexpression of *mcl-1* anti-apoptotic gene. These findings highlight the potential participation of galectin-1 in the physiopathology of PV, suggesting an interaction of this lectin with Bcl-2 family members in PV.

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0330

NO EVIDENCE THAT MPL, NPM1 OR FLT3 CONSTITUTIONAL HAPLOTYPES PREDISPOSE TO THE ACQUISITION OF ACQUIRED MUTATIONS IN MYELOID DISORDERS

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Background. We and others have previously shown that the constitutional JAK2 46/1 haplotype predisposes to the acquisition of JAK2 V617F in myeloproliferative neoplasms, and to a lesser extent it also predisposes to the acquisition of JAK2 exon 12 and MPL mutations. It is not known whether this association between germline and somatically acquired factors reflects something unique about the JAK2 locus, or whether other acquired driver mutations in cancer also arise on specific inherited haplotypes. *Aims.* To investigate the relationship between acquired mutations and haplotypes in MPL, FLT3 and NPM1 in MPN and AML. *Methods.* For each gene, regions of low genetic recombination were determined firstly by visual inspection of HapMap data and then minimal regions of high linkage disequilibrium were selected for detailed analysis using the programs LDMapper and PHASE along with SNP data generated by the Welcome Trust Case Control Consortium (WTCCC) from normal blood donors. From the list of haplotypes generated, a minimal number of SNPs for each gene locus was identified that could capture at least 85% of the genetic variation in that region. Pyrosequencing, a technique that provides a quantitative readout of SNP allele ratios, was used for genotyping and also detected allele skewing of heterozygous SNPs bought about by acquired uniparental disomy at 1p (MPL) or 13q (FLT3). For cases carrying homozygous MPL W515X and FLT3-ITD mutations, haplotypes associated with mutant alleles could be determined. Allele frequencies of patient subgroups were compared to data from either the WTCCC UK blood donor cohort (n=1500), the 'German Kooperative Gesundheitsforschung in der Augsburg' cohort (KORA; n=1805), and the Italian 'Invecchiare in Chianti' cohort (inCHIANTI; n=1200) depending on the ethinicity of the patient population. The allele frequencies of each patient subgroup were compared to controls using Fisher's exact test (2-tailed). *Results.* There were no significant deviations from the expected allele frequencies of matched control populations for three MPL SNPs genotyped in 139 cases with a diagnosis of MPL W515X positive MPN. Likewise, for the 96 cases with a diagnosis of AML carrying a mutation in NPM1, there were no significant differences in allele frequency for two SNPs in NPM1. For FLT3, a total of three SNPs were genotyped in 144 AML cases (FLT3-ITD; n=91, FLT3-TKD; n=43, FLT3-ITD and TKD; n=10), but again there were no statistically significant deviations from allele frequencies found in matched controls. *Summary.* These findings show myeloid-specific mutations in MPL, FLT3 and NPM1 appear to arise randomly on different haplotypes. Whilst this does not preclude the possibility that somatic mutations in other genes might occur preferentially on particular haplotypes, it does suggest that the association between JAK2 46/1 and somatically acquired mutations in JAK2 and MPL is not a general phenomenon.

0331

FIRST YEAR ACHIEVEMENTS OF MPN&MPNR-EURONET (COST ACTION BM0902), A NEW EUROPEAN NETWORK DEDICATED TO THE DIAGNOSIS OF MYELOPROLIFERATIVE NEOPLASMS AND HEREDITARY ERYTHROCYTOSIS AND THROMBOCYTOSIS

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Background. The MPN&MPNr-EuroNet network, created in 2009, is supported by the European program COST (CoOperation in Science and Technology). It is open to all colleagues active in the fields of myeloproliferative neoplasms (MPN) and related hereditary diseases (MPNr: hereditary erythrocytosis and thrombocytosis). *Aims.* To facilitate and improve the diagnosis of MPN and hereditary erythrocytosis and thrombocytosis in Europe. *Methods.* MPN&MPNr-EuroNet has formed 4 working groups (WG): WG 1 focuses on JAK2-mutated MPN; WG 2 is dedicated to thrombocythemia and myelofibroses without mutation of JAK2 and includes subgroups specialized in hereditary thrombocy-

tosis and in histopathology; WG 3 is dedicated to hereditary erythrocytosis; WG 4 is responsible for scientific cooperation and the diffusion of scientific knowledge. *Results.* During the first year of activity of MPN&MPNr-EuroNet, colleagues from 17 countries participated in the four WG, resulting in the achievements listed below. WG 1: determination of the best JAK2V617F assays, a joint MPN&MPNr-EuroNet/LeukemiaNet project; on-going study of MPN cases with low JAK2V617F burden; on-going study of MPN cases with multiple JAK2 mutation; WG 2: list of laboratories responsible for the diagnosis of MPL and THPO mutations in Europe; first international quality test of the detection of MPL mutations; on-going study of new THPO and MPL mutations in cases of hereditary thrombocytosis (HT); on-going study of the histopathology of MPN without mutation of JAK2 and of HT; WG 3: list of laboratories responsible for the diagnosis of hereditary erythrocytosis (HE) in Europe; consensus on a diagnostic algorithm for the diagnosis of HE; close interaction with COST Action TD0901 - HypoxiaNet - to facilitate discovery of new genes of interest for the diagnosis of HE; exchange of positive control samples for the main mutations responsible for HE; WG 4: MPN&MPNr-EuroNet's website (www.mpneuronet.eu); organization of two annual meetings (2011 meetings: April 6-8, La Baule, France; Sept. 14-16, Münich, Germany); support for short term scientific missions for exchange between participating institutions; and creation of two annual training schools: a spring training school dedicated to the molecular detection of JAK2 and MPL mutations (Nîmes, France), and a fall training school dedicated to hereditary erythrocytosis (Coimbra, Portugal). *Summary/Conclusions.* MPN&MPNr-EuroNet will enable European researchers to define new diagnostic tools and exchange technologies. MPN&MPNr-EuroNet is open to all interested physicians and scientists and we invite new members to join us. Scholarships are available to finance participation to meetings and training schools, and to facilitate exchanges between participating institutions (see www.mpneuronet.eu).

0332

ROLE OF METALLOPROTEASES IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA ERYTHROID DIFFERENTIATION

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Background. Several hypotheses have tried to explain how a unique mutation is related to three different phenotypes in myeloproliferative neoplasms (MPN). Metalloproteases are a group of proteins involved in matrix remodelling, migration and differentiation processes. Our group has found differential expression of Matrix metalloproteinase-14 (MMP14) and CD44 in PV and ET JAK2V617F positive samples (E. Albizua *et al.*, Ann Hematol. 2011Feb). *Aims.* To analyze the phenotypic divergence between PV and ET by proteomic screening, and validation of previously MMP14 and CD44 gene expression results by protein expression analysis and cultures methods, with the objectives of identifying alternative routes for targeted therapy. *Methods.* Fifty-nine MPN were included in the study: 24 PV, 24 ET and 11 PMF. An additional 24 healthy donors were used as controls. Granulocytes from whole venous peripheral blood were isolated and the corresponding cytosolic protein fraction was extracted. PV and ET cytosolic proteomes were analyzed using 2D-DIGE gels followed by MALDI-TOFTOF mass spectrometry analysis of the spots of interest. Results were analyzed with DeCyder v7.0 (GE) and Mascot software. Leukocytes were obtained and analyzed by flow cytometry (FCM) using anti-MMP14, anti-CD44 and anti-CD45 antibodies (BD). Bone marrow biopsies were selected to perform immunohistochemistry (IHC) with anti-MMP14 and anti-CD44 antibodies (R&D). Finally a culture study was performed. Mononuclear cells from patients were extracted and seeded in Methocult with IL-3, SCF and EPO (Stem Cell). MMP14 was inhibited by the drug Marimastat at 100µM, 50µM and 10µM (TOCRIS); and CD44, with anti-CD44 antibody at 10mg/ml, 1mg/ml and 0.1mg/ml. Results were analyzed by BFU-E count, viability study by trypan blue and FCM employing antibodies anti-CD45, anti-CD71, anti-CD44, anti-MMP14 and Annexin (BD). The Mann-Whitney non-parametrical statistical hypothesis test was used to assess the statistical significance of our results. *Results.* 2D-DIGE analysis found 50 spots with statistically significant differences

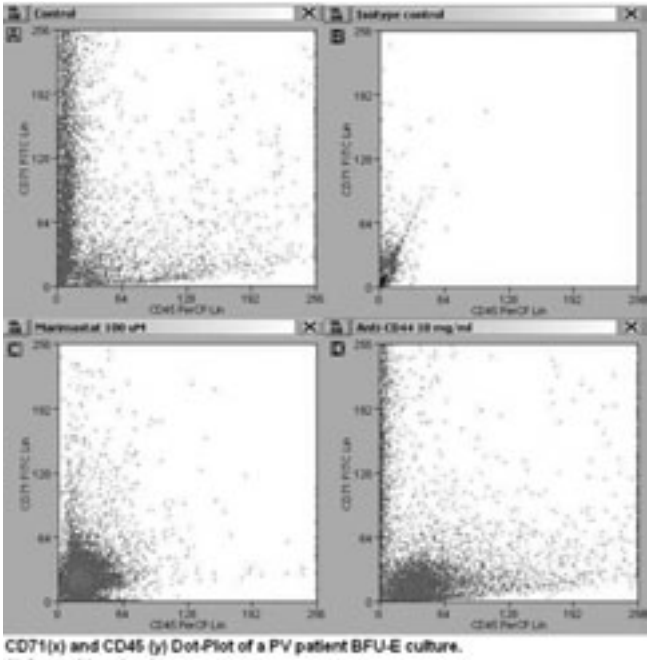

Figure 1. FCM dot-plot of PV patient BFU-E culture.

in protein expression between PV and ET samples. Three spots were especially interesting in the context of our model. These corresponded to HSPA1A, a chaperone related with GATA-1 and erythroid differentiation. FCM of the granulocytes showed over-expression of CD44 in PV population versus ET (P=0.004). FCM CD44 results were confirmed by bone marrow biopsies IHC in granulocytes (P=0.039); IHC also showed MMP14 differential expression between ET, PMF or PV compared to healthy biopsies in megakaryocites, over-expressed in MPN. However, ET and PV did not show MMP14 differential expression. Significant differences of inhibition BFU-E growth and cell proliferation were found between treatment groups (Marimastat -100µM and 50µM- and anti-CD44 -10mg/ml and 1mg/ml-) versus group without treatment (P=0.05 and P=0.037 respectively). FCM of BFU-E cultures pointed to a significant decrease of CD71 (erythroid) and increase of CD45 (leukocyte-common antigen) with both treatments. *Conclusions.* Our results suggest that MMP14 and CD44 could play a role in erythroid and myeloid differentiation. Differences between ET and PV may be caused by both molecules which may contribute to phenotypic divergence. Our results suggest that MMP14 and CD44 could be future therapeutic targets. Other molecules that could contribute to phenotypic divergence, such as chaperone HSPA1A, are under study.

0333

JAK1 AND STAT3 LOW MRNA EXPRESSION IS ASSOCIATED TO RESPONSIVENESS TO IFN-α THERAPY OF ESSENTIAL THROMBOCYTHEMIA

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Background. Interferon-α (IFN-α) is a useful and nonleukemogenic treatment of ET, able to normalize platelet counts in 80% of treated patients, to induce molecular response and to keep patient off therapy. This cytokine suppresses megakaryocyte (MK) formation of both murine and human marrow cells by inhibition of TPO induced MK growth. IFN-α is a type I IFN and its receptor is associated with Janus kinase proteins, Tyk2 and Jak1. IFN binding to this receptor results in tyrosine cross-phosphorylation and auto-phosphorylation of the JAKs proteins. These phoshothyrosines recruit STAT proteins whose phosphorylation and activation are in turn regulated by Jak1 and Tyk2. The STATs activated in response to type I IFNs include STAT1, STAT2, STAT3 and STAT5; these initiate transcription of SOCSs proteins,

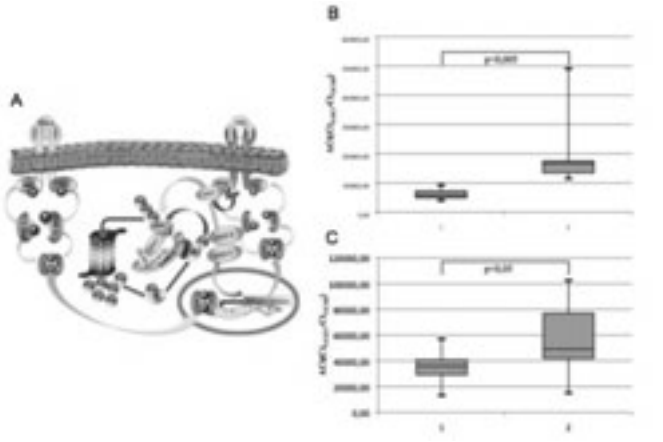

Figure 1. JAK1 and STAT3 mRNA expression is lower in IFN-α r.

whose role is to extinguish cytokine signaling by inhibition of JAKs. The SOCSs KIR blocks JAK kinase-activity directly, instead, the SOCS-box motif promotes the polyubiquitination and proteasomal degradation of SOCS binding partners, like Jak2. In summary, IFN-α induces SOCS expression which inhibits TPO mediated signaling through Jak2 double inhibition. This allows IFN-α and the TPO pathway to cross-talks by means of the JAK-STAT-SOCS cascade. *Aims.* In order to predict IFN treatment responsiveness, we evaluated the expression of specific genes involved in the IFN-α receptor pathway, which signal cross-talks with the JAK-STAT pathway under the TPO receptor. In particular we evaluated the mRNA expression of JAK1, TYK2, STAT1, STAT3, SOCS1 and SOCS3. *Methods.* Among the 60 IFN-α treated patients we considered eligible the 21 who had not received previous therapy and were treated with the same schedule (IFN-α-2b 3 million units 3 times a week for at least 6 months). Informed consent for the study was obtained from all patients. Two patient groups were selected on the basis of clinical response to therapy: Responders (R) (n=12) achieved a reduction of platelet count below 400x109/L, whereas the No Responders (NR) (n=9) group failed to show this hematological response. Target genes mRNA expression was explored by RT q-PCR, using SYBR Green detection, on bone marrow samples. Data were normalized as follows: [mRNA normalized copy number (NCN)= mRNA target gene/mRNA GUSB*104]. Methylation-specific PCR was performed to investigate the methylation status of the promoter regions of the SOCS3 genes. *Results.* STAT3 expression showed a significant lower levels in R in relation to NR (p=0,01); JAK1 expression also was lower in BM cells from R compared with NR (p=0,0008). No differences were found for other gene expressions and for the methylation of SOCS3 promoter between R and NR. Response was not influenced by age (p=0,06), gender (p=0,37), baseline values of hematocrit (p=0,57), platelet (p=0,52), WBC (p=0,08), spleen volume (p=0,09), JAK2V617F mutation (p=0,33). *Conclusions.* JAK1 and STAT3 gene expression may be used as predictor marker of response to IFN-α in ET patients. Thus, patients with low levels of JAK1 and STAT3 mRNA could be also candidate for lower IFN-α doses, which are better tolerated. Moreover, patients with JAK1 mRNA increased levels could be addressed for JAK1/JAK2 inhibitors therapy.

0334

JAK2V617F ALLELE BURDEN IS A CONTRIBUTING FACTOR IN PROTHROMBOTIC MECHANISMS ACTIVATION

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Introduction. Myeloproliferative neoplasms (MPN) are associated with thrombohemorragic diathesis which, in some extent, can be consequence of platelet and leukocyte activation. Different groups suggested an association between JAK2V617F mutation and thrombosis in MPN patients. *Objective.* Evaluate haemostatic activation parameters and their relationship with JAK2V617F allele burden and thrombosis in a group of PV and ET patients. *Methods.* 28 PV and 46 ET patients (median age 65 and 75y, respectively) and a control group of 47 healthy volunteers (median age 30y) were studied. All patients are clinically stable and under HU treatment. With patients' informed written consent, aspirin was withdrawn for 10 days prior the studies. Using flow cytometry we evaluated: platelet P-selectin (CD62P) and granulophysin (CD63), platelet dense granules (mepacrine uptake/release test), platelet-leukocyte aggregates (PLA), leukocyte CD11b and monocyte Tissue Factor (TF) expression. JAK2V617F allele was quantified by Allele Specific qRT-PCR (JAK2MutaQuant, Ipsogen). Presence of MPL exon 10 mutations were screened by SSCP and identified by direct sequencing. *Results/Discussion.* 28 PV (100%) and 28 ET (61%) with JAK2V617F mutation and 1 ET with MPL W515L mutation; 7 PV and 16 ET patients had thrombosis at diagnosis. All patients have increased baseline CD62P and CD63 expressions ($p<0.01$), increased response to arachidonic acid ($p<0.01$) and diminished response to TRAP6 stimulation ($p<0.01$); 77% of PV and 50% of ET present a storage pool disease. Leukocyte CD11b and monocyte TF expressions were increased in all patients ($p<0.01$). PLA were found increased in all patients ($p<0.01$) vs controls, nevertheless platelet-neutrophil (PMN) aggregates were significantly increased in ET vs PV ($p<0.01$). Patients with JAK2V617F>50% present a significantly increase of CD11b expression and platelet-PMN aggregates comparing to JAK2V617F<50% ($p<0.01$). PV patients present increased levels of TF when comparing with ET ($p<0.01$), and was higher in PV JAK2V617F>50% ($p<0.01$). In ET JAK2V617F mutation was statistically correlated with thrombosis and with JAK2V617F allele >50% (no association in PV). Regarding the allele burden and platelet function studies no association was found (p=ns). These results show, with statistically significance, that PV and ET patients have circulating activated platelets and leukocytes and increased PLA. Activation parameters were higher in patients with JAK2V617F allele burden >50% comparing to those with JAK2V617F<50%, which is consistent with the influence of JAK2V617F allele burden in leukocyte activation. *Conclusions.* Our data demonstrate that JAK2V617F mutation drives mechanisms that favor thrombosis, namely, neutrophil and monocyte activation, increased monocyte TF expression and platelet-leukocyte aggregates.

0335

C-CBL MUTATIONS IN V617FJAK2 POSITIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background. BCR-ABL1 negative chronic myeloproliferative neoplasms (CMPNs) are a heterogeneous group of clonal hematological malignancies. The most frequent aberration is V617FJAK2, present in most of PV and over 50% of ET and MF. In last years, different groups have described mutations in other genes as C-CBL (Casitas B-lineage Lymphoma), that encodes for an E3 ubiquitin ligase involved in negative regulation of several tyrosine-kinases, as EGFR, FGFR or SYK. These mutations are present in different myeloproliferative neoplasms, especially in CMML and JMML, often in patients negative for other frequent mutations as V617FJAK2. Several groups have demonstrated that mutations in the RING finger domain of C-CBL result in deregulation of downstream targets of this protein. *Methods.* We have used dHPLC to detect sequence mutations on samples from 377 BCR-ABL1 negative CMPN patients (145 V617FJAK2 negatives and 232 positives) and 20 samples from healthy individuals as controls. We analyzed the proliferative response induced by the C-CBL mutants in 32D cell line, previously transfected with FLT3 in four independent experiments, using the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay. *Results.* We have found five mutations in C-CBL in six patients, four of them non-described previously. Three of these mutations were located in the RING finger domain, but two were found in the proline-rich domain (one of them was recurrent). All the mutations promoted a significant increase in the rate of proliferation induced by cytokines. Three of the mutations (two of the RING finger and one in proline-rich region) were found in four V617FJAK2 positive patients. *Conclusion.* Our results suggest that mutations in C-CBL and JAK2 genes are not exclusive events. In addition, proline-rich region mutants can induce the same proliferative effect than RING finger domain mutants, so this region of C-CBL must also be considered for mutation analysis of this gene in CMPN. This work has been funded with the help of the Spanish Ministry of Science and Innovation (SAF 2007-62473), the PIUNA Program of the University of Navarra and the Caja Navarra Foundation through the Program "You choose, you decide" (Project 10830). PA has a predoctoral grant from the Government of Navarra.

0336

INCREASED PHOSPHO-MTOR EXPRESSION IN AN *EX VIVO* MEGAKARYOCITIC UNILINEAGE SYSTEM DERIVED FROM CD34-POSITIVE CELLS ISOLATED FROM PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND MYELOFIBROSIS

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Background. The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that functions as a key regulator of cell growth, protein translation and metabolism. Consistent with its role as a growth-promoting factor, numerous studies have found increased mTOR signaling in a broad spectrum of human cancers. Essential thrombocythemia (ET) and myelofibrosis (MF) are BCR-ABL-negative chronic myeloproliferative disorders characterized by megakaryocytic bone marrow hyperplasia and a sustained elevation of platelet number with a tendency for thrombosis and hemorrhage. However, the molecular mechanisms underlying the pathogenesis of these diseases are still poorly understood, delaying the development of effective targeted treatments. *Aims.* To evaluate phospho-mTOR expression in megakaryocytic cultures derived from the peripheral blood of healthy individuals or from patients diagnosed with ET or MF. *Methods.* We have developed a liquid megakaryocytic (MK) unilineage system that reproduces, *ex vivo*, all the stages of megakaryopoiesis generating morphologically and functionally mature platelets. Human CD34-positive cells were purified by positive selection. Unilineage MK differentiation was induced in each sample (healthy individuals and ET or MF patients) by cultivating CD34-positive cells for 14 days in the presence of thrombopoietin. Purity of the selected population was evaluated immediately after purification and during MK differentiation (days 0, 3 e 12) by flow-cytometry using anti-CD34 and anti-CD61 antibodies and morphological analysis after May-Grünwald-Giemsa staining. Phospho-mTOR expression was analyzed on day 3 and 12 of the differentiation process by flow-cytometry, immunofluorescence and immunohistochemistry (carried out on slides from 35 bone marrow samples: 14 ET and 21 MF patients). *Results.* mTOR activation was increased during MK differentiation in both ET and MF patients compared to healthy donors where mTOR staining was barely detectable. Immunohistochemical analysis of phospho-mTOR confirmed high expression levels in ET and MF patients in contrast with the negative staining observed in healthy individuals. *Conclusions.* Taken together, our data suggest that induction of the mTOR pathway is involved in the MK differentiation of samples derived from ET and MF patients. Our findings suggest that mTOR could represent an attractive molecular target for the treatment of ET and MF patients failing previous lines of treatment.

0337

MOLECULAR PROFILING OF PERIPHERAL BLOOD CELLS FROM PATIENTS WITH POLYCYTHEMIA VERA AND RELATED NEOPLASMS IDENTIFIES SIGNIFICANT DEREGULATION OF INFLAMMATION GENES

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Background. Essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF) are haematopoietic stem cell neoplasms, in which the JAK2 V617F mutation is observed in >95% of PV patients and in about 50 % of patients with ET and PMF. All diseases may be associated with autoimmune or chronic inflammatory disorders. Gene-expression profiling studies have yielded divergent results which might be explained by different platforms used and differences in the cell types being profiled (granulocytes, CD34+ cells) and their origin (bone marrow samples, peripheral blood). Aberrant expression of genes involved in inflammatory responses has also been reported, mainly being performed on granulocytes or CD34+ cells. *Aims.* In order to achieve a "global"characterization of aberrant genes of significance for inflammation and immune function, we have performed gene expression profiling of whole blood to obtain an integrated transcriptional signature of cells involved in inflammation, immune surveillance and function. *Methods.* Gene expression microarray studies have been performed on control subjects (n = 21) and patients with ET (n = 16), PV (n = 36), and PMF (n = 9). Patients on interferon-alpha therapy were excluded from the study. Gene expression profiles were generated using Affymetrix HG-U133 2.0 Plus microarrays recognizing 54,675 probe sets (38,500 genes). Total RNA was purified from whole blood and amplified to biotin-labeled RNA and hybridized to microarray chips. *Results.* We identified 23,657, 25,567, and 17,417 probe sets which were significantly differentially expressed between controls and patients with ET, PV, and PMF, respectively (false discovery rate (FDR) adjusted p values < 0.05). 122 genes involved in inflammation and immune regulation were studied and 52, 73, and 60 were significantly deregulated in ET, PV and PMF, respectively. A total of 98 genes were significantly upregulated. The most significantly upregulated genes included in ET patients CCL7, CCL25, FGB, and ITGB3, in PV patients CCR1, CXCL10, IL1R1, and ORM1, and in PMF patients C5, CXCL2 CXCL3, ORM1, PTX3, and VEGFA. In the whole group of patients, 87 genes were significantly downregulated. The C5 and IL5 genes were progressively and significantly upregulated in patients with ET, PV, and PMF, whereas the genes, CCR3, CCR6, CCR9, CD40LG IL10RA, and SELPLG were progressively and significantly downregulated from ET over PV to PMF, respectively. *Summary/Conclusions.* Our findings of significantly deregulated genes involved in inflammation and immunoregulation with progressive deregulation for particular genes from ET, over PV to PMF may reflect chronic inflammation to be of pathogenetic importance for the progression of these neoplasms towards the myelofibrotic endstage. In this context, the aberrant inflammatory and immunoregulatory pathways may be deregulated as part of the clonal evolution. Irrespective of the underlying mechanisms, the deregulated (upregulated or downregulated) genes may drive clonal myeloproliferation. It is intriguing to consider if chronic inflammation or a chronic aberrant autoimmune process might also elicit clonal evolution and the emergence of a myeloproliferative neoplasm. If so, the abnormal immune homeostasis may be involved in the progression of the disease consequent to defective immune surveillance.

0338

THE ACTIVATING G537R MUTATION IN HIF2-ALPHA IS ASSOCIATED WITH IN VITRO HYPERSENSITIVITY OF ERYTHROID PROGENITORS TO ERYTHROPOIETIN

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Background. Polycythemias/erythrocytoses in childhood are rare and may be a result of various pathophysiological conditions. Primary polycythemias were reported in children with acquired mutations in JAK2 and with congenital mutations in erythropoietin receptor (EPOR). Recessive mutations in gene encoding von Hippel-Lindau (VHL) protein lead to polycythemia, which exhibits features of both primary and secondary. Recently described polycythemic patients with mutations in PHD2 and HIF2-alpha (HIF2A), were reported to have elevated serum EPO levels; the information on *in vitro* sensitivity of erythroid progenitors to EPO is missing or not complete. *Aims.* We aim to characterize, at cellular and molecular level, selected pediatric patients diagnosed with polycythemia in Czech Republic (1990 - 2010). Part of this cohort (6 patients) was previously presented.[1] *Patients and Methods.* We studied a group of 9 children diagnosed at the centers of Pediatric Hemato-Oncology in Czech Republic with signs of primary polycythemia. Their hemoglobin levels at the time of diagnosis were in the range of 18-22.3 g/dL. Four patients were clinically asymptomatic, five patients had plethora. Hematopoietic colony assay was used to determine *in vitro* sensitivity of erythroid progeni-

tors to EPO. Mutation analysis of known candidate genes was performed and included sequencing of JAK2 exons 12 and 14, EPOR exons 7 and 8, HIF2A and HIF1A exon 12 and all exons of VHL and PHD2. *Results.* All patients exhibited *in vitro* hypersensitivity of erythroid progenitors to EPO, seven samples were also positive for the growth of endogenous erythroid colonies (EECs). A known heterozygous 5967insT mutation in EPOR[2] was found in two unrelated patients; this result was already presented.[1] Recently, we detected another previously reported mutation, heterozygous 1609G>A substitution which changes glycine 537 for arginine in HIF2A,[3] in the other two unrelated and clinically asymptomatic patients. The erythroid progenitors of both were *in vitro* hypersensitive to EPO, one was also EEC-positive. Surprisingly, their EPO levels were not elevated, however only one single measurement for each from two different biochemical laboratories is available. Therefore EPO levels need to be re-evaluated in one center using another rigorous measurement. The remaining five patients were negative for a mutation in all analyzed genes. *Conclusions.* We present a group of pediatric patients with primary polycythemia, and mild clinical sings in 5/9 of the cases. The disease causing mutation was detected in 4/9 patients. The presence of the same G537R mutation in two our patients in combination with independent cases reported by others confirm the suggested mutational hotspot in HIF2A gene. We show for the first time that in addition to VHL-associated polycythemia also erythroid progenitors of patients with HIF2A mutation are *in vitro* hypersensitive to EPO. We hypothesize that endogenous erythroid EPO production is stimulated by this activating G537R HIF2A mutation leading to augmented activation of EPO/EPOR pathway and excessive erythroid proliferation.

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0339

ANALYSIS OF THE MUTATIONAL STATUS OF IL-3, IL-5 AND GM-CSF RECEPTORS IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background. Over the last few years several genetic aberrations have been described to be the cause of some cases of chronic myeloproliferative neoplasms (CMPNs). Among these aberrations V617F mutation in JAK2 is the most frequent one, having been found in 55% of ETs, almost 100% of PVs and 65% of IMFs. In spite of these findings, an important number of CMPNs still have an unknown molecular origin. *Aims.* Current knowledge of CMPNs has revealed the importance of JAK2 and the JAK/STAT pathway in these diseases. This encouraged our team to study cytokine receptors signaling through JAK/STAT pathway. We focused our work in the screening for mutations on the genes coding for the receptors for interleukin (IL)-3, IL-5 and GM-CSF. *Methods.* We analyzed a series of DNA samples from 44 patients with *BCR-ABL1* and V617F*JAK2* negative CMPNs. We used denaturing High Performance Liquid Chromatography to carry out a screening for mutations on the coding exons of genes *IL3RA*, *IL5RA*, *CSF2RA* and *CSF2RB*. Samples with an elution profile different to a healthy control were sequenced. For the exons with alterations the study was extended to other series of CMPNs patients, either V617F*JAK2* positive or negative, and a longer series of samples from healthy donors was analyzed for the recurrent ones. No matched sample from other tissue was initially available in any case. *Results.* We found nine sequence changes not previously described as mutations nor polymorphisms in dbSNP or 1000genomes.org in *CSF2RB*, *CSF2RA* and *IL3RA*. Five were found in *CSF2RB* (p.D312N, p.A328T, p.P509S, p.P513L, and p.R517C); three in *CSF2RA* (p.R164Q, p.P166S and p.Y167D); and one in *IL3RA* (p.W226X). R164Q in *CSF2RA* was found to be recurrent as it appeared in nine patients, but also in three healthy donors. In one case with R164Q*CSF2RA* a buccal swab sample was found to carry the same sequence change, showing that it was germinal. In addition, D312N in *CSF2RB* and Y167D in *CSF2RA* appeared in one healthy donor each. *Summary.* We have found some sequence changes in the genes we studied. Three cases (R164Q and Y167D in *CSF2RA*, and D312N in *CSF2RB*) were also present on healthy samples. These changes seem to be rare variants, although they could contribute to the effect of other mutations. For the six remaining cases the analysis of 20 control samples was negative. These sequence variants (1.10% (4/362) in *CSF2RB*, 0.55% (1/183) in *CSF2RA*, and 0.64% (1/156) in *IL3RA* in V617F*JAK2* negative CMPNs patients) could be rare variants or singletons but could also be oncogenic mutations. In fact, random mutagenesis experiments have shown an oncogenic effect for some *CSF2RB* mutations. In addition, W226X, found in *IL3RA* produces a truncated protein. We are currently studying the impact of the sequence changes we found on cell cultures. Anyway mutations in these genes, if existing, would be infrequent events in these diseases. Although considering all our data, almost 2% of CMPNs cases show alterations in these genes.

Non-Hodgkin lymphoma - Biology

0340

CD20 DIRECTS CELL POSITIONING IN SECONDARY LYMPHOID ORGANS

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Background. Human CD20 is a B cell restricted transmembrane molecule and the most successful monoclonal antibody targeted antigen, used worldwide to treat patients with B cell non-Hodgkin's Lymphoma (B-NHL). Unfortunately, the majority of B-NHL patients develops resistance to anti-CD20 therapy, resulting in relapse of the disease. Anti-CD20 antibody resistance is poorly understood and a current focus of investigation. Antibody resistance may be related to the interaction of CD20 with tumor cells and the microenvironment. However, although the CD20 molecule was discovered 30 years ago, its exact function is still unknown. *Aim.* The aim of this study is to gain insight in the role of the CD20 molecule in relation to the microenvironment. *Methods.* To explore the function of CD20, we used a system that isolates human CD20 from other B cell surface molecules by retrovirally transferring the human CD20 gene into normal human T cells. Then these CD20-positive T cells were used in *in vivo* mouse models and in *in vitro* functionality assays. *Results.* Initial *in vitro* assays comparing peptide-specific CD20-positive T cell clones with nontransduced parental clones demonstrated no altered proliferative activity or cytokine production associated with CD20 transduction. We then injected a T cell population containing 20% CD20-positive T cells into immune deficient RAG2-/-γc-/- mice (n=10) to compare the distribution of the cells into the organs with the distribution of normal human T cells (control mice n= 10) and human B cells (control mice n=5). Immunohistochemical staining of the organs revealed a remarkable phenomenon in all 10 spleens of the mice that received the transduced T cells: while normal T cells were scattered throughout the spleen, the CD20-positive T cells had positioned themselves periarteriolar in the same way as the human B cells do (figure 1).

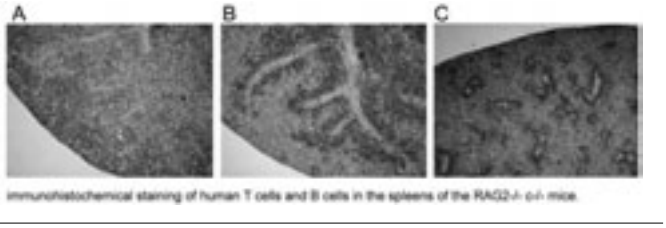

Figure 1.

In the other organs, like the gut, liver and lungs, in contrast, the distribution of the CD20-positive T cells did not differ from the normal T cells. To confirm the hypothesis that the migration capacity of T cells is altered following expression of the CD20 molecule, we studied the influence of CD20 expression on T cells in *in vitro* transwell migration assays. In these assays CD20-positive T cells exhibited a 50% decreased migration capacity towards stroma cells compared to the normal T cells (p=0.0075). *Summary/Conclusions.* These data demonstrate that the CD20 molecule directs the positioning of cells in secondary lymphoid organs. This indicates that CD20 holds back further migration of the cells in order to take a periarteriolar position, which may be the optimal site for the physiological (T cell independent) B cell antigen recognition. These findings help to understand the role of CD20-positive cells in their microenvironment, which opens up new ways to conquer anti-CD20 antibody resistance in the treatment of B-NHL.

0341

CD137 EXPRESSION IS INDUCED BY EPSTEIN-BARR VIRUS (EBV) INFECTION AND ACTIVATES NF-?B CONTRIBUTING TO THE DEVELOPMENT OF EBV-POSITIVE T/NK-CELL LYMPHOPROLIFERATIVE DISEASE

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Background. There are EBV-positive T/NK-cell lymphoproliferative diseases (EBV-T/NK-LPD), such as extranodal NK/T-cell lymphoma nasal type (ENKL), aggressive NK-cell leukemia, and chronic active EBV infection (CAEBV). In these diseases clonally proliferating cells are EBV-positive T or NK cells and it has been suspected that EBV may contribute to the development of these disorders. However, why and how EBV infects T or NK cells, whether EBV itself induces these malignancies, and the mechanism of action responsible for these malignancies have not been clarified to date. *Aims.* To clarify the molecular mechanism underlying the development of EBV-T/NK-LPD, we focused on costimulatory receptor CD137, which is expressed on the surface of activated T cells and plays a pivotal role in their proliferation, survival, and differentiation. *Methods.* Four EBV-positive T and NK cell lines, SNT8, SNK6, SNT15 and SNT16, were obtained for examination. These cell lines had been established from primary lesions of ENKL patients (SNT8 and SNK6) and peripheral blood of CAEBV patients (SNT15, 16). Clinical samples were obtained from CAEBV patients, diagnosed according to the criteria suggested by Okano M. *et al.* (Am J Hematol 80:64-9, 2005). To detect and isolate EBV-infected cells, T and NK cells were separated using magnetic beads from peripheral blood mononuclear cells. *CD137* gene and protein expression were analyzed by RT-PCR and flow cytometry respectively. For *in vitro* EBV infection, EBV was prepared from the culture medium of B95-8 cells and added to MOLT4 cells as previously described (PNAS 100:7836-40, 2003). NF-κB activation was examined by western blotting and electrophoretic mobility shift assay. Apoptotic cells were detected by $DiOC_6$ staining. The study was approved by the ethical committee and written informed consent was obtained from each patient. *Results.* We detected significantly high expression of *CD137* gene and protein in four EBV-infected T or NK cell lines compared to EBV-negative T and NK cell lines. *In vitro* EBV infection of MOLT4 cells resulted in induction of endogenous CD137 expression. Transient expression assay demonstrated that the EBV-encoded protein, LMP1, induced endogenous *CD137* gene expression. Next we validated the results using clinical samples. Sixteen patients (aged 8-72 years; 6 males, 10 females; infected cell types CD4: 4, CD8: 5, γδ: 1, and CD56: 6) were diagnosed with CAEBV. EBV-infected T or NK cells derived from these patients revealed significantly higher *CD137* gene expression than cells from healthy donors. CD137 protein expression was also upregulated in peripheral mononuclear cells from CAEBV patients in the presence of Interleukin-2, but not in cells from healthy donors. VP16-induced apoptosis of these cells was significantly suppressed through the stimulation of CD137 on the cell surface by the human CD137L stably expressed on Chinese Hamster Ovary cells. Moreover, NF-κB activation downstream of CD137 was shown. *Summary.* These results indicate that upregulation of CD137 expression by EBV in T/NK cells induces the anti-apoptotic intracellular signaling pathway through NF-κB activation and may contribute to the development of EBV-T/NK-LPD.

0342

DEREGULATION OF MULTIPLE CHEMOKINE RECEPTORS DURING GASTRIC MALT LYMPHOMAGENESIS - MALIGNANT TRANSFORMATION IS ACCOMPANIED BY LOSS OF CXCR4-SDF-1? SIGNALLING

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Background. Chemokine receptors play a crucial rule in development and progression of lymphoid neoplasms. *Aims and Methods.* Because the chemokine receptor expression profile in gastric MALT lymphoma is unknown, we performed an expression analyses of 19 chemokine receptors on gastric tissue samples derived from Helicobacter pylori (HP) associated gastritis, gastric MALT lymphoma and extranodal diffuse large B cell lymphoma (eDLBCL) originating from MALT lymphoma

(transformed MALT lymphoma) by using a semi-quantitative RT PCR approach. In addition five chemokine receptors (CCR8, CCR9, CXCR4, CXCR6, CXCR7) and the CXCR4 ligand SDF-1α were analysed by immunohistochemistry. *Results.* In the model of the stepwise development from a non-neoplastic lesion to the HP associated gastritis, we observed a de novo expression of CCR7, CXCR4, CXCR5 and CXCR7 and a loss of XCR1. Following malignant transformation from HP to MALT lymphoma, an up-regulation of CCR7 and CXCR7, de novo expression of CXCR3 and a loss of CXCR4 were detected. Additionally, we showed that the chemokine receptor expression profile of gastric MALT lymphomas differs substantially compared to eDBLCL with a higher expression of CCR1, CCR5, CCR7, CCR8, CXCR3, CXCR6 and CXCR7 and de novo expression of CCR9 and XCR1 in eDLBCL. Continuous expression of all B-cell homeostatic chemokine receptors was found in both lymphoma entities except for CXCR4, which was entirely lacking following transformation to MALT lymphoma. SDF-1α expression, the ligand for CXCR4 and CXCR7, was found in epithelial, endothelial and dendritic cells, macrophages, in lymphoma cells of gastric MALT lymphomas and with a significant higher number of positive lymphoma cells in eDLBCL. Comparing CXCR4 expression between gastric MALT lymphoma and nodal marginal B cell lymphoma and between gastric eDLBCL and nodal DLBCL, CXCR4 mRNA transcripts were exclusively found in nodal lymphomas. Furthermore, the proliferation rate of gastric MALT lymphomas and gastric eDLBCLs correlated with expression of CCR9 and CXCR7. *Conclusions.* Our results support a model of a stepwise progression of gastric MALT lymphoma from a non neoplastic event to HP associated gastritis, to MALT lymphoma, and finally to overt eDLBCL, is guided by differentially expressed B-cell homeostatic and activation-dependent chemokine receptors.

0343

JUNCTIONAL ADHESION MOLECULE C (JAM-C) INFLUENCES SELECTIVELY THE HOMING OF NORMAL AND MALIGNANT B-CELLS TO DIFFERENT LYMPHOID ORGANS

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Background. Homing of malignant B-cells to bone marrow (BM) and secondary lymphoid organs is of critical importance in disease progression of lymphoproliferative syndromes. Junctional adhesion molecule C (JAM-C) is an adhesion molecule with restricted expression to precise differentiation stages in B-cell maturation, allowing a clear classification into two types of B-cell malignancies: JAM-C positive lymphomas, with supposed origin in the marginal zone, and JAM-C negative lymphomas, with supposed origin in the germinal center. In the current study, we investigated the role of JAM-C in the proliferation and migration of normal and malignant B-cells. *Methods.* Human B-cells from tonsils and from peripheral blood of healthy donors and lymphoma patients were activated *in vitro* with interleukins (IL-2, IL-4, IL-10) and CD40L, with or without JAM-C antibodies, then proliferation and JAM-C expression were assessed by flow cytometry. To study the role of JAM-C in B-cell migration, B-cells were injected i.v. into NOD/SCID mice and homing of cells to lymphoid organs (BM, spleen, lymph nodes) was analyzed one hour later by flow cytometry and immunohistochemistry. In parallel experiments, B-cells were incubated with anti-JAM-C, anti-VLA4 or combinations of both antibodies prior to injection into mice. *Results.* Activation of B-cells led to a 50% decrease in JAM-C expression and incubation with anti-JAM-C antibodies significantly reduced proliferation of normal and malignant JAM-C pos B-cells by 33%. *In vivo* studies demonstrated that JAM-C pos B-cells from mantle cell lymphoma and marginal zone lymphoma have a reduced capacity to home to lymph nodes (decrease of 77% in number of cells compared to normal B-cells). Furthermore, blocking JAM-C with anti-JAM-C antibodies reduced the homing of normal and lymphoma JAM-C pos B-cells to BM and spleen by 50-60%. This contrasted to the reduced homing of B-cells into BM and lymph nodes but not into spleen when the cells were incubated with anti-VLA4 antibodies prior to injection. Interestingly, combination of both antibodies resulted in inhibited homing into the three lymphoid organs. *Conclusion.* Our results show for the first time a functional role of JAM-C in B cell proliferation and in the homing to lymphoid organs. Targeting JAM-C could thus constitute a new therapeutic strategy, with JAM-C blocking as a treatment to prevent lymphoma cells from reaching supportive microenvironments in BM and spleen.

0344

DEEP SEQUENCING REVEALS A COMPLEX PATTERN OF CLONALITY IN FOLLICULAR LYMPHOMA BY ANALYSIS OF THE HEAVY CHAIN OF THE IMMUNOGLOBULIN GENE IN DIFFERENT TUMOR SUB-POPULATIONS

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Background. We previously demonstrated the existence of a common progenitor cell (CPC) linking follicular lymphoma (FL) and transformed FL (t-FL) by analyzing the somatic mutation (SHM) of the heavy chain of the immunoglobulin gene (IgH-VH). *Aims.* We investigated IgH-VH using the Roche 454 GS FLX Titanium platform and DNA extracted from flow-sorted subpopulations, corresponding to 4 stages of B-cell maturation (i.e. pro/pre germinal centre (PP-GC), centroblasts (CB), centrocytes (CC) and memory enriched (ME)) in sequential biopsies from lymphoma cases showing a pattern of direct or divergent evolution, in order to determine the full extent of clonal diversity in FL and identify compartment specific changes that could explain these two models of progression. *Methods.* 20-45 ng of DNA (approximately 3500-7000 cells) from 7 lymph nodes cells suspension (6 FL/1 t-FL) corresponding to 3 patients (2 having a pattern of direct and 1 of divergent evolution) and 24 samples from the corresponding flow-sorted subpopulations, were investigated. The subpopulations were purified using IgD, CD38, CD10 and CXCR4 cell surface markers. Amplicons were prepared using IgH-VH3 primers (known to detect the tumor clone) modified with unique tags and the germline IgH from A375β6 melanoma cell line as control. Libraries were pooled and sequenced, twice, in both the directions. *Results.* We generated 937,000 high quality reads (average per library: 26,000; range 17,647 - 57,848). Among the sequences, tumor related sequences were 52%-77% in total biopsies and 9%-75% in subpopulations. The majority were identical to the major clone, except for the PP-GC and ME populations. A complex degree of clonality was observed, with the number of haplotypes ranging from 11 (in a ME sample) to 221 (in a CC) and many clones detected at a frequency <0.001. Most haplotypes were closely related to the major clone (only 2-3 different SHM) and typically showed a more mutated IgH-VH locus. Comparison of the samples from patients with a direct and divergent evolution did not show any difference regarding the number of haplotypes, level and type of SHM. In the samples from the patient with a CPC pattern it was possible to identify reads identical to the 2 major clones (each having 8 and 22 unique SHMs) in the other total biopsy and selected subpopulations, at frequencies from 0.02 to 0.0009. Sequences similar to the predicted CPC were not detected, suggesting that or is absent or it exists at a level below the assay's sensitivity. *Summary/Conclusions.* The investigation of the IgH-VH SHM in FL subpopulations using GS FLX Titanium platform unravels an unexpected complex level of clonality; the haplotypes' analysis shows a high variability among the different subpopulations, independently of the inferred model of evolution. This variability is more related to the individual than to the samples and similar profiles are observed when biopsies from the same patient are considered. Further investigation of the relationship and the maturation of these clones will better highlight the evolution of these FL clones.

0345

THE KINETICS OF SYSTEMIC CELLULAR IMMUNOSUPPRESSION IN PATIENTS WITH POOR-RISK DIFFUSE LARGE B-CELL LYMPHOMA DURING TREATMENT WITH 'CHOP-R': A PROSPECTIVE STUDY FROM THE ALLG

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Background. The immune system plays a pivotal role in the pathogenesis of lymphoma. Within the malignant lymph node, a variety of mech-

anisms have been identified by which the lymphomatous B-cell evades the host immune response. Conversely, the mechanism(s) by which systemic immunosuppression is mediated are poorly understood. Consistent with previous reports, in 122 consecutive patients with diffuse large B-cell lymphoma (DLBCL) treated with rituximab-CHOP therapy (median follow-up 31 mo.), lymphopenia (≤650mm3/L) was an adverse prognostic factor independent of IPI (p=0.03). Understanding the biology underpinning this observation is a pre-requisite to developing novel immunotherapeutic strategies in DLBCL. *Aim.* To perform a highly-detailed prospective functional characterisation of systemic cellular immunosuppression in DLBCL. *Method.* We collected 30mls peripheral blood in twenty-three HIV- patients with newly diagnosed poor-risk DLBCL (mean age =53 years, range32-71, 2F:M13). Poor-risk was defined as IPI>1 and/or bulky (≥7.5cm) disease. Serial samples were taken at specified time-points: prior to therapy ('baseline') and twenty-one days after cycle four CHOP-R therapy ('interim'). All patients received pegylated G-CSF at day +2. 20 age-matched healthy controls were used for comparison. All participants provided informed consent. The study was performed by the Australasian Leukaemia Lymphoma Group. *Results.* In patients with DLBCL, at baseline (versus controls) circulating CD3+ T-cells but not CD19+ B-cells are decreased. This was due to lower CD4+ counts (p<0.001) with normal CD8+ T-cells and CD3-CD56+ NK-cells. Interim CD4+ T-cells remained reduced. To put this into context, we quantified conventional cellular immune evasion mechanisms. However, we observed no difference in patient levels of either CD4+CD127-CD25hi regulatory T-cells or CD14-HLADR-CD33+ myeloid derived suppressor cells. By contrast, CD14+ monocytes were strikingly elevated in patients at baseline relative to control subjects (p<0.0001). In paired analysis, levels of monocytes rose further at interim. However, the surface marker composition of circulating monocytes markedly changed, with total CD14+HLA-DR-/lo monocytes increased at baseline compared to interim. A paired comparison of CD14+HLA-DRhi / CD14+HLA-DR-/lo monocytes between baseline and interim showed the ratio to be higher in baseline patient samples (p<0.01). The CD14+HLA-DR-/lo phenotype is a newly identified monocyte subset proposed to mediate systemic immunosuppression, including modulating lymphocyte proliferation, effector T-cell activity, and impairing responsiveness to anti-tumour vaccines. As circulating monocytes can differentiate into dendritic cells (DC) *in-vivo*, (and serve as a reservoir of DC for immunomodulation), we quantified the numbers of mature monocyte derived DC (moDC) that differentiate from adherent PBMC. Concordant with the decline in CD14+HLA-DR-/lo monocytes, *in-vitro* differentiation to moDC was improved at interim relative to baseline. *Summary.* There is growing interest in stimulating host immunity in poor-risk DLBCL. The optimal timing of immunomodulation is unknown, but will be influenced by circulating effector cells, immunosuppressive monocytes, and the timing of concurrent therapy. To our knowledge we present the first study outlining the kinetics of lymphocyte subsets and CD14+HLA-DR-/lo monocytes in DLBCL. We show that CD14+HLA-DR-/lo monocytes are elevated at baseline but normalize after 4 cycles of CHOP-R. CD14+HLA-DR-/lo monocytes appear inversely proportional to CD4+ T-cells, and have reduced ability to differentiate into moDCs.

0346

BONE MARROW STROMA CD40 EXPRESSION IN SPLENIC MARGINAL ZONE LYMPHOMA IS ASSOCIATED WITH PROMINENT MAST CELL INFILTRATION AND CORRELATES WITH SHORTER TIME TO PROGRESSION

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Background. Splenic marginal zone lymphoma (SMZL) is an indolent mature B-cell malignancy. However, nearly one-third of patients display a rapidly progressive disease and a dismal outcome. Risk stratification has been recently proposed based on the assessment of clinical and laboratory parameters on diagnosis. Biological prognostic factors are still lacking and their identification might prove of great value for identifying patients at high risk of unfavorable disease. In SMZL, bone marrow (BM) infiltration is almost invariably observed on diagnosis and the BM microenvironment may play an important role in the disease progression. *Aims.* Aim of this study was to characterize the BM microenvironment associated with SMZL infiltrates in order to identify

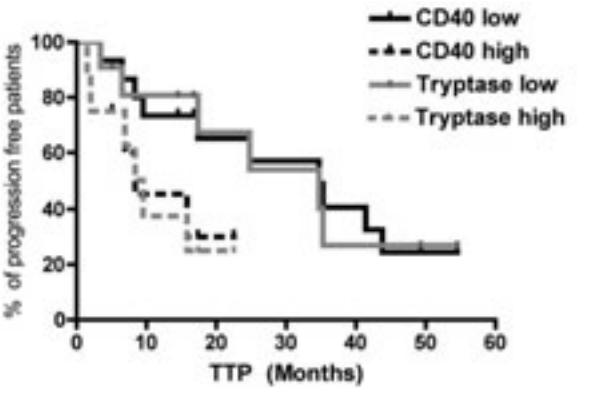

Figure 1.

potential influences of the stroma on the biology and natural history of this lymphoma. *Methods.* Routinely processed BM biopsies (BMB) of thirty-five consecutive cases of SMZL diagnosed at our Institution between 2001 and 2010 were collected. All patients had a BMB at the time of diagnosis. Besides the expression of CD20, CD3, CD5, CD43, CD23, bcl-2 and bcl-6, density and distribution of stromal elements were immunohistochemically evaluated on BMBs by semiquantitative analysis of the markers CD10 (adventitial reticular cells), CD31 (blood vessels), and CD40 (activated stromal cells and endothelia). Moreover, the amount of immune cells infiltrating the SMZL lymphoid aggregates was estimated by counting the number of CD68+ (macrophages), DC-Sign+ (myeloid DCs), CD4+ (Th cells), and tryptase+ cells (mast cells). Time to progression (TTP) was used as clinical endpoint. TTP was calculated as the time interval between diagnosis and progressive disease (PD). PD was defined either as an increase in size of previously documented disease greater than 25%, or as the appearance of disease at any new site or even the shift to a more aggressive histotype. *Results.* We found a significant correlation between the amount of infiltrating tryptase+ mast cells and TTP (Fig. 1). Patients with elevated numbers (above mean value) of mast cells (41.4%) had a mean TTP of 11.1 months, as compared with patients with low mast cell numbers (58.6%) that showed a TTP of 30.8 months (p=0.05). A strong positive correlation was observed between CD40 stromal expression and the number of tryptase+ mast cells (p=0.0001). Multivariate analysis was performed including, along with tested immunohistochemical variables, clinical and laboratory features of the SMZL prognostic score system, such as hemoglobin, LDH, and albumin levels. Notably, Cox proportional hazard revealed that the amount of tryptase+ mast cells populating SMZL BM infiltrates, and CD40 expression in the BM stroma were significant and independent predictors of a shorter TTP (p=0.05; p=0.03) in our SMZL patients. *Conclusions.* Here we demonstrated that BM microenvironment-related features, namely the presence of tryptase+ mast cells and CD40 stromal expression, could have a role in determining prognosis of SMZL patients. Our preliminary results point out a possible functional link between mast cells and BM stromal cells that may impact on neoplastic clone survival and proliferation. Further investigations will address the functional significance of such interactions.

0347

MANTLE CELL LYMPHOMA: USING THE TRANSCRIPTION FACTOR SOX11 AS BIOMARKER FOR MCL VIA A HIGHLY SENSITIVE AND SPECIFIC QPCR ASSAY

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Background. In both leukemia and malignant lymphomas balanced chromosomal translocation are seen as tumor markers in a significant part of the patients. These translocations characterize the molecular phenotype of the disease, which provides for a mean to diagnose accurately using PCR and can be used as biomarkers to determine minimal residual disease (MRD) using real-time quantitative PCR (qPCR). MRD quantification with markers specific for the malignant cells makes more risk based treatment strategies possible. The translocation t(11;14) is the hallmark of Mantle cell lymphoma (MCL) and results in cyclin D1 (CCND1) overexpression but it only gives limited information on the course of MCL. Some patients suffer an aggressive course with large tumor burdens while other resembles CLL, which may not require treatment. The gene encoding the transcription factor SOX11 was in

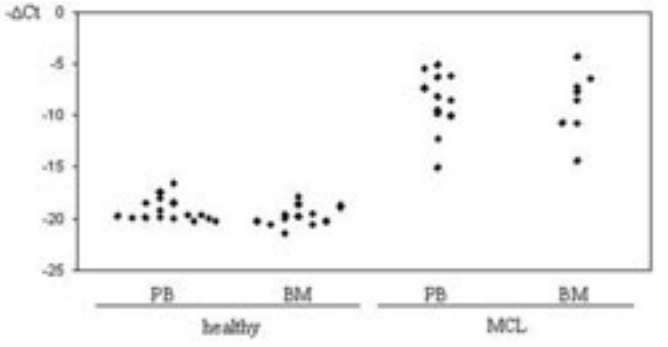

Figure 1. SOX11 expression.

1995 described to play a part in CNS development and later as a potential tumor marker for MCL as it is overexpressed in this subset of hematological malignancy and the SOX 11 expression seems to give information on the clinical and biological behavior in MCL. *Aims.* We wished to establish a very sensitive and specific qPCR assay for detection and quantification of SOX11 mRNA in order to use this gene as biomarker for MCL. As SOX11 is an intronless gene the challenging task was to establish a sensitive and specific qPCR assay without the risk of contamination from genomic DNA (gDNA). Furthermore we wished to compare the expression patterns of SOX11 and CCND1 in a cohort of MCL patients. *Methods.* Mononuclear cells were obtained from peripheral blood (PB) and bone marrow (BM) samples from healthy individuals and diagnostic PB and BM samples from MCL patients. The cell line Granta 519 was used as positive control for SOX11 and CCND1 expression. Total RNA was prepared and cDNA was synthesized using anchored oligo(dT) primers avoiding amplification of gDNA in the downstream qPCR. We overcame the risk of gDNA contamination by the design of a polyA specific reverse primer together with an LNA modified TaqMan probe ensuring efficient amplification and detection of SOX11 cDNA. GUS and B2M were used as control genes. *Results.* We find a highly significant differences in expression of SOX11 between healthy individuals and MCL patients (PB: $p<10^{-4}$, BM: $p=10^{-4}$, calculated using Wilcoxon rank-sum test). Figure 1 depicts the SOX11 expression levels in healthy individuals and diagnostic MCL PB and BM evaluated as -ØCt (ØCt = Ct(SOX11)-(average Ct control genes)) with an assay sensitivity of 10^{-4}. Ct = 40 was used as cut off between SOX11 negative and positive samples. *Conclusions.* A highly sensitive and specific qPCR assay without risk of contaminating gDNA was established enabling SOX11 to be used as biomarker for following MRD in MCL. This potent qPCR assay will be used in the ongoing longitudinal study of MRD in MCL patients in order to demonstrate a connection between treatment response and load of MRD in the heterogeneous group of MCL patients.

0348

ROLE OF GLYCOLYSIS IN THE PATHOGENESIS OF NON-HODGKIN LYMPHOMA

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Background. Non-Hodgkin lymphoma is a set of heterogeneous diseases with different pathology and malignant behaviors, varied a lot from indolent to aggressive, and chemosensitive to chemoresistant, thereby highlighting the importance of determine the underlying molecular mechanisms and biomarkers for the evaluation of treatment efficacy and prognosis. *Aims.* To investigate the relationship between metabolomic, biologic, and genomic phenotypes of Non-Hodgkin lymphoma, identify the pathogenesis biomarker and the corresponding mechanism, and provide a target for therapy. *Methods.* C57BL/6Nr mice were administered either corn oil or the environmental carcinogen dibenzo-[a,l]pyrene (DB[a,l]P) via gavage to establish the lymphoma-bearing mouse models. Tumors were developed in various organs including liver, spleen, lymphnode, thymus, small intestine, mammary gland, ovary, kidney and bone marrow, demonstrated by histopathology and immunohistochemistry. Urine and serum samples were collected and metabolomic analysis was performed using Ultra Performance Liquid Chromatography coupled to Time-of-Flight Mass Spectrometry (UPLC- QTOFMS). Biomarker extraction was undertaken using SIMCA-P+ and Random Forests. Non-

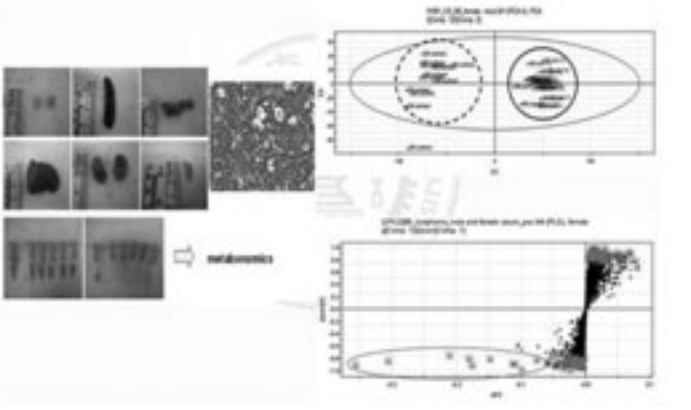

Figure 1. Metabolomics study of lymphoma-bearing mice.

Hodgkin lymphoma cell lines Namalwa, Raji, SU-DHL-4 and Jeko-1 cells were maintained. Cell proliferation and cytotoxicity were detected using Cell Counting Kit-8. Real-time PCR were performed to determine the molecular mechanisms. *Results.* Principal Component Analysis (PCA) revealed good separation of lymphoma and control groups, and biomarkers contributed to the separation were identified as metabolites of energy metabolism, especially the glycolysis pathway. The abnormal accumulation of lactate, final product of the glycolysis pathway under hypoxia, and succinate, the intermediate metabolite of mitochondrial tricarboxylic acid cycle, suggested the activation of glycolysis and dysfunction of mitochondrial respiration. Real-time PCR demonstrated a significant upregulation of glycolysis enzyme genes including hexokinase 1, glucose-6-phosphate isomerase, phosphofructokinase, aldolase A, fructose-bisphosphate, riosephosphate isomerase 1, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase 1, phosphoglycerate mutase 1, enolase 1, pyruvate kinase, and lactate dehydrogenase A genes in lymphoma cell lines. Furthermore, the upregulation levels were positive correlated with the malignant behaviors of those cell lines as Namalwa > Raji > SU-DHL-4 > Jeko-1 cells. Glycolysis inhibitor 2-Deoxyglucose (2-DG) exhibited cytotoxic effect in Namalwa cells, together with a significant decrease in the production of lactate and transcription of glycolysis enzyme genes, suggesting glycolysis as a target for lymphoma therapy. Summary/conclusions: In the present study, metabolomic analysis clearly distinguished lymphoma-bearing mice from control mice. The underlying mechanisms of the fundamental metabolic alteration were identified as an activation of glycolysis in the lymphoma cells, resulting in the consumption of more glucose to get sufficient ATP supply for their active proliferation. Inhibition of glycolysis may have potential therapeutic implications due to the preferential killing of lymphoma cells. Further metabolomic studies using human biofluid samples are needed to determine whether those markers would have clinical utilities.

0349

ACTIVATION AND STABILIZATION OF P53 BY NUTLIN-3A LEADS TO DOWNREGULATION OF HSP90 AND SYNERGISTIC EFFECTS WITH 17-AAG IN ANAPLASTIC LARGE CELL LYMPHOMA

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Background. The tumor suppressor gene p53 is frequently expressed but rarely mutated in ALK+ anaplastic large cell lymphoma (ALCL) (Rassidakis *et al*, Leukemia 2005, 19:1663), an aggressive type of T-non Hodgkin lymphoma. Recently, a small molecule that functions as a potent Mdm2 inhibitor, nutlin-3a, has been developed, which disrupts the interaction between p53 and Mdm2 resulting in stabilization and activation of p53. In a recent study, we showed that nutlin-3a induces cell cycle arrest and apoptosis through activation of p53 in ALK+ ALCL cells carrying wild -type (wt) (SUPM2) or mutated (mt) but partially functional p53 gene (DEL) (Drakos *et al*, Leukemia 2009, 23:2290). *Aim.* The aim of the study was to identify the proteomic profile of ALK+ ALCL before and after stabilization and activation of wild-type (wt) p53. *Methods.* Mass spectrometry-based proteomics were used to identify the proteomic profile of ALK+ALCL cell lines before and after treatment with

nutlin-3a in an effort to uncover new targets of activated p53 protein in our *in vitro* system. Cell viability and proliferation assays were performed. Expression levels of proteins were analyzed by Western blot. *Results.* Our preliminary proteomic data revealed that Hsp90 protein levels were significantly decreased following nutlin 3a treatment in SUP-M2 and DEL. This finding was confirmed using Western blot analysis and whole lysates of the same ALK+ ALCL cell lines. Combined treatment of ALK+ALCL cells with nutlin-3a and 17-AAG, a potent inhibitor of Hsp90, which is already used in investigational clinical trials in patients with aggressive lymphomas, resulted in significant decrease of cell viability assessed by trypan blue exclusion assay. In addition, proliferation of viable cells assessed by MTS assay and total cell numbers was substantially reduced following nutlin 3a treatment in these ALK+ ALCL cell lines. Analysis of these data also demonstrated synergistic effects in cell death and proliferation of cells treated with nutlin-3a and 17-AAG as compared with the results for each agent alone. *Conclusions.* Our preclinical findings support the rationale for combined use of targeted therapies such as nutlin 3a and 17-AAG in aggressive lymphoma types.

0350

ANALYSIS OF T-CELL SUBSETS BY FLOW CYTOMETRY IN LYMPH NODE BIOPSIES IDENTIFIES PATIENTS WITH GOOD PROGNOSIS IN FOLLICULAR LYMPHOMA (FL)

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Background. Tumor microenvironment plays an important role in the outcome of the patients with FL. By gene expression and immunohistochemistry, an increase in macrophages has been associated with poor outcome, whilst an increase in T-cells is associated with better prognosis. The quantification of immunohistochemical staining is time consuming and poorly standardized. The use of flow cytometry could help in identifying the different groups of risk in FL patients. *Patients and Methods.* Lymph nodes at diagnosis from 73 patients (35M/38F, median age 59, range 29 to 81) with FL were processed by standard flow cytometry. The percentage of CD3, CD4, CD8, CD57, and germinal center (GC) CD4 cells (CD4+CD57+), as well as the ratio B/T, CD4/CD8, CD4/CD3, CD8/CD3 and GC-CD4/CD4 were correlated with the main initial features and the clinical outcome of the patients. Histological grade 1 and 2 was observed in 58 patients, grade 3a in 12 and grade 3b in 1. Low-risk FLIPI was observed in 52%. 61 patients have received polychemotherapy, including rituximab in 36. After a median follow-up of 6 years, 23 patients have died, with a 5-year overall survival (OS) of 76%. *Results.* The mean (±SD) percentage of B-cells, CD3, CD4, CD8 and GC-CD4 was 59.7% (±15.1), 35.2% (±15.4), 26.4% (±12.3), 8.7% (±5.5), and 3.6% (±2.9), respectively. Age >60 years was associated with higher percent of CD3, CD8 cells and higher CD3/B-cell ratio. Grade 3 histology was associated with higher CD3 and CD8 cells and lower CD4/CD8 than grades 1 and 2. Response to treatment was not related to lymphocyte subpopulations. FLIPI, among other clinical variables, was able to predict OS. Patients with a CD4/CD8 ratio 4.4 had better OS than the remainder (5-year OS: 100% vs. 68%, respectively; p=0.01). Patients with high GC-CD4/CD4 ratio (0.19) showed a better OS than the others (5-year OS: 100% vs. 65%, respectively; p=0.02). A multivariate analysis identified GC-CD4/CD4 (p=0.03), CD4/CD8 (p=0.01) and FLIPI (p=0.04) as the most important variables to predict OS. *Conclusion.* flow cytometry allows the identification of a microenvironment pattern associated with good prognosis in patients with FL.

0351

ACQUIRED IGH TRANSLOCATIONS IN SPLENIC MARGINAL ZONE LYMPHOMA TARGET MISCELLANEOUS PARTNER GENES, INCLUDING MYB

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Background. Splenic marginal zone lymphoma (SMZL) is a rare low-grade B-cell lymphoma that has been recognized as a separate entity in the WHO classification. Chromosomal abnormalities are present in more than 70% of cases, most frequently gains of 3q and 12q, and loss of 7q sequences. In rare SMZL cases, immunoglobulin (*IG*)-mediated translocations affecting *BCL6*/3q27, *MUM1*/6p25, *PAX5*/9p13 and *BCL3*/19q13 have been described. We have collected four cases of SMZL characterized by del(7q) and t(*IGH*), either in the stem line, or in a subclone. In one of these cases, occurrence of the t(*IGH*) coincided with clinical progression of the disorder. *Aims.* Our project aims to identify partner genes affected by t(*IGH*) in SMZL and to unravel their role in the pathogenesis/progression of these disorders. *Methods.* Fluorescence *in situ* hybridization (FISH) with a set of probes for candidate partner genes was applied. In one case with unbalanced t(*IGH*), additional array comparative genomic hybridization (aCGH) analysis was performed with the Agilent 244K platform. Transcript levels of candidate genes were analyzed by QRT-PCR. *Results.* All four presented SMZL cases showed del(7q) and one of the following translocations: t(6;14)(q23;q32) (case 1), der(14)t(14;18)(q12;q32) (case 2), t(14;19)(q32;q13) (case 3) and an unknown t(*IGH*) detected by interphase FISH (case 4). FISH with probes for 7q and *IGH* was performed to determine the prevalence of both aberrations in each sample. The t(6;14) of case 1 was absent in the diagnostic sample, but appeared as a subclonal aberration at the time of clinical progression. FISH mapped the 6q23 breakpoint in the region of *MYB*. The unbalanced t(14;18) of case 2 showed a breakpoint centromeric to *BCL2* and *MALT1*. FISH and aCGH analysis narrowed down the breakpoint to the region harboring *DCC*, *MBD2* and *TCF4*. Further molecular studies of both cases are ongoing. The t(14;19) of case 3 showed involvement of *BCL3*, and in case 4 a normal FISH status of the candidate partner genes *BCL3*, *BCL6*, *MYB* and *PAX5* was found. *Conclusion.* Our preliminary data suggest that *IGH* translocations are recurrent secondary events acquired during the clinical course of SMZL. These translocations target miscellaneous partner genes, including *BCL3* and *MYB*. *MYB* is a proto-oncogene that encodes a transcription factor important for lineage commitment, proliferation and differentiation of hematopoietic progenitor cells. So far, involvement of *MYB* in the pathogenesis of B-cell malignancies has not been reported.

0352

COMPARISON OF FLOW CYTOMETRY AND BONE MARROW BIOPSY IN THE DETECTION OF LYMPHOID INFILTRATION IN NON-HODGKIN'S LYMPHOMAS

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Background. Bone marrow biopsy (BMB) is mandatory for non-Hodgkin's lymphoma's (NHL) staging, being also useful for assessing treatment response and for re-staging in relapse. Immunophenotypical analysis by multiparametric flow cytometry (MFC) has been increasingly used in hematological malignancies due to its high applicability and sensitivity at diagnosis and for detection of minimal residual disease (MRD). However, the use of MFC in the routine clinical settings for NHL staging is not yet well established. *Aims.* To compare the value of trephine biopsies (BMB) and multiparametric flow cytometry (MFC) in the assessment of bone marrow infiltration in NHL. *Methods.* 494 diagnostic (n=282) and follow up (n=212) specimens, simultaneously analysed by BMB and MFC, from non Hodgkin's lymphomas (NHL) patients were included in the study. Most patients (87%) had B-cell NHL: diffuse large B cell lymphoma (DLBCL) 35% (n=172), follicular lymphoma (FL) 27% (n=135), mantle cell lymphoma (ML) 10% (n=49), marginal zone lymphoma (MZL) 9,7% (n=48), Burkitt's lymphoma (BL) 2.2% (n=11), small lymphocytic lymphoma (LLC/SLL) 1.8% (n=9) and lymphoplasmacytic lymphoma (LPL) 1.4% (n=7); the rest of cases (12.8%) were classified as T/NK lymphomas (T/NK NHL). BMB infiltration was categorized as nodular, interstitial, mixed and diffuse. Selected panels of monoclonal antibodies were used for MFC study, using four-colour direct immunofluorescence technique and according to previously well described methods, aimed to identify and characterize B and/or T cells in BM. *Results.* Concordant results between BMB and MFC were found in 396 samples (80%), being both techniques negative in 83% (330/396) and positive in 17% (66/396) of cases. Discrepant results were found in 98 cases (20%), with a similar distribution among cases BMB+MFC- (42%; 41/98 cases) and BMB-MFC+ (58%; 57/98 cases). Considering histology, discrepant results were found more frequently in T/NK NHL (33%; 21/63 cases) in which MFC

was a little more sensitive than BMB, since 62% of discrepant cases (13/21) corresponded to BMB-MFC+ cases, whereas 38% (8/21) corresponded to BMB+/MFC- cases. In ML 20% were discrepant cases (10/49), also being MFC more sensitive than BMB (90% of discrepant cases were BMB-/MFC+). In MZL 23% (11/48) cases were discrepant, with a similar frequency of cases BMB-MFC+ (45%) and BMB+MFC- (55%). In FL and DLBCL discrepancies were more uncommon, with 17% (23/135 cases) and 15% (26/172 cases) of discrepancy, respectively. In FL, BMB was lightly more sensitive than MFC (13/23 -56%- being BMB+MFC-), and, by contrast, in DLBCL MFC was more sensitive (15/26 -58%- being BO-MFC+). Although with low number of cases, all discrepancies in LPL (2/7) were BMB+CMF- whereas in BL (2/11) and LLC/SLL (3/9) all discrepancies were BMB-MFC+. *Summary/Conclusions.* According with our results, we can conclude that MFC can detect a subgroup of patients with bone marrow involvement in which BMB is negative. Therefore both techniques complement each other and both should be used for the detection of bone marrow infiltration in B cell disorders.

0353

FREQUENT DELETION OF THE TUMOR SUPRRESSOR TNFAIP 3 IN SEZARY SYNDROME SAMPLES

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The genetic background of Sezary syndrome, a disseminated form of cutaneous T-cell lymphomas (CTCL), is still a matter of discussion. To unravel genetic imbalances, high density comparative genome hybridization was performed on leukemia samples derived from 12 patients. We identified bi- and monoallelic deletions of the tumor necrosis factor alpha induced protein 3 gene (TNFAIP3; A20) in a high proportion of SS patients as well as biallelic A20 deletion in the SS-derived cell line SeAx. Furthermore, we demonstrate that inhibition of A20 activates the NF-κB pathway thereby increasing the proliferation of normal T lymphocytes. On the other hand, the reconstitution of A20 expression slowed down the cell cycle in SeAx cells. Recently A20 inactivation has been reported in various B-cell lymphomas. In this study we show that A20 is also a putative tumor suppressor in the T-cell malignancy - Sézary syndrome.

0354

PATIENTS WITH B-CELL NON HODGKIN LYMPHOMA SHOW INCREASED FREQUENCIES OF REGULATORY T CELLS AND CD8+ T-CELL EXPANSIONS

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Background. Although most non Hodgkin lymphomas (NHL) take origin from the B-cell lineage, several studies suggest that any impairment involving the different branches of the immune system may play a role in their pathogenesis. Moreover the cross-talk among lymphoma cells and other cell types, such as for instance T-lymphocytes and antigen presenting cells, within the peritumoral microenvironment seem to deeply influence the onset and evolution of NHL. *Aim.* In order to explore the possible impact that the degree of activation of the T-cell immune system and the balance among different T-cell populations may have on the NHL pathogenesis, we analysed the T-cell receptor (TCR) repertoire and the distribution of different T-cell subsets -including regulatory T-cells (Treg)- in patients with NHL. *Methods.* Our study was based on a flow cytometric analysis performed on the peripheral blood of 15 patients (6 with indolent NHL and 9 with diffuse large B-cell lymphoma, DLBCL) and 15 age-matched controls. We first determined the frequency of CD3+, CD4+, CD8+ and CD16-56+ T-cells. Treg were then identified by considering the CD4+ cell fraction characterised by a very high (>2 log) expression of CD25 and by a very low (<2 log) expression of CD127, as well as by determining the expression of FoxP3 and CD152. TCR repertoire analysis was based on a panel of 24 beta variable (BV) family-specific antibodies. A BV expansion was defined as any value of BV family expression higher than the mean + 3 standard deviations calculated in normal controls. *Results.* We first showed that patients had reduced frequencies of CD16+ CD56+ natural killer cells (14% vs 21%) when compared with normal controls, while CD3+, CD4+ and CD8+ frequencies were similar. Patients also showed a higher frequency of Treg than controls (mean 2.35% vs 1.14%), although this increase was mainly confined to patients with DLBCL (mean 2.79%) rather than in patients with indolent NHL (mean 1.70%). Finally we determined the frequency of expanded T-cell subpopulations expressing the same TCR BV subfamilies, showing in patients and controls a similar frequency of expansions in CD4+ cells (1,3% vs 1,5%), besides an increased frequency of CD8+ expansions in patients (5,7% vs 3,2%). When we looked at the possible influence of several disease-related factors, such as WHO lymphoma subtype, IPI score, presence of constitutional symptoms, bone marrow involvement and stage, only a diagnosis of DLBCL rather than indolent NHL was associated with a trend towards an increased frequency of CD8+ lymphocyte expansions (6,2% vs 4,8%). *Conclusions.* Our preliminary data suggest that the T-cell branch of the immune system in patients with NHL show features which can be distinguished from those observed in normal controls. In particular, NHL patients seem to show an increased degree of activation of the TCR repertoire along with a higher frequency of Treg, which are both even more pronounced in patients with aggressive NHL.

0355

PRIMARY EFFUSION LYMPHOMA CELL ADHESION TO EXTRACELLULAR MATRIX PROTEINS

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Background. Primary effusion lymphoma (PEL) is a KSHV-associated B-cell non-Hodgkin lymphoma developing as malignant effusions in serosal cavities. The extracellular matrix (ECM) of serosal cavities contains type IV collagen, fibronectin (FN) and laminin (LN). *Aims.* To investigate the ability of PEL cells to adhere to ECM proteins and to identify the integrin receptors mediating cell-ECM interactions. *Methods.* The expression pattern of integrins was determined in eight PEL cell lines using membrane immunostaining and flow cytometry analyses. Their ability to attach to human ECM proteins versus human serum albumin used as control was assessed in cell-ECM adhesion assays, with or without preincubation with specific blocking monoclonal antibodies to various integrin subunits or isotype-matched immunoglobulins, and competing RGD peptides. *Results.* All PEL cell lines were found to express CD29/β1 and CD49f/α6 integrins. All but one (ISI-1) were found to be CD49d/α4-positive. CD49b/α2, CD49c/α3, CD49e/α5, α9β1, CD104/β4 and αVβ3 were found to be expressed in three (BBG-1, BCP-1, CRO-AP/3), two (BCP-1, ISI-1), five (BC-1, BCBL-1, BCP-1, HBL-6, ISI-1), four (BC-1, BC-3, CRO-AP/3, ISI-1), five (BC-1, BC-3, BCP-1, CRO-AP/3, ISI-1) and four cell lines (BC-1, BC-3, CRO-AP/3, ISI-1), respectively. All PEL cell lines were found to lack expression of CD49a/α1 and CD18/β2. Cell-ECM adhesion assays showed a significant binding of BC-3 (11.23 ± 6.09%), CRO-AP/3 (14.20 ± 0.93%) and BCP-1 (28.14 ± 15.66%) to FN, and a significant binding of BC-3 (12.67 ± 3.12%), BBG-1 (21.66 ± 8.35%) and ISI-1 (30.94 ± 9.02%) to LN. BCBL-1, BC-1 and HBL-6 did not show any significant attachment to ECM proteins. No significant binding to type IV collagen was observed. LN binding was reduced by 62% for BBG-1 (8.57 ± 5.76%, P=0.002), 55% for BC-3 (5.71 ± 2.16%, P=0.02), and 76% for ISI-1 (7.50 ± 4.64%, P=0.009) by anti-CD29/β1 mAb, and by 78% for BBG-1 (4.82 ± 2.24%, P=0.01), 59% for BC-3 (5.16 ± 4.64%, P=0.02) and 50% for

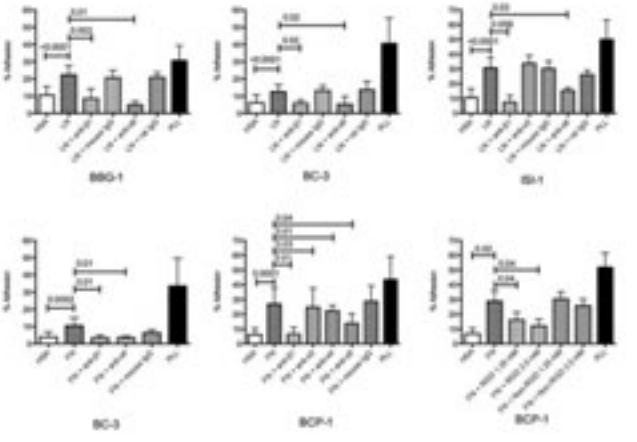

Figure 1.

ISI-1 (15.31 ± 2.09%, P=0.03) by anti-CD49f/α6 mAb. Incubation with anti-CD49c/α3, anti-α9β1, anti-αVβ3 and anti-CD104/β4 mAbs did not inhibit PEL cell adhesion to LN. FN binding was reduced by 68% for BC-3 (3.33 ± 1.70%, P=0.01) and 76% for BCP-1 (6.49 ± 4.62%, P=0.01) by anti-CD29/β1. BC-3 adhesion to FN was also reduced by 67% (3.49 ± 1.04%, P=0.01) by anti-CD49d/α4, whereas BCP-1 adhesion to FN was moderately although significantly reduced by anti-CD49c/α3 (24.76 ± 13.06%, P=0.03), anti-CD49d/α4 (22.23 ± 3.80%, P=0.01) and anti-CD49e/α5 (13.76 ± 6.64%, P=0.04) mAbs, with 8%, 18% and 49% inhibition, respectively. Moreover, competing RGD peptides significantly reduced BCP-1 attachment to FN, in a dose-dependent manner, suggesting a predominant involvement of α5β1/VLA-5. *Conclusions.* All PEL cell lines express the LN receptor α6β1/VLA-6 and at least one FN receptor, mostly α4β1/VLA-4. BBG-1, BC-3 and ISI-1 cell attachment to LN is predominantly α6β1/VLA-6-dependent. BC-3 binding to FN is mostly α4β1/VLA-4-dependent, whereas BCP-1 binding to FN is mediated by α3β1/VLA-3, α4β1/VLA-4 and α5β1/VLA-5, with a predominant involvement of α5β1/VLA-5. These integrin receptors may represent interesting targets for the development of novel therapeutic strategies for patients with PEL.

0356

HANS' CLASSIFIER IN PATIENTS TREATED WITH DOSE ADJUSTED EPOCH-R (DAEPOCH-R) FOR ADVANCED DIFFUSE LARGE CELL B LYMPHOMA (DLBCL)

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Purpose. Analysis of the Progression Free Survival (EFS) and overall survival (OS) of diffuse large cell B lymphoma treated with R-EPOCH utilizing Han's classifier. *Material and Methods.* We use R-EPOCH as described by Wilson *et al*.[1] No radiation therapy was applied over bulky disease. We included only patients diagnosed of diffuse with a IPI score higher than 3 or IPI adjusted<65 higher than 2. Han's classifier was applied as reported by Hans *et al*.[2] Cases were considered positive for CD10,BCL6 and MUM when the number of cells stained were higher than 30% of the tumor cells. Cases were classified as Germinal centre when CD10 was positive, or if CD10- cases when BCL6+ and MUM1- concurrently. *Patients.* 33 Patients were treated with R-EPOCH. Median age was 55 (28-77); 15 patients (45%)were older than 60 year old. 20 patients (60%) were male. Ann Arbor stage was: IIB: 2 patients; IIIA:, IIIB: 2, IV: 28. Median LHD was 811 (256-5413 u.i/L; normal 480 u.i/L). Median Beta 2 microglobulin was 3.07 (1,3-7,3 mg/dl; control 2). Adjuted age IPI (aaIPI) mean was 3,55; IPI mean was 4,03. *Results.* Median follow-up is 28 months (1.4- 73.3). 26 patients are alive. Treatment outcomes were: 27 achieved CR; 1 uCR; 3 (9%) patients were resistant or progressed during treatment. Three patients died during treatment. 5 patients relapsed at a median follow-up of 7.5 months (6-40,867). Median OS or EFS have not been reached. OS at 5 years was 72% and EFS was 60.58%. Three relapsed/resistant patients were successfully salvaged with Autologous stem-cell transplantation, one patient obtained complete response with R-ESHAP. Bcl6 were positive in 95% of the cases; Bcl2 in 55%; CD10 in 25%; MUM-1 in 75%. 33% of the patients were Germinal-Center type according to Han's classifier. In univariate analysis only high LDH was correlate with poor outcome (p=0.05). We have not found correlation with outcome with age, beta-2-microglobulin, stage and expression of bcl-2, bcl-6, MUM1 and Hans classifier. *Conclusion.* DA EPOCH-R overcomes poor prognosis derived from biological markers, including Bcl6. We not confirm the negative impact of Bcl-6 negativity observed by Wilson *et al*1. Our results compared favorably to R-CHOP or R-CHOP plus transplant in high risk DLCBL. These results are even better than the results obtained by Wilson *et al* in patients with worse prognosis1 and confirms the results of Garcia-Suarez *et al*.[3]

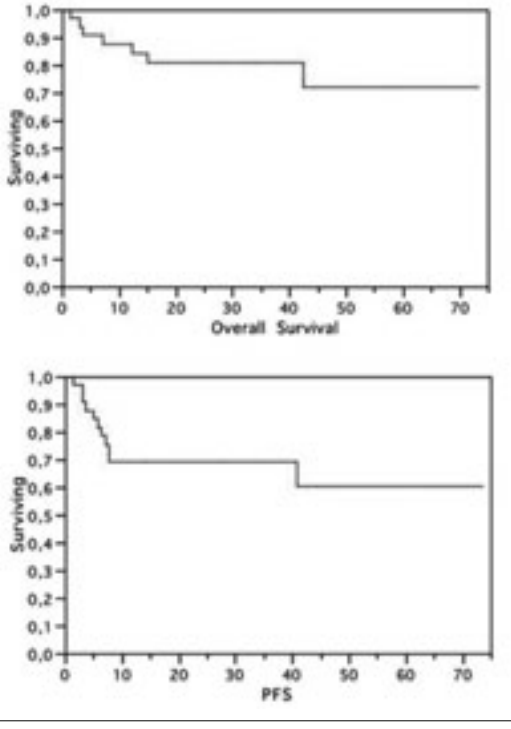

Figure 1.

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0357

MANTLE CELL LYMPHOMA CELLS EXPRESS B7 FAMILY MOLECULES AND B7-H1 EXPRESSION ARE UP-REGULATED AFTER INTERFERON-GAMMA AND LPS EXPOSURE VIA MEK-DEPENDENT PATHWAY IN MANTLE CELL LYMPHOMA CELLS

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Background. Mantle cell lymphoma (MCL) is a distinct subtype of B-cell non-Hodgkin lymphomas characterized by a specific t(11;14)(q13;q32) translocation, causing over-expression of cyclin D1. Recent studies demonstrated that B7 family molecules were not only expressed on antigen presenting cells but also on various hematopoietic malignancies and solid tumors, and may play important roles in tumor immunology. Many cytokines could upregulate the expression of B7 molecules, however, the molecular mechanism of regulating expressions of B7 molecules in mantle cell lymphoma are still unknown. *Aims.* We detect the expression of B7 family molecules in mantle cell lymphoma cells and investigate the expression of B7 family molecules in mantle cell lymphoma cells after interferon-gamma and LPS exposure and study the cell signaling pathway involved. *Methods.* RNA isolation, RT-PCR, quantitative real-time polymerase chain reaction, Flow cytometry, Cellular lysate preparation, Western blot analysis and Signal transduction analyses. *Results.* RT-PCR and flow cytometry demonstrated that MCL patients and cell lines express B7 family molecules. After interferon-gamma and LPS stimulation, B7-H1 expression were upregulated detected by flow cytometry in MCL patients and cell lines. When we knocked down TLR4, LPS stimulation did not up-regulate B7-H1 expression. Pretreatment and coincubation of MCL cells with the MEK1/2 inhibitor UO126 reduced interferon-gamma and LPS induced B7-H1 expression, indicating that the MEK pathway was crucial for B7-H1 expression in MCL cells. To confirm that LPS and interferon-gamma induced B7-H1 expression through a MEK pathway in MCL cells, we stimulated MCL cells with LPS or interferon-gamma and analyzed the phosphorylation of ERK1/2 at different time. ERK1/2 phosphorylation were significantly up-regulated following LPS or interferon-gamma treatment. We confirmed that pretreatment of the cells with MEK inhibitor U0126 inhibited LPS or interferon-gamma induced phosphorylation of ERK1/2. *Summary/Conclusions.* In conclusion,

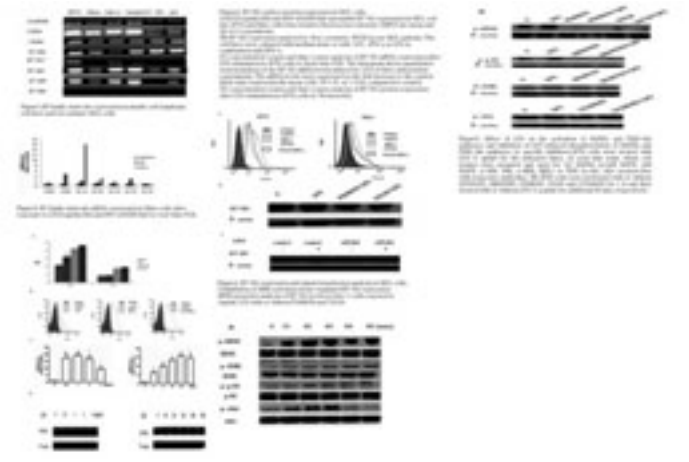

Figure.

our study demonstrated that mantle cell lymphoma cells express B7 family molecules. B7-H1 expression were up-regulated after interferon-gamma and LPS exposure via MEK-dependent pathwaysin MCL cells.

0358

DECREASED NUMBER OF CIRCULATING T REGULATORY CELLS AMONG PATIENTS WITH NEWLY DIAGNOSED DIFFUSE LARGE B CELL LYMPHOMA

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Introduction. T regulatory cells (Tregs) are a subset of lymphocytes contributing to immune evasion by maliganacies. However, in contrast to solid tumors their role in promoting diffuse large B cell lymphoma (DLBCL) has not been clearly established. So far the presence of Tregs was demonstrated in involved tissues with conflicting results with regard to prognosis. The goal of our study was to prospectively analyze the number of Tregs in peripheral blood of patients with newly diagnosed DLBCL. Results were correlated with clinical characteristics. *Methods.* Blood samples from 19 patients with median age of 58,5 (22-79) years were collected and analyzed with the use of multiparameter flow cytometry for the presence of CD3+CD25+FoxP3+ (phenotype of "natural"Tregs) cells including subtypes characterised by expression of CD45RA (naïve Tregs), HLA-DR (marker of activation), CTLA4 (marker of costimulation), CD39 (selectin P), CD62L (marker of homing to inflamed regions). Results were compared with those achieved for 12 healthy individuals. *Results.* The absolute number of circulating Tregs, including both Tregs expressing CD45RA and CD45RA-negative was significantly lower among patients compared to controls (Table 1). The same was found for subpopulations of HLA-DR+ and CD62+ Tregs. The proportion of CD45RA+ cells among Tregs was also reduced for DLBCL group. Among patients with DLBCL the number of circulating Tregs was higher for patients in good performance status (ECOG=0) compared to those with ECOG=1 or 2: 46 (23-107) x10e6/L vs. 10 (4-48) x10e6/L, p=0.004.The number of Tregs did not correlate with age, LDH level, clinical stage, or the presence of general symptoms. *Conclusions.* The number of circulating Tregs is significantly decreased in patients with DLBCL. The reduction is associated with poorer performance status. Our observations together with previously published studies suggest that Tregs migrate from peripheral blood to involved areas and therefore may play a role in the pathogenesis of DLBCL.

Table 1.

Subpopulation	DLBCL n=19	Controls N=12	p
CD4+CD25+FoxP3+ (x10e6/l)	19 (4-107)	44 (19-104)	0.02
CD45RA (% Treg)	18 (7-60)	37 (13-56)	0.04
CD4+CD25+FoxP3+CD45RA+ (x10e6/l)	3 (0-28)	12 (4-52)	0.004
CD4+CD25+FoxP3+CD45RA- (x10e6/l)	17 (2-88)	30 (10-52)	0.04
HLA-DR+ (% Treg)	27 (8-63)	25 (14-49)	0.76
CD4+CD25+FoxP3+HLA-DR+ (x10e6/l)	5 (1-35)	11 (5-30)	0.03
CD39+ (% Treg)	60 (8-89)	45 (25-63)	0.16
CD4+CD25+FoxP3+CD39+ (x10e6/l)	7 (1-79)	17 (8-48)	0.06
CD62L+ (% Treg)	89 (7-97)	90 (71-94)	0.75
CD4+CD25+FoxP3+CD62L+ (x10e6/l)	13 (2-94)	36 (17-97)	0.02
CTLA4+ (% Treg)	81 (55-97)	90 (84-90)	0.58
CD4+CD25+FoxP3+CTLA4+ (x10e6/l)	20 (2-101)	67 (54-93)	0.1

0359

DNA MICROARRAY ANALYSIS IN DLBCL DEPENDENT ON SKP2 EXPRESSION

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Background. The heterogeneity of diffuse large B-cell lymphoma (DLBCL) has prompted the search for new markers that can accurately separate prognostic risk groups. We previously showed in multivariate analysis that high Skp2 expression by immunohistochemical method was a strong predictor of poor outcome in DLBCL. To better characterize the molecular mechanisms, we performed the DNA microarray study on DLBCL patients. *Material and Methods.* To investigate which genes are aberrantly expressed in DLBCL cells, we performed cDNA microarrays and compared gene expression profiling of either group with high (n=4) or low Skp2 DLBCL cells (n=4). *Results.* We selected 633 genes, 311 upregulated and 322 downregulated, which showed significant differences with a P-value of <0.01 (lima). IPA (Ingenuity pathway analysis) showed clearly the network composed of cell cycle regulators, which mediate cell cycle progression during the G1/S checkpoint. A computer-assisted approach was used to procure specific molecular signalling pathways that were aberrantly expressed in DLBCL cells. Several genes related to cyclins and cell cycle regulation and to the MAPK, JAK/Stat, WNT, tumor growth factor β and Myc mediated apoptosis signalling pathways were altered in DLBCL cells when compared with Skp2 levels. In addition, MTA3 (Metastasis -associated protein 3), which was direct corepressor WNT4 pathway, was a high average increase (1.16-lima/fold change, respectively) in high Skp2 DLBCL. MTA3 are reportedly highly expressed in high-growth fraction lymphomas, such as Burkitt Lymphoma, DLBCL. *Conclusion.* These genes may play a significant role in the pathogenesis of DLBCL and deserve further investigation as candidates for new therapeutic targets.

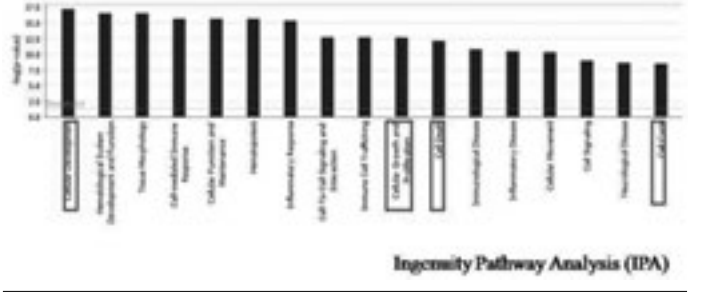

Figure 1. Functional classification (High Skp2 vs Low Skp2).

0360

EXPRESSION OF CHEMOKINE RECEPTORS CCR1 AND CCR2 IN B-CELL LYMPHOMA CELL LINES AND ON CD10-POSITIVE B-CELLS IN PERIPHERAL BLOOD OF PATIENTS WITH B-CELL LYMPHOPROLIFERATIVE DISORDERS

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Chemokines - chemokine receptors (CRs) signal transduction network controls the proper function of the immune system. Previously we have identified the cluster of CR genes that includes CCR1 and CCR2, on human 3p21.31 within the malignancy-related chromosome

instability region (MCIR). Genes within MCIR are often silenced in cancer cells by DNA rearrangements and/or by epigenetic mechanisms (methylation). This 3p21.3 region was the most frequently deleted (LOH was detected in 83% of informative cases) in human solid tumors from different tissues (576 tumors from 10 tissues were analyzed) (Petursdottir *et al*., 2004). EBV is associated with endemic Burkitt's lymphoma (BL) and post-transplant lymphoproliferative disease. EBV infection leads to B-cell activation and transformation. Upon infection viral proteins induce interferon pathway, cell-surface adhesion molecules, activation antigens, chemokines and CRs (CCR6, CCR10, and CCR7). The aim of this study was to examine expression of CCR1 and CCR2 in long-time cultivated CD10+ B-cell lines both, EBV-negative (EBV-) and EBV-positive (EBV+), and also in peripheral blood (PB) circulating CD10+ B-cell subset of primary patients (prior specified diagnosis and treatment) with B-cell lymphoproliferative disorder (LPD). Twenty three B-cell lines (18 BLs (11 EBV+ and 7 EBV-), 3 PB B-cell lymphomas (BcL), and 2 diffuse histiocytic lymphomas (DHL)) were assayed by duplex RT-PCR for CRs (CCR1, CCR2, CCR5) from 3p21.31 region, CXCR4, CD markers (CD10, CD30, CD34, CD38, CD77), and EBV genes (EBNA1, EBNA2, LMP1) as well. Eleven cell lines (all BL) that transcribed CCR1 (among them 7 transcribed also CCR2), and PB of 8 patients were analyzed by polychromatic flow cytometry (FC), using monoclonal antibodies CD19-PerCP-Cy5.5, CD10-PE, CD191-Alexa-Fluor647 and CD192-Alexa-Fluor647. All cell lines were negative for CD34 and CCR5 transcripts, and were positive for CXCR4 transcript. In all 12 EBV- cell lines CCR2 transcript was not found, but CCR1 transcript was detected in two. On the contrary, among 11 EBV+ cell lines seven were CCR2-transcript positive and nine were CCR1-transcript positive. Notably that by polychromatic FC CCR2 was only found in about 10% of cells in 3 EBV+ cell lines, but CCR1 was present in the range of 4 - 36% in 9 out of the 11 EBV+ and in the range of 6 - 10% in two EBV-cell lines. In 8 samples of primary patients with CD10+ B-cell LPD CCR1 and CCR2 were observed on PB circulating CD10+ B-cell subset in the range of 98 - 100% and 76 - 99% respectively. Our results tentatively suggest that the lack of CCR2 in PB circulating CD10+ B-cell subset might be associated with progression of immature B-cell malignancy. Obviously, further extensive studies are necessary for the verification of our hypothesis. Petursdottir *et al*., Genes Chromosomes Cancer, 2004; 41:232-242.

Non-Hodgkin lymphoma - Clinical 1

0361

BORTEZOMIB-RITUXIMAB RESULTS IN IMPROVED PFS AND RESPONSE RATES VERSUS RITUXIMAB, AND QUALITY OF RESPONSE IS ASSOCIATED WITH IMPROVED OUTCOMES, IN PATIENTS WITH RELAPSED FOLLICULAR LYMPHOMA (FL)

B Coiffier,[1] E Osmanov,[2] X Hong,[3] A Scheliga,[4] J Mayer,[5] F Offner,[6] S Rule,[7] A Teixeira,[8] J Walewski,[9] M Crump,[10] O Shpilberg,[11] R Hermann,[12] DL Esseltine,[13] E Zhu,[14] C Enny,[14] P Theocharous,[14] H van de Velde,[15] Y Elsayed,[14] PL Zinzani[16]

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Background. In patients with FL, quality of response to first-line therapy has been linked with improved survival. Additional active treatment options are required for patients with relapsed FL to improve outcomes. Rituximab is approved for relapsed/refractory FL; bortezomib has shown activity alone and in combination with rituximab in relapsed FL. The international, multicenter, phase 3 LYM3001 study compared bortezomib-rituximab with rituximab alone in patients with rituximab-naïve/-sensitive relapsed FL. *Aims.* This analysis was conducted to determine the impact of quality of response to treatment on outcomes. *Methods.* Patients with grade 1/2 measurable, relapsed FL (time to progression[TTP] ≥6 months for prior rituximab-containing therapy) were randomized (1:1) to five 35-day cycles of bortezomib (1.6 mg/m^2, days 1, 8, 15, 22, all cycles) plus rituximab (375 mg/m^2, days 1, 8, 15, 22, cycle 1, and day 1, cycles 2-5), or rituximab alone. All patients provided written informed consent. The primary endpoint was progression-free survival (PFS). Response/progression were assessed by an independent radiology committee using modified International Workshop Response Criteria. PFS, duration of response (DOR), TTP, time to next lymphoma treatment (TTNT), and treatment-free interval (TFI) were assessed in patients achieving complete response (CR)/unconfirmed CR (CRu) (verified by bone marrow and lactate dehydrogenase), partial response (PR), or no response (NR). *Results.* A total of 676 patients were enrolled to receive bortezomib-rituximab (n=336) or rituximab alone (n=340). Baseline characteristics were generally well balanced between arms; median age was 57 (range 24-83)/57 (range 21-84) years, 51%/40% were male, 41%/41% had high (≥3) FLIPI score, and 43%/44% had received prior rituximab in the bortezomib-rituximab/rituximab arms. After a median follow-up of 33.9 months, median PFS was 12.8 months with bortezomib-rituximab versus 11.0 months with rituximab (HR 0.822, p=0.039); overall response rates (CR/CRu+PR) were 63% versus 49% (p<0.001), including 25% versus 18% CR/CRu. In both arms, PFS was significantly longer in patients who achieved CR/CRu versus PR versus NR (bortezomib-rituximab: 32.6, 13.6, 4.5 months, respectively; rituximab: 33.2, 14.1, 4.7 months; p≤0.001 for all comparisons). Similarly, higher quality of response was associated with longer DOR, TTP, TTNT, and TFI in both treatment arms. Median DOR was 16.0 and 13.8 months, with 50%/32% of patients in the bortezomib-rituximab arm and 38%/23% in the rituximab arm having responses durable for 6/12 months. Median TTP was 13.3 versus 11.3 months (HR 0.808, p=0.027), median TTNT was 23.0 versus 17.7 months (HR 0.799, p=0.024), median TFI was 17.7 versus 13.0 months, and 1-year OS rate was 90.1% versus 90.5%, with bortezomib-rituximab versus rituximab. In the bortezomib-rituximab and rituximab arms, 46%/21% of patients had grade

≥3 adverse events (AEs), 18%/11% had serious AEs, and 17%/1% had peripheral neuropathy (3% and 0% grade ≥3), respectively. *Conclusions.* Bortezomib-rituximab resulted in improvements in PFS, response rates, DOR, and other outcomes compared with rituximab alone, with acceptable additional toxicity. Achievement of CR/CRu versus PR versus NR was associated with greater clinical benefit in both arms. The longer PFS/TTNT in the bortezomib-rituximab arm was driven by the additional responses with bortezomib-rituximab, which were as meaningful as those on rituximab alone.

0362

BENDAMUSTINE FOR PATIENTS WITH INDOLENT LYMPHOMA - A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS (RCT)

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Background. Survival of patients with indolent lymphoma has improved in the recent decade. While it is clear that the addition of rituximab to induction chemotherapy improves survival of these patients, it is unclear which is the best chemotherapy to combine with rituximab. None of the chemotherapy regimens that had been compared in randomized controlled trials (RCTs), were superior in terms of overall survival (OS). A number of RCTs have examined the effect of bendamustine in patients with indolent lymphoma. Progression free survival (PFS) was similar or prolonged with bendamustine compared to other chemotherapy and an OS benefit has not been shown. *Aims.* We performed a systematic review and meta-analysis to evaluate the effect of bendamustine on the OS of patients with indolent lymphoma. *Methods.* We included RCTs that compared bendamustine to other chemotherapy regimens for patients with indolent lymphoma. In December 2010 we searched The Cochrane Library, MEDLINE, LILACS, conference proceedings, and databases of ongoing trials. The primary outcome was all cause mortality. Relative risk (RR) for dichotomous data and hazard ratio (HR) for time to event data were estimated and pooled. *Results.* We identified 4 trials, conducted between the years 1994 and 2010 randomizing 1251 adult patients with a mean/median age of 58 - 68. The rate of patients with follicular lymphoma ranged between 40% to 52%, and mantle cell lymphoma 20% to 22% in the 3 trials that included patients with those types of lymphoma. One trial included only patients with chronic lymphocytic leukemia (CLL). The comparisons were between bendamustine, vincristine, prednisone to cyclophosphamide, vincristine, prednisone (COP); bendamustine-rituximab to cyclophosphamide, adriamycin, vincristine, prednisone, rituximab (RCHOP); bendamustine-rituximab to fludarabine-rituximab; and bendamustine to chlorambucil. Patients treated with bendamustine had an improved OS compared to controls, RR for death 0.80; 95% CI 0.67 - 0.97, I2 = 0 (Figure). After excluding the trial with only CLL patients the RR is 0.82; 95% CI 0.67 - 1.01. PFS was improved with bendamustine, HR 0.47; 95% CI 0.39 - 0.57. The rate of complete remission improved with bendamustine compared to controls, RR 2.31; 95% CI 1.07 - 4.96, random effects model, I2 = 88%. The rate of grade 3/4 adverse events was unaffected RR 1.21; 95% CI 0.99 - 1.48. *Conclusions.* This meta-analysis shows for the first time that bendamustine improves OS and PFS of patients with indolent lymphoma and CLL compared to other chemotherapy. These results should be interpreted cautiously due to the wide clinical heterogeneity of patients and treatments. Further trials of a more homogenous group should be performed to explore the role of bendamustine in various lymphoproliferative neoplasms.

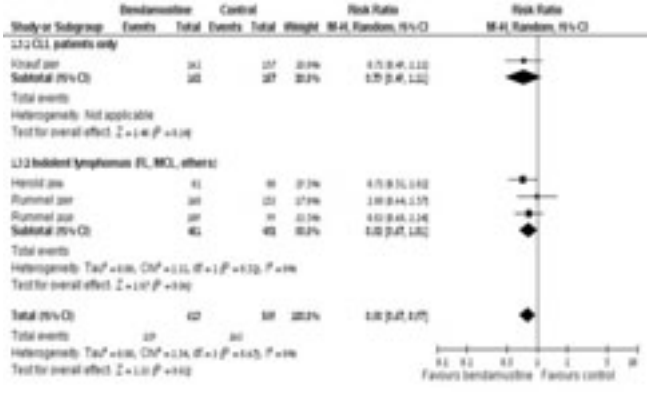

Figure 1. All cause mortality in patients with indolent lymphoma treated with bendamustine compared to other chemotherapy. CI = confidence interval; CLL = chronic lymphatic leukemia; FL = follicular lymphoma; MCL = mantle cell lymphoma.

0363

PRALATREXATE REVERSES THE TREND IN PROGRESSIVE RESISTANCE WITH SUCCESSIVE CHEMOTHERAPY REGIMENS IN THE TREATMENT OF RELAPSED OR REFRACTORY PERIPHERAL T-CELL LYMPHOMA (PTCL)

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Background. It is well documented that objective response rate (ORR) and progression-free survival (PFS) decrease with each subsequent chemotherapy regimen in the treatment of most cancers, a hallmark of acquired drug resistance. Although not reported, this trend often defines the natural history of patients with PTCL. The most common first-line treatment in PTCL is cyclophosphamide/doxorubicin/vincristine/prednisone (CHOP); however, despite high response rates, most patients progress within 6 to 12 months and require further salvage systemic therapy. Pralatrexate (FOLOTYN®) was granted accelerated approval in the United States for the treatment of relapsed/refractory PTCL based on results of the pivotal PROPEL study. As part of patients' medical history, data were collected on the ORR and PFS documented in previous therapies. Patients had a median of 3 prior therapies (range, 1-12). *Aims.* This retrospective analysis was conducted to evaluate whether progressive resistance was observed in patients with relapsed/refractory PTCL and to assess activity of pralatrexate compared with patients' previous treatments. *Methods.* Using investigator assessment of response, analyses were conducted according to the patients' number of prior systemic therapies. PFS and ORR for pralatrexate were compared with PFS and ORR for the most recent therapy prior to pralatrexate (-1); PFS and ORR of the most recent prior therapy (-1) were compared with those of the second prior therapy (-2); and PFS and ORR of second prior therapy (-2) were compared with the third therapy prior to pralatrexate (-3). *Results.* Results indicated a trend of reduced PFS and ORR with prior successive therapies, which appeared to be reversed by pralatrexate. Of the 109 evaluable patients in the PROPEL study, all had ≥1 prior therapy. For example, for the 57 patients who had ≥3 prior therapies in the -3 versus -2 analyses, the hazard ratio (HR) was 0.660, median PFS was 213.5 days, and ORR was 56% for the -3 therapy, compared with a PFS of 140 days and ORR of 33% for -2 therapy. These same patients had a further decrease in median PFS and ORR (95 days and 30%, respectively) with their -1 therapy. The trend was reversed with pralatrexate, which produced a median PFS of 134 days and ORR of 40% (Figure). Similarly, for the 86 patients who had ≥2 prior therapies in the -2 versus -1 analysis, the HR was 0.785, median PFS was 144 days, and ORR was 38%. In the full population of 109 patients with ≥1 prior therapy, the HR further increased to 1.051 for the -1 prior therapy when compared with pralatrexate; median PFS

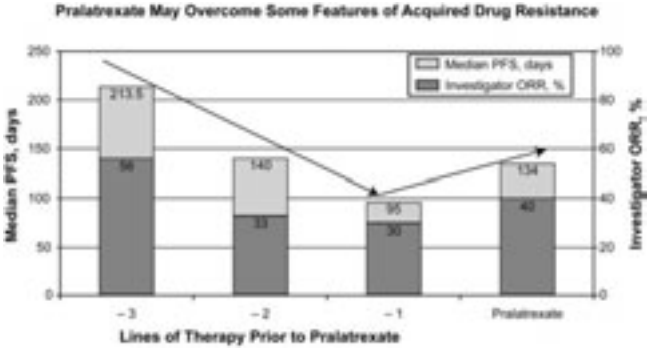

Figure 1.

was 114 days and ORR was 38% with the immediately previous line of therapy, as compared with a median PFS of 121 days and ORR of 39% for pralatrexate. *Conclusions.* This analysis demonstrated that patients with PTCL exhibit progressive resistance to treatment in which outcomes worsened with successive therapy. This trend was reversed with pralatrexate. Pralatrexate demonstrated higher responses and longer PFS than would be expected in a later line of therapy, thus reversing the trend of progressive resistance.

0364

DICE AS SALVAGE TREATMENT IN PATIENTS WITH RELAPSED OR REFRACTORY EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE

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Background and Aims. Recently, non-anthracycline-based chemotherapy was shown to be effective in extranodal NK/T-cell lymphoma, nasal type. Thus, a prospective phase II study was conducted in our institution to evaluate the efficacy and safety of DICE regimen in patients with untreated or relapsed disease. Here, we reported the treatment outcomes of DICE in the salvage setting. *Methods.* Thirty-eight patients with relapsed or refractory extranodal NK/T-cell lymphoma, nasal type were enrolled and received DICE (dexamethasone 40 mg, ifosfamide 1200 mg/m^2, cisplatin 20 mg/m^2 and etoposide 75 mg/m^2 on days 1 to 4) as salvage treatment. After chemotherapy, radiotherapy could be considered for patients with localized stage or residue disease if possible. *Results.* The median age of the patients was 38 years (range, 21 to 79). Other major patient characteristics were shown in Table 1. Of the 18 patients with stage IV disease, skin involvement was most frequent and developed in 10 patients (55.6%). In terms of prior treatment, 27 patients (71.1%) received radiotherapy and 31 patients (81.6%) had chemotherapy exposure which was mostly anthracycline-based (77.4%). The median cycles of DICE was four (range, 1 to 6). Twenty-one patients responded to DICE with an overall response rate of 55.3%. The complete and partial response rates were 23.7% and 31.6%, respectively. Radiotherapy was given to 13 patients with localized stage or residue disease after chemotherapy. Among 11 patients of them, who did not achieve complete response with chemotherapy, 6 patients (54.5%) were rendered disease-free by radiotherapy. Hematological toxicities were remarkable, but easily managed including dose-reduction in 17 patients (44.7%). All patients experienced grade 3/4 neutropenia and 10 patients (26.3%) had grade 3 neutropenic fever. The grade 3 and 4 anemia rates were 10.5% and 7.9%, respectively. Seven patients (18.4%) experienced grade 3 thrombocytopenia. Grade 3/4 non-hematological toxicities were uncommon except nausea/vomiting. There was no chemotherapy-related death. With a median follow-up time of 22 months (95% CI, 8.4 to 35.2), 17 patients died and 7 of them were complicated by hemophagocytic syndrome. Two-year progression-free and overall survival (OS) rates were 42.9% and 58.7%, respectively. Sub-group analysis demonstrated that chemo-sensitivity significantly correlated with survival. Two-year OS rates for patients with complete response, partial response and no response were 77.8%, 66.7% and 35.3% (P = 0.019). Through multi-variate analysis, response status after chemotherapy was also found to be the sole independent prognostic factor (RR 1.74, 95% CI 1.15 - 2.62; P = 0.009). *Conclusions.* In the present study, DICE was proved to be effective with manageable toxicities in patients with relapsed or refractory extranodal NK/T-cell lymphoma, nasal type. Response after chemotherapy had a significant impact on survival outcome. Efforts need to be done in an attempt to improve chemo-sensitivity which might be achieved by integrating novel agents with DICE. e-mail address: medoncol@gmail.com (Ye Guo, MD).

Table 1. Major patient characteristics.

Characteristics	No.	%
Gender		
Female	6	15.8
Male	32	84.2
Performance status		
0	3	7.9
1	35	92.1
Stage		
I	16	42.1
II	4	10.5
IV	18	47.4
B symptom		
Absent	17	44.7
Present	21	55.3
LDH level		
Normal	27	71.7
Elevated	11	28.9

0365

LONG-TERM FOLLOW-UP OF RITUXIMAB AND INFUSIONAL CYCLOPHOSPHAMIDE, DOXORUBICIN, AND ETOPOSIDE (CDE) IN COMBINATION WITH HAART IN HIV-RELATED NON-HODGKIN'S LYMPHOMAS (NHL)

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Background. The combination of Rituximab plus chemotherapy (CT) is more effective than CT alone in the treatment of high grade NHL. *Objective.* To report the long-term follow-up of CDE plus Rituximab in HIV-NHL. *Methods.* In June 1998, we started a phase II study using infusional CDE (Cyclophosphamide 187.5 mg/m2/day, Doxorubicin 12.5 mg/m2/day and Etoposide 60 mg/m2/day) administered by continuous intravenous infusion for 4 days every 4 weeks and Rituximab 375 mg/m2 i.v. on day 1. HAART was given concomitantly with CT. *Results.* Seventy-four patients (pts) have been enrolled. The median CD4+ cell count was 161 (range 3-691) and the median Performance Status was 1 (range 0-3). Diffuse large B-cell NHL was diagnosed in 72% of pts and Burkitt in 28%. Seventy per cent of pts had advanced stage (III-IV) disease and 57% of pts had an age-adjusted international prognostic index >2. Fifty-two out of 74 pts (70%) achieved a complete remission (CR), 4/74 (5%) had a partial remission and 18 pts progressed. With a median follow-up of 61 months, only 17% of CRs have relapsed and 41/74 pts are alive. The overall survival, disease free survival and time to treatment failure (TTF) at 5 years were 56%, 81% and 52%, respectively. Four cases of secondary tumours have been observed. No case of late pulmonary or cardiac toxicity has been reported. *Conclusions.* The combination of Rituximab and CDE in HIV-NHL treated concomitantly with HAART is very active. CR rate (70%) and TTF at 5 years (52%) are comparable to those observed in high grade NHL of the general population. Our data confirm that in HAART era a high proportion of HIV-NHL can be cured.

0366

NAVITOCLAX (ABT-263) SAFETY AND EFFICACY IN PATIENTS WITH RELAPSED OR REFRACTORY LYMPHOID MALIGNANCIES: PRELIMINARY PHASE 2 RESULTS FROM A PHASE 1/2A STUDY

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Background. Bcl-2 family proteins are associated with tumor initiation and are frequently over-expressed in lymphomas. Navitoclax, a novel, oral, small molecule BH3 mimetic with clinical activity in lymphoid malignancies, binds with high affinity (Ki ≤1 nM) and inhibits Bcl-2, Bcl-Xl and Bcl-w, which regulate survival of lymphocytes, platelets and spermatocytes. *Methods.* This is a phase 2a safety-expansion portion of a phase 1/2a, single-agent, international study of patients with relapsed/refractory lymphoid malignancies, ≥1 prior chemotherapy regimen and ECOG status ≤1. The Phase 1 results have been reported previously. Following a 7 to 14-day lead-in dose-titration of 150 mg/day oral navitoclax, patients with platelet count ≥50,000/mm^3 proceeded to

21/21-day continuous dosing at 250 mg/day on C1D1 followed by possible dose titration to 325 mg/day, based on safety data from the initial 11 patients. Safety, efficacy, and pharmacokinetics (PK) were evaluated in Arm A (relapsed/refractory follicular lymphoma [FL]) and Arm B (other indolent B-cell lymphoid malignancies). Preliminary efficacy analyses included tumor response (IWG, NCI-WG criteria) and progression-free survival (PFS). Adverse events (AEs) were graded by NCI CTCAE v3. *Results.* Twenty-six patients (median age 62 years, range 42-86) are enrolled in the phase 2a study. Eleven patients enrolled on Arm A (FL), and 15 on Arm B (CLL [6], SLL [2], mantle-cell lymphoma [2], lymphoplasmacytic lymphoma [2], marginal-zone lymphoma [1], low-grade B-cell lymphoma nos [1], and transformed prolymphocytic leukemia [1]). Tumor response for patients in Arm A: 1 CRu, 4 SD, 4 PD, 2 incomplete data; and in Arm B: 6 PR, 6 SD, 1 PD and 2 incomplete data. For CLL/SLL patients in Arm B, 4 had PR (2 nodular), 2 SD, 1 PD; 1 incomplete data; and 7 had >50% reduction in absolute lymphocyte count. Overall 7 (27%) patients had objective responses. Median PFS [95% CI] for all patients was 6.4 months [3.1, not reached (NR)]; Arm A 3.0 months [1.5, 3.8]; and Arm B 6.4 months [NR, NR]. The most common navitoclax-related AEs (any grade) were diarrhea (81%), nausea (50%), and thrombocytopenia (42%); the most common Grade 3/4 AE was thrombocytopenia (31%). Two patients had serious AEs: bacterial sepsis, tumor lysis (1 each), and 7 patients had AEs (mainly thrombocytopenia) leading to dose reduction. Navitoclax exposure appeared consistent across cycles. *Conclusions.* Navitoclax was reasonably well tolerated with most toxicity due to on-target effects. Thrombocytopenia was predictable and manageable. Following a lead-in dose of 150 mg/day oral navitoclax, patients with platelet counts ≥50,000/mm^3 proceeded to 21/21-day continuous dosing at 250 mg/day on C1D1 followed by possible dose titration to 325 mg. As observed in the phase 1 study, CLL/SLL showed the best tumor responses. In other tumor types, navitoclax should be tested in combination with other agents.

0367

RESULTS OF A PHASE 2 STUDY OF AME-133V (LY2469298), AN FC-ENGINEERED HUMANIZED MONOCLONAL ANTIBODY, IN LOW AFFINITY FCGRIIIA PATIENTS WITH PREVIOUSLY TREATED FOLLICULAR LYMPHOMA

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Background. AME-133v is a humanized monoclonal antibody that via Fc engineering has greater CD20 affinity and antibody-dependent cell-mediated cytotoxicity (ADCC) potency than rituximab *in vitro*. A Phase 2 study was conducted to assess the safety and efficacy of AME-133v in patients with previously treated follicular lymphoma (FL) who expressed a low affinity variant of FcγRIIIa. The Phase 1 dose-escalation component of a Phase 1/2 clinical trial of AME-133v at doses ranging from 2 - 375 mg/m2 demonstrated that AME-133v was safe and tolerable at all dose levels tested, and 375 mg/m2 was chosen for further assessment. *Aims.* The aims of the Phase 2 component of this study were to determine the safety and tolerability of four, weekly infusions of AME-133v at 375 mg/m2, and to determine the pharmacokinetic (PK) profile, objective response rate, and duration of response in patients who are F-carriers in one or both alleles that encode amino acid position 158 of the FcγRIIIa gene. *Methods.* After obtaining informed consent, 50 patients were enrolled in the Phase 2 study. Based on the Phase 1 dose escalation results, AME-133v was administered at the highest previously tested dose of 375 mg/m2 intravenously every week for 4 weeks. Six patients were treated at 375 mg/m2 during dose-escalation and are included in this analysis as pre-specified in the protocol. Safety, PK, response, and progression free survival were assessed. Response was also assessed by an independent central reviewer. *Results.* The median age was 61 (39-83) years and the median number of prior therapies was 2 (1 - 9). The majority of patients (54%) received prior rituximab [(median number of doses was 8 (0-24)]. There were 22 patients with V/F- and 28 with F/F FcγRIIIa alleles. Forty-nine patients reported mostly Grade 1/2 adverse events (AEs) with the most common related AEs being chills, fatigue, nausea, and pyrexia. Grade 3/4/5 AEs were observed in 13 patients and SAEs were reported by 11 patients. Possibly related SAEs included neutropenia, abdominal pain, nausea, vomiting, and aspiration pneumonia. Three patients discontinued the study due to AEs and four patients experienced a dose-limiting toxicity (DLT). Two patients died during the study; one due to life-threatening oesophageal achalasia and one due to aspiration pneumonia. Investigator-assessed responses were observed in 15 (30%) patients, including 4CRs and 3 CRus. The centrally assessed response rate was 32%. The median progression free survival (PFS) was 38.3 weeks as assessed by the investigators. The pharmacokinetic profile of AME-133v was similar to rituximab. Lymphocyte subset analysis showed a significant and selective reduction of B-cells during and after AME-133v treatment. *Summary/Conclusions.* AME-133v was safe and well tolerated at the recommended Phase 2 dose of 375 mg/m2. Clinical responses, including 14% with CR/CRu, were observed. These data demonstrate that AME-133 is active in previously-treated FL patients who are F-carriers at amino acid position 158 in the FcγRIIIa gene.

0368

INTERIM RESULTS FROM A PHASE IB STUDY OF THE ANTI-CD20 ANTIBODY OBINUTUZUMAB (GA101) IN COMBINATION WITH FC OR CHOP IN RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA (FL)

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Background. Obinutuzumab (GA101) is the first type II, glycoengineered, humanized monoclonal anti-CD20 antibody. Phase II Single-agent results in FL have previously been reported (Salles, ASH 2010) *Methods.* 56 patients with relapsed or refractory FL received either 4-6. cycles of G-FC q28d (n=28) or 6-8 cycles of G-CHOP q21d (n=28). Patients in each chemotherapy arm were randomized between two doses of GA101, either 400 mg on Days 1, 8, of cycle 1 and 400 mg for subsequent cycles or 1600 mg GA101 on Days 1, 8, of cycle 1 and 800 mg for subsequent cycles. Responding patients were then offered maintenance treatment with GA101 at the randomized dose received previously, on a 3 monthly schedule for a maximum of 2 years or until progression. All patients gave informed consent. The primary objective was to investigate the safety of GA101 in combination with chemotherapy. Preliminary efficacy data was a secondary/exploratory objective (end of treatment response assessed according to Cheson *et al*. 1999 criteria, but CRu downgraded to PR). *Results.* Patients baseline characteristics are reported in Table 1. At this interim analysis, all 28 G-FC patients and 17 of 28 G-CHOP patients had completed induction treatment. Incidence, as well as severity of AEs, was similar in both treatment dose groups. The most common adverse events (AE) during induction treatment were infusion related reactions (IRR); 64% and 79% of the patients for G-CHOP and G-FC respectively, mainly during the first infusion. Seven percent of the patients in both arms experienced Grade 3 / 4 IRR, all during first infusion. Currently no patient has withdrawn from G-CHOP, 22/28 patients completed all scheduled cycles on G-FC. Reasons for 6 early withdrawals in the G-FC arm were: AEs (n=5) and insufficient response (n=1). Grade 3/4 toxicities included neutropenia (n=10 and n=14), infections (n=4 and n=5) for G-CHOP and G-FC patients respectively. Two deaths were reported on G-FC arm, secondary to progression in one patient and from underlying Parkinson's disease in the other. Of 181 doses of G-CHOP, 6 doses in 5 patients were delayed for an AE (3 doses by 2 weeks, 3 doses by 1 week). Vincristine was reduced for neuropathy in 5 patients, and one patient each had a reduction of prednisone, cyclophosphamide, and cyclophosphamide/doxorubicin. Of the 137 doses of G-FC delivered, 10 doses in 9 patients were delayed for an AE (5 doses by 2 weeks, and another 5 by 1 week), 7 of these patients also had a dose

Table 1.

Characteristic	CHOP (n=28)	FC (n=28)
Age, median (range)	62.5 (32–75)	61.0 (45–77)
Clinical stage at progression I-II/III-IV, n (%)	9 (32%)/9 (68%)	5 (18%)/23 (82%)
FLIPI, n (%) Low Intermediate High	8 (29%) 15 (54%) 5 (18%)	8 (29%) 7 (25%) 13 (46%)
Prior treatment lines, median (range)	1.0 (1–3)	2.0 (1–7)
Prior rituximab treatment lines, median (range)	1.0 (0–2)	1.0 (0–3)
Bone marrow involvement, n (%	7 (25%)	7 (26%)*
Bulky >7cm, n (%)	9 (32%)	5 (18%)

* Bone marrow results available for n = 27

reduction, and an additional 3 patients had a dose reduction only. At the time of interim analysis, end of treatment response were 94% (8 CR, 8 PR) in G-CHOP arm and 93% (14 CR and 12 PR) in G-FC arm. *Conclusion.* These preliminary results indicate that G-CHOP and G-FC combinations may be delivered safely and are highly effective treatments in patients with relapsed FL. Importantly, G-CHOP can be delivered at the pre-specified three-weekly intervals without need for dose reductions or delays. GA101 is now being investigated in first-line patients in combination with CHOP and bendamustine.

0369

RESOLUTION OF MALIGNANT CUTANEOUS LESIONS WITH BRENTUXIMAB VEDOTIN (SGN-35) IN PATIENTS WITH RELAPSED OR REFRACTORY SYSTEMIC ANAPLASTIC LARGE CELL LYMPHOMA

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Background. Brentuximab vedotin (SGN-35) is an anti-CD30 antibody conjugated to the highly potent antimicrotubule agent, monomethyl auristatin E (MMAE), by a plasma-stable linker. Brentuximab vedotin binds to CD30 on the cell surface, internalizes, and releases MMAE inside the cell via lysosomal degradation. Binding of MMAE to tubulin disrupts the microtubule network within the cell, induces cell cycle arrest, and results in apoptotic death of the CD30-expressing tumor cell. In a phase 2 study of brentuximab vedotin in 58 patients with relapsed or refractory systemic anaplastic large cell lymphoma (sALCL), an objective response rate of 86% was observed by independent review. *Aims.* To describe the experience with brentuximab vedotin in relapsed or refractory sALCL patients with malignant cutaneous lesions who participated in a phase 2, single-arm, multicenter study. *Methods.* Brentuximab vedotin 1.8 mg/kg was administered every 3 weeks as a 30 minute outpatient IV infusion for up to 16 cycles of treatment. Informed consent was obtained for all patients. Determination of antitumor efficacy was based on objective response assessments by an independent review facility according to the Revised Response Criteria for Malignant Lymphoma (Cheson 2007). Resolution of cutaneous lesions was assessed by the investigator. *Results.* 15 patients with relapsed or refractory sALCL who participated in the phase 2 study had malignant cutaneous lesions at baseline. Among these patients, the median age was 57 years (range 33-70), and ECOG performance status was 0 or 1. Median number of prior therapies was 2 (range 1-5) and 4 patients (27%) had an autologous stem cell transplant prior to the study. The majority of patients (80%) had ALK-negative disease. Complete resolution of malignant cutaneous lesions was achieved in 93% of patients (14 of 15) with a median time to resolution of all lesions of 4.9 weeks (range 2.6-36). Objective responses were achieved by all patients (12 CR, 3 PR). Median duration of objective response was not reached (range 0.3+ to 45.3+ weeks). At the time of the analysis, the median follow up in the study was approximately 6 months. Patients received a median 7 cycles of treatment (range 1-16), with 4 remaining on treatment at the time of the analysis. The most common adverse events (≥30%) of any grade among the 15 patients were diarrhea, pyrexia, constipation, nausea, peripheral sensory neuropathy, and decreased appetite. Adverse events ≥ Grade 3 that occurred in more than 1 patient were neutropenia (4 patients), anemia, diarrhea, and thrombocytopenia (2 patients each). *Summary/Conclusions.* In a phase 2 trial of brentuximab vedotin in relapsed or refractory sALCL, local and systemic responses were observed among 15 patients who had malignant cutaneous lesions at baseline: 93% of patients had complete resolution of cutaneous lesions and 100% had objective responses. Adverse events were manageable, and the safety profile was comparable to that observed among patients without cutaneous involvement. These results warrant further study of brentuximab vedotin in patients with CD30-positive cutaneous and systemic lymphomas. A trial of brentuximab vedotin in patients with CD30-positive cutaneous T-cell lymphomas (CTCL) is planned.

0370

PHASE II STUDY OF INTRATHECAL LONG ACTING LIPOSOMAL CYTARABINE IN THE PROPHYLAXIS OF LYMPHOMATOUS MENINGITIS IN HIV-RELATED NON-HODGKIN'S LYMPHOMA

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Background. Around 5% of patients (pts) with aggressive non-Hodgkin's lymphoma (NHL) develop central nervous system (CNS) progression or relapse during the course of their disease. Pts with HIV-related NHL often develop CNS progression despite the use of adequate prophylaxis. Liposomal cytarabine has show a significant activity in lymphomatous meningitis but there are limited data in the prophylactic setting. *Methods.* Since May 2006, we are running a prospective phase II study of intrathecal liposomal cytarabine at the dose of 50 mg in 48 pts with HIV-NHL with the aim to evaluate the feasibility and activity of this drug in the prevention of lymphomatous meningitis. *Results.* Forty-two pts were males and the median age was 44 years (range 18-69). As far as the histological subtype of NHL, 47% of pts had a diffuse large B-cell (DLBC) NHL and 40% Burkitt NHL. Stage III-IV was diagnosed in 80% of ps and 68% of DLBC were age-adjusted IPI 2 or more. An extranodal involvement was diagnosed in 70% of pts (gastrointestinal 30%, bone 27%, spleen 10%, liver 22%, bone marrow 17%). Liposomal cytarabine was well tolerated with headache grade I to III being the most frequent side effect in only 32% of pts. Less common toxicity (all grade I) included cortical changes (4%), fever (2%), vomiting (2%), hypertension (2%), chills (2%). With a median follow up of 15.5 months only one pt (2%) with Burkitt lymphoma developed a combined systemic and meningeal relapse. Moreover, in our experience previously the present study, we used methotrexate as practical use in 426 HIV-NHL with a meningeal progression or relapse of 14% (p=0.09). The use of a liposomal formulation allowed to significantly reducing the number of lumbar injections in comparison to the standard schedules (approximately of 50%) with an improvement of quality of life of pts and with a reduction of professional exposure risk for health care staff. *Conclusions.* In this first prospective study on prophylaxis of lymphomatous meningitis in HIV-NHL reported in the literature, liposomal cytarabine seems safe and active and it reduces of approximately 50% the number of lumbar punctures and exposure risk for health staff as well.

0371

LENALIDOMIDE PLUS RITUXIMAB-CHOP21 IS SAFE AND EFFECTIVE IN ELDERLY UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): RESULTS OF PHASE I PART OF REAL07 TRIAL OF ITALIAN LYMPHOMA FOUNDATION (FIL)

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Background. R-CHOP21 is the standard treatment in newly diagnosed DLBCL of the elderly, however 30-40% of the patients failed.

Lenalidomide monotherapy in relapsed/refractory DLBCL was tested, with promising results; preclinical studies demonstrated a synergism with Rituximab. On these basis, FIL is running a prospective multicenter dose finding phase I-II trial with the aim to evaluate toxicity and efficacy of Lenalidomide plus R-CHOP21 (LR-CHOP21) for elderly untreated DLBCL patients (clinicaltrials.gov, NCT00907348). The primary end-point for the phase I was to define the Dose Limiting Toxicity (DLT), the maximum dose inducing any grade ≥ 3 non-hematologic toxicity, or a > 15 days delay of planned cycle. *Patients and Methods.* Inclusion criteria were: age 60-80; untreated CD20+ DLBCL; Ann Arbor stage II/III/IV; International Prognostic Index (IPI) at low-intermediate/intermediate-high/high (LI/IH/H) risk. Treatment plan was: 6 R-CHOP21 every three weeks in association with Lenalidomide for days 1-14 at the established dose level. Phase I was planned to define the Maximum Tolerated Dose (MTD), that is the dose that achieves a DLT in 33% or less patients; evaluation was planned after three LR-CHOP21. The study was designed with the Continual Reassessment Method (CRM), a Bayesian memory design that uses, as dose allocation rule of the sequentially incoming patients, the re-estimated probability of toxicity based on the results obtained for the patients already observed. Four doses of Lenalidomide were tested: 5, 10, 15 and 20 mg. At the end of each cohort, the dose level associated with an updated DLT probability closest to 33% was recommended to be administered to the next cohort. Results. From May 2008 to February 2010, 21 patients were enrolled in the phase I part of the study. Clinical characteristics were: median age 68 (61-77); stage III/IV 81%; PS > 1 81%; IH/H IPI risk 52/24%. Patient allocation by Lenalidomide dose (on days 1-14 of each LR-CHOP21 courses) was: 5 mg/day in nobody, 10 mg/day in nine patients, 15 mg/day in nine and 20 mg/day in three. DLTs in the first three courses of LR-CHOP21 were recorded in seven patients; according to CRM, these events determined Lenalidomide 15 mg/die as the MTD in association to R-CHOP21. Of 115 LR-CHOP21 courses performed in the series of 21 patients, hematological toxicity was mild: grade III/IV thrombocytopenia occurred in 10% of courses, anemia in 4% and neutropenia in 28%. Extra-hematological toxicities were moderate: grade IV increase of CPK in one patient, grade III cardiac in one, grade III neurological in three and grade III infections in four (two pneumonias, one febrile neutropenia with diarrhea and one diarrhea). At the end of six LR-CHOP21, complete remission was achieved in 76% patients. *Conclusions.* MTD for Lenalidomide in association to R-CHOP21 is Lenalidomide 15 mg, administered on days 1-14 of each courses. LR-CHOP21 is safe and feasible in elderly DLBCL, with promising preliminary efficacy results. The ongoing phase II part of the trial is aimed to test the efficacy of 15 mg of Lenalidomide in association with R-CHOP21.

0372

PRIMARY MEDIASTINAL LARGE B CELL LYMPHOMA (PMBL). LONG TERM RESULTS AND LATE TOXICITY IN PATIENTS TREATED WITH MACOP-B WITH OR WITHOUT RITUXIMAB PLUS INVOLVED MEDIASTINAL RADIATION THERAPY

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Background. Primary mediastinal large B cell lymphoma is a distinct subtype of diffuse large B cell lymphomas that is more common in younger females. The combination of chemotherapy (CHT) together with involved field radiation therapy (IFRT) is considered the standard treatment. In the pre-Rituximab era third generation regimens such as MACOP-B have improved survival in PMBL patients (pts) over CHOP, but the introduction of Rituximab has abrogated this difference. The real need of consolidation mediastinal IFRT is still debated in view of the risk of secondary cancers and cardiac complications. We report the long-term results on a large series of PMBL pts treated at a single center. *Method.* 107 patients (pts) with PMBL were treated between June 1991 and September 2006 at our institute; 80 pts had stage II and 27 stage IIE-IV; 75% had elevated LDH; bulky disease was present in 95 pts including 58 (55%) with clinical evidence of superior vena cava obstruction. Median age was 34 yrs (15-61) and 71% were females. The aaIPI score was 0-1 in 60 pts and 2-3 in 47. Ninety-two pts were treated with the standard MACOP-B regimen and 15 pts with a Rituximab/MACOP-B regimen since March 2004. Overall, 101/107 pts (94%) received IFRT at a dose of 30-36 Gy. The response was evaluated in all pts at the end of CHT and of IFRT. *Results.* At the end of the program, a CR/CRu was obtained in 76 pts (71%), a PR in 23(21%), NR 1(1%),while 7 pts were not evaluable (6 pts received an early intensification for progressive disease and 1 died for CHT related toxicity). At the end of the program: 14 PR pts obtained a CR/CRu after IFRT with an overall CR/CRu rate of 89%; 9 pts relapsed within 10 months and 4 of them died of progressive disease. After a median follow-up of 111 months (1-238) the 10-yrs OS, PFS and EFS were 88%, 85% and 83% respectively. No statistically significant difference in terms of PFS and OS and toxicity was recorded for pts treated with or without Rituximab. Patients with an IPI 0-1 had a significantly better PFS (p=0.020) and OS (p= 0.015). In our cohort of pts, 1/107 developed a secondary cancer (acute myeloid leukemia) after 164 months from the end of therapy and no breast cancer occurred. Four of 107 pts presented late severe cardiotoxicity (3 congestive heart failures and 1 arhythmic sudden death). *Conclusions.* This is the largest reported series of pts with PMBL treated with a uniform strategy at a single center. MACOP-B +/- Rituximab plus IFRT is highly effective and devoid of severe long-term toxicities. Future randomized trials should evaluate the real need of a mediastinal IFRT in pts who obtain a PET-negative CR after a R-chemotherapy regimen to reduce unexpected late toxicities.

0373

CLINICAL AND PROGNOSTIC SIGNIFICANCE OF APOPTOTIC PROFILE IN PATIENTS WITH NEWLY DIAGNOSED NODAL DIFFUSE LARGE B CELL LYMPHOMA (DLBCL)

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Background. Apoptosis-related proteins might play an important role in the pathogenesis of lymphoma and sensibility to chemotherapy in patients with non-Hodgkin's lymphoma (NHL). Aim of our study was to analyzed the relationship between expression of two proapoptotic (CD95, caspase 3) and four antiapoptotic proteins (c-FLIP, bcl-2, survivin and XIAP) and clinical outcome of nodal DLBCL patients. *Methods.* We have analyzed lymph node biopsy specimens obtained from 78 patients with newly diagnosed nodal DLBCL. The expression of apoptotic parameters was analyzed using the standard imunohistochemical method (antibodies against caspase 3, CD95, c-FLIP, XIAP, survivin and bcl-2) on formalin-fixed and routinely processed paraffin-embedded lymph node specimens. The expression of immunohistochemical parameters has been evaluated semiquantitavely as a percentage of tumor cells. *Results.* Caspase 3, CD95, c-FLIP, survivin, XIAP and bcl-2 immunoexpression has been found in 48(61.5%), 39(50%), 45(57.7%), 41(52.6%), 43(55.12%) and 39(50.0%) patients, respectively. The therapy response was achieved in 53(67.9%) patients. Besides numerous clinical parameters survivin and XIAP positivity along with CD95 negativity were found to be unfavorable factors for therapy response and shorter survival in univariate analysis. According to this finding, an "apoptotic score"which includes unfavorable apoptotic parameters has been defined. In multivariate analysis only IPI and apoptotic score remained independent prognostic predictors for the chance to reach the complete remission (p=0.003 and p=0.044, respectively) and longer overall survival (p=0.002 and p=0.046, respectively). Significantly the better response to immunochemotherapy in comparison to chemotherapy has been achieved in patients with expression of caspase 3, c-FLIP and survivin and in patients without the immunoexpression of XIAP. In addition, immunochemotherapy was superior to chemotherapy in both bcl-2 positive and bcl-2 negative patients. *Conclusion.* The results of this study showed that the disregulation of apoptosis can appear on different places of apoptotic cascade in DLBCL. Apoptotic score is more useful tool in predicting therapy response and overall survival of patients with DLBCL than single apoptotic parameters and along with IPI could help to identify a high risk group of newly diagnosed nodal DLBCL.

0374

PATIENT-REPORTED OUTCOMES DURING AND FOLLOWING TREATMENT WITH BORTEZOMIB PLUS RITUXIMAB OR RITUXIMAB ALONE IN PATIENTS WITH RELAPSED FOLLICULAR LYMPHOMA (FL): RESULTS FROM A PHASE 3 STUDY

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Background. FL, an indolent, incurable non-Hodgkin's lymphoma subtype, can adversely affect patients' health status, thus adversely affecting patient-reported outcome (PRO) scores on these measures. An important aim of treatment is to maintain patients' functional status through active treatment options offering improved outcomes with favorable toxicity profiles. Bortezomib-rituximab resulted in improved progression-free survival, response rates, and other long-term outcomes versus rituximab alone in patients with relapsed FL in the international, multicenter, phase 3 LYM3001 study. Assessment of PROs was an exploratory endpoint. *Aims.* To evaluate changes from baseline in PRO scores during/following treatment with bortezomib-rituximab or rituximab using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30). *Methods.* Patients were randomized to receive five 5-week cycles of bortezomib-rituximab (N=336) or rituximab (N=340), as previously described (Coiffier *et al*, ASH 2010). All patients provided written informed consent. The primary endpoint for the PRO analyses was the Global Health Status (GHS) subscale of EORTC QLQ-C30; other endpoints included the other subscales, comprising five functional scales, three symptom scales, and six single items. Patients completed EORTC QLQ-C30 at baseline, on day 1 of each cycle, at the end-of-treatment visit, and every 10 weeks post-treatment/pre-progressive disease (PD). *Results.* 333 and 335 patients in the bortezomib-rituximab and rituximab arms, respectively, provided evaluable EORTC QLQ-C30 data at baseline; as expected, these numbers decreased over time, to 293/289 at the end of treatment, and 23/21 at end of data collection. At baseline, observed mean GHS scores were 62.94 and 63.11 in the bortezomib-rituximab and rituximab arms, respectively; changes are shown in the figure. For analyses of changes from baseline and comparisons between arms, a joint modeling approach was used to address the potential issue of non-random missing values; values deviate from observed means due to incorporation of informative dropout and repeated measures. For bortezomib-rituximab, mean GHS was not statistically different from baseline through week 25 (end of treatment) and statistically improved at weeks 30-120; mean changes ranged from 3.57 at week 30 (mean 66.02) to 6.55 at week 80 (mean 69.00). With rituximab, mean GHS was statistically improved from baseline at weeks 5-120; mean changes ranged from 1.93 at week 5 (mean 64.92) to 7.91 at week 70 (mean 70.89). Between-group comparisons showed significantly lower mean scores with bortezomib-rituximab versus rituximab at weeks 10 (mean change -0.69 vs 2.78), 15 (mean change -1.44 vs 2.75) and 20 (mean change -1.98 vs 1.95); from week 25 there were no significant differences between arms. Changes from baseline and differences between arms were generally not sufficiently large to be considered clinically meaningful (≥5 points). Findings for changes in functional scores were similar to those for GHS. Minimal differences between arms in symptoms and side-effect scales were consistent with the adverse event profile of bortezomib. *Conclusions.* Rituximab resulted in slightly better PRO scores compared with bortezomib-rituximab during treatment, reflecting additional adverse events associated with bortezomib. However, post-treatment/pre-PD scores were similar between arms and slightly higher compared to baseline. Differences/changes from baseline were not clinically significant.

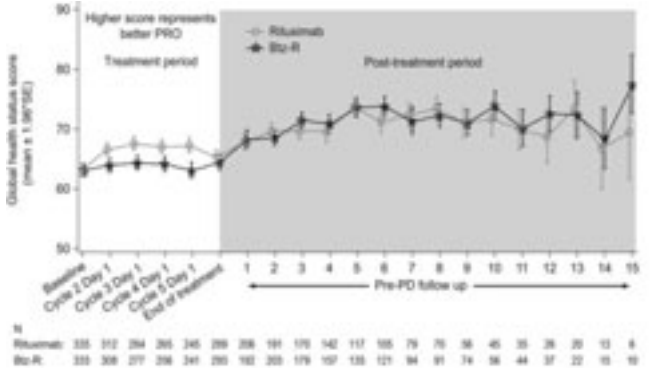

Figure 1.

0375

COMPREHENSIVE GERIATRIC ASSESSMENT-ADAPTED CHEMOTHERAPY IN ELDERLY PATIENTS (> 70 YEARS) WITH DIFFUSE LARGE B-CELL NON-HODGKIN'S LYMPHOMA (DLBCL): FINAL RESULTS AND LONG TERM FOLLOW-UP

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Background. R-CHOP is the standard treatment for elderly patients (pts) with DLBCL. Many pts aged 70 years (yrs) or more are unable to receive R-CHOP and the majority of them are excluded from clinical trials. Comprehensive geriatric assessment (CGA) is a useful instrument to predict the clinical outcome of elderly pts with cancer. Within the GOL (Gruppo Oncoematologico Linfomi) we started a phase II study aiming to evaluate feasibility and activity of a CGA-driven chemotherapy for elderly pts with DLBCL. *Material and Methods.* Pts with no comorbidity received CHOP/R-CHOP; pts with mild cardiopathy received epirubicin instead of doxorubicin; in pts with moderate/severe cardiopathy the use of anthracyclines was omitted; pts with diabetes did not receive prednisone; in pts with neuropathy vincristine was omitted. The dosage of chemotherapy was decided according to CGA: pts with a good score (ADL=6 and IADL>6) received full doses of CT; pts with an intermediate score (ADL=5 and IADL>4) received 75% of the dose; pts with a poor score (ADL<5 and IADL<5) received 50% of the dose. *Results.* One hundred pts (41 males and 59 females) have been treated. The median age was 75 yrs and stages III-IV were diagnosed in 51% of pts. Sixty-one percent of pts received full doses of CT; 25% received 75% of dose and 14% received 50% reduced dose; 86% of pts received an anthracycline and 54% rituximab. Toxicity was quite acceptable. Grade 3-4 neutropenia was observed in 30% of pts, mucositis in 12%, and peripheral neuropathy in 9%. Four toxic deaths were observed. Overall, 81% of pts achieved complete remission; with a median follow-up of 50 months, 20% of them have relapsed. The 5 yr-OS, DFS, EFS are 58%, 78% and 50%. It is remarkable that the 5-year specific survival is 72%. *Conclusions.* Our results demonstrate that a CGA-driven approach is feasible in elderly pts with DLBCL. This strategy allows offering a curative approach to all pts with aggressive NHL, avoiding to under treating pts with a potentially cured disease or over treat pts with severe comorbidities.

0376

A NEW PROGNOSTIC SCORING MODEL FOR PERIPHERAL T-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED

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Background. Peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) comprises a clinically and histopathologically heterogeneous group of lymphomas which do not fit into the definition of any other identified subtype of PTCLs. Most of the cases are characterized by aggressive behavior and a dismal prognosis. *Aim.* To assess the clinical backgrounds and the prognosis of PTCL-NOS patients, the Hokkaido Hematology Study Group (HHSG) conducted a multicenter retrospective survey in Hokkaido, Japan. *Methods.* We reviewed 508 mature T-cell and NK-cell neoplasms diagnosed according to the 4th edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues at the HHSG, which includes 30 hematology/oncology or pediatrics departments of 23 institutes, from

January 2002 to December 2009. One hundred seven (21%) PTCL-NOS patients were extracted and further analyzed with regard to their clinical backgrounds, treatments, remission or relapse rates, survival, and prognostic factors. The overall survival (OS) and progression free survival (PFS) were estimated using the Kaplan-Meier method and compared by a log-rank test. The risk factors at diagnosis for OS were evaluated either in a univariate analysis by the chi-squared test, or in a multivariate analysis by the Cox proportional hazards model. *Results.* The median follow-up of the patients was 24 months (range 1-95). The patients included 70 males and 37 females with a median age of 67 years (range 9-94). Chemotherapy (ChT) was selected in 90% (96/107) patients as the primary treatment. CHOP-like regimens were chosen for 91% (86/96) of patients as the primary ChT. A total of 48 (52%) of the 92 evaluable patients achieved a CR after the primary treatment, in which 46% (22/48) relapsed. The estimated 5 year-OS and -PFS of all patients was 35%, and 28%, respectively. The risk factors at diagnosis accosiated with OS by the univariate analysis were age>60 (p=0.027), presence of B-symptom (p=0.006), an advanced clinical stage (p=0.002), a high LDH level (p=0.007), sIL2R>3000U/ml (p=0.001), platelets<10x10^4/μl (p=0.002), lymphopenia (p=0.040), bulky disease (p=0.042), and bone marrow involvement (p=0.013). Extranodal involvement sites>1 (p=0.116) and a poor perfomance status (p=0.119) were not significant risk factors for a deceased OS. In a multivariate analysis, three independent risk factors for OS bulky disease (hazard ratio; HR=5.324, p=0.019), age>60 (HR=3.015, p=0.025), and platelets<10x10^4/μl (HR=3.999, p=0.036), were identified. Three risk groups for OS were defined by the numbers of these 3 risk factors: score 0, low risk; score 1, intermediate risk; score 2-3, high risk. The OS curves of the PTCL-NOS patients were more significantly stratified into three risk groups by using our scoring model (p=0.0005, Figure 1), compared to the results obtained by using the prognostic index for the PTCL-unspecified (PIT) scoring model (p=0.019). *Conclusions.* We demonstrated that the OS for PTCL-NOS patients was clearly stratified into 3 risk groups according to new prognostic scoring model by using three risk factors; bulky disease, age>60, and thrombocytopenia. Although further verfication by a prospective analysis is needed, these findings may provide valuable information to help predict the prognosis or to select effective therapeutic strategies for PTCL-NOS patients.

0377

COMPARISON OF PROGNOSTIC MODELS FOR PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA: THE NEED FOR A REVISION IN THE RITUXIMAB ERA

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Background. Several revisions of International prognostic index (IPI) have been proposed for patients with diffuse large B cell lymphoma (DLBCL) after the introduction of rituximab. Expanding evidence suggests that baseline absolute lymphocyte count (ALC) is also an independent factor for outcome prediction. *Aims.* We investigated the optimal prognostic model for these patients in the rituximab era. *Methods.* The study enrolled 274 consecutive patients with DLBCL receiving first-line CHOP-based chemotherapy with rituximab between 2003 and 2009. Five factors within IPI and ALC were entered for Cox regression analysis. Overall survival (OS) was calculated for different risk groups of models. Efficacy of models was compared by the value of Akaike information criterion (AIC). *Results.* Revised IPI (R-IPI) and ALC/R-IPI, but not IPI, were informative to discriminate between different risk groups of patients with DLBCL. In multivariate analysis for individual factors of the prognostic models, however, only performance status > 1 [odds ratio (OR) 3.59], Ann Arbor stage III or IV (OR 2.24), and ALC less than 1x109/L (OR 2.75) remained their significance. A modified score based on the three factors divided patients into four risk groups and the 5-year OS rate was 81%, 74%, 36%, and 9% respectively. By comparing AIC values in the Cox proportional hazards analysis, the modified 3-factor model had superior prognostic value followed by ALC/R-IPI, R-IPI and IPI scores. *Conclusions.* Addition of the novel factor, ALC, interacts with other established factors in outcome prediction for DLBCL. Development of a new score is needed for a better risk stratification in the rituximab era and would be helpful in the design of future clinical trials. The proposed 3-factor model should be validated in large scale studies.

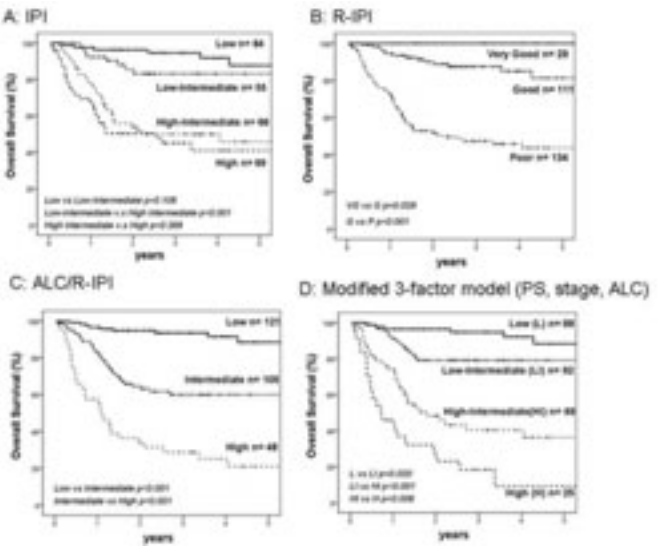

Figure 1. Overall survival by four prognostic models.

0378

R-CHOP21 VS R-CHOP14 IN 200 DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS: RESULTS FROM A RETROSPECTIVE STUDY

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Background. Diffuse large B cell lymphoma (DLBCL) is one of the most common types of non-Hodgkin's lymphoma. R-CHOP21 (C21) is considered the standard therapy but a large number of studies tested R-CHOP14 (C14). *Aims.* The aim of our study was to evaluate retrospectively a cohort of patients (pts) treated with C21 or C14. *Methods.* All pts with diagnosis of DLBCL or follicular grade IIIb lymphoma, treated with curative intent were accrued. *Results.* From january 2002 to december 2009, 99 pts were treated with C21 and 101 were treated with C14. The two cohorts of pts were balanced for all clinical characteristics a part for age (<65 or >64 years) with more aged pts in C21 arm (p 0.002) and Beta2 mycroglobulin value (evaluable in 137 patients) with more pathologic values in C21 arm (0.007). After induction therapy 158 pts (79%) obtained a complete remission: 76/99 (77%) after C21 and 82/101 (81%) after C14. After a median period of observation of 31 months 23 pts relapsed, 8 (11%) in the C21 arm and 15 (18%) in the C14 arm. Considering the two therapies, C21 vs C14, no differences were reported in OS, PFS and DFS: 61% vs 68%, 59% vs 58% and 74% vs 61% respectively. In univariate analysis OS was lower in older pts (p: 0.02), advanced stage (p:0.02), symptomatic disease (p:0.05), elevated LDH (p: 0.001), bone marrow infiltration (p: 0.02) and intermediate or high risk IPI (p: 0.000); PFS was lower in advanced stage (p: 0.002), symptomatic disease (p: 0.009), elevated LDH (p: 0.001), bone marrow infiltration (p: 0.001) and intermediate high risk IPI (p: 0.000). In multivariate analysis OS was significantly better in low-intermediate IPI risk pts (p:0.000) and in pts treated with C14 (p:0.02); the PFS was better in low-intermediate IPI risk pts (p:0.000). Considering only pts with low or low-intermediate IPI we observed that OS was significantly superior in the group treated with C14 (90% vs 64% p:0.03), moreover in young pts (< 65 years) OS was better in pts treated with C14 (81% vs 58% p:0.05). As expected hematological grade III/IV toxicity was more frequent in pts treated with C14, all pts but three (3%) completed the therapy without delay or dose reduction. No differences in extra-hematological toxicity were observed. *Conclusions.* In conclusion our results confirm that C14 do not improve the results of the standard C21 in the whole lymphoma population but in a subset of pts, young and low/intermediate risk pts, the C14 scheme semms to improve the OS. Further prospective randomized studies are needed to verify this preliminary observations.

0379

HIGH RESPONSE RATE IN SPLENIC MARGINAL ZONE LYMPHOMA IN PATIENTS TREATED WITH RITUXIMAB, EITHER AS MONOTHERAPY OR IN COMBINATION WITH CHEMOTHERAPY

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Background. Splenic marginal zone lymphoma (SMZL) is an uncommon indolent B-cell non-Hodgkin's lymphoma usually presenting with marked splenomegaly and bone marrow (BM) and/or peripheral blood (PB) involvement. Splenectomy has been the treatment of choice in symptomatic patients. Systemic treatment is required in patients with widespread disease, who are at high risk from surgery, or who have relapsed after splenectomy. Rituximab has shown encouraging results in SMZL, with sustained responses. *Aims.* To assess, retrospectively, response to treatment, toxicity and survival after rituximab in SMZL. *Methods.* Twenty-nine patients from two different centers, diagnosed with SMZL between 1982 and 2011, received one or more treatments with rituximab. Eighteen patients received rituximab alone and 20 combined with chemotherapy. Thus 9 patients received each of these sequentially, to improve response, with or without interim relapse. The median age at diagnosis was 62 years (range 37-89 years); the male:female ratio was 2:3; B symptoms were present in 12 patients; ECOG performance status was 0-2 in 28/29 patients. All presented with splenomegaly, with involvement of the BM in 27 patients, PB in 24, lymph nodes in 11 and extranodal involvement in 7 patients. Diagnosis was made according to the WHO 2008 classification by spleen histology (n=12), BM histology (n=19), PB morphology (n=12) and immunophenotype (n=13). Rituximab monotherapy was administered at 375 mg/m2/weekly x4 weeks. In combination with fludarabine-based regimens (n=14), or other regimens including CHOP (n=6), rituximab was administered on day 1 of each cycle. Responses were assessed according to the Response Criteria Guidelines for SMZL (Matutes, Leukemia 2008). Toxicity was graded according to the CTCAE v3.0. The Fisher exact test was used to compare best responses between groups. Survival was estimated using the Kaplan-Meier method. *Results.* All patients responded to rituximab monotherapy and/or to a combination treatment. At least one complete response (CR) was achieved in 20/29 patients (69%). This compares with a CR in only 4/17 (24%) of these same patients after any prior therapy (p=0.005): 11 patients had received splenectomy, with or without chemotherapy and 6 had received chemotherapy only. There was no difference in the CR rate between rituximab monotherapy (71%) versus rituximab combinations (68%). The most common adverse event was grade 3-4 neutropenia (n=7, 24%). Two patients had grade 3-4 infections. Anemia (n=3) and thrombocytopenia (n=2) were grade 1-2 only. Four cases presented with histological transformation prior to rituximab, all achieving CR with rituximab combination therapy. After a median follow-up of 24 months from rituximab treatment (range 4-102 months), the median overall survival was not yet reached. Survival at 2 years after rituximab was 100%. Only one death, due to lung cancer, occurred at 48 months follow-up. *Conclusions.* In patients with SMZL, the CR rate after rituximab, either alone or in combination with chemotherapy, was significantly better than the CR rate after other prior treatments in the same patients, with manageable toxicity. No difference in the CR rate between rituximab monotherapy or combination therapy was observed. Rituximab, with or without splenectomy, should be considered in the therapeutic scenario of SMZL.

0380

SALVAGE CHEMOTHERAPY WITH RITUXIMAB, IFOSFAMIDE AND ETOPOSIDE (R-IE) IN PATIENTS WITH PRIMARY CNS LYMPHOMA RELAPSED OR REFRACTORY TO HIGH-DOSE METHOTREXATE-BASED CHEMOTHERAPY

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Background. Conventional upfront high-dose methotrexate (HD-MTX)-based chemotherapy ± radiotherapy is associated with a high complete remission rate in immunocompetent patients with primary CNS lymphoma (PCNSL); however, 35-60% of responsive patients experience relapse within a few months and an additional 10-15% is refractory to primary chemotherapy. Often, salvage therapy results in a second remission with consequent symptomatic and survival improvement; however, only a few active drugs suitable for salvage therapy exist and studies addressing new drugs and combinations in relapsing patients should be encouraged. Thus, patients with PCNSL relapsed or refractory to HD-MTX/HD-cytarabine-based chemotherapy ± radiotherapy were treated with a combination of rituximab, ifosfamide and etoposide (R-IE regimen) at nine Italian centres. The choice of the drugs was based on their capability to cross the blood-brain barrier and their efficacy to treat extra-CNS aggressive lymphomas. *Aims.* To evaluate feasibility and activity of R-IE chemoimmunotherapy regimen in patients with relapsed or refractory PCNSL. *Methods.* HIV-negative patients with ≤75 years old, ECOG PS ≤3 with PCNSL relapsed or refractory to HD-MTX/HD-cytarabine-based chemotherapy ± radiotherapy were considered. R-IE regimen consisted of four courses of rituximab 375 mg/m^2 day 0; ifosfamide 2 g/m^2/d days 1-3; etoposide 250 mg/m^2 day 1. *Results.* Twenty patients (median age 60 ys, range 39-71; M/F ratio: 1.2) were treated with R-IE, as second-line treatment in 15 patients, as third line in three and as fourth line in two patients. Thirteen patients had refractory PCNSL (progressed during previous treatment) and seven had relapsing disease. Ten patients had received whole-brain radiotherapy as part of previous treatments. Fifty-one (64%) of the 80 planned courses were actually delivered. R-IE was interrupted in 14 patients due to lymphoma progression (n=12), toxicity (n=1) and patient's refusal (n=1); treatment is ongoing in one patient. G4 hematologic toxicity (neutropenia 50%; thrombocytopenia 25%; anemia 15%) was common but manageable; no G4-nonhematologic toxicity was observed; one patient died of pulmonary aspergillosis. Response after R-IE was complete in six patients and partial in one (ORR= 35%; 95%CI: 14%-56%), with a median response duration of 11+ months (4+ - 19+). Five responsive patients successfully collected autologous stem cells after the 2nd course of R-IE, and three of them received consolidation with BCNU 400 mg/m2 day -6, thiotepa 5 mg/kg days -5 -4 and ASCT day 0. At a median follow-up of 11 months, no responder experienced relapse, while 12 patients experienced lymphoma progression, with a 2-yr PFS of 23% ± 11%. Nine patients are alive, nine died of lymphoma, one of pulmonary aspergillosis and one patient died of neurological impairment while in remission, with a 2-yr OS of 30% ±12%. The number of previous lines of treatment, prior irradiation and relapsed or refractory disease did not influence response nor survival rates. *Conclusions.* R-IE is a feasible and active combination for patients with relapsed or refractory PCNSL. This regimen allowed autologous stem cell collection, and consolidation with high-dose chemotherapy supported by ASCT resulted in long-term remission.

0381

WEEKLY INFUSION OF RITUXIMAB AND BORTEZOMIB IS EFFECTIVE AND SAFE IN RELAPSED/REFRACTORY INDOLENT AND MANTLE CELL LYMPHOMA: LONG TERM ANALYSIS OF A PHASE II TRIAL OF ITALIAN LYMPHOMA FOUNDATION (FIL)

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Background. Gene-profiling studies demonstrated a constitutive activation of the NFκB signalling pathway in Mantle Cell Lymphoma (MCL) and Marginal Zone Lymphoma (MZL). Bortezomib, an inhibitor of the proteasome, is effective in relapsed MCL and it is synergistic with Rituximab to enhance apoptosis and NFkB depletion. On these basis, the FIL conducted a phase II multicenter study aimed to evaluate safety and efficacy of Bortezomib in association with Rituximab in relapsed/refractory non-follicular Lymphoma (Linfocytic, LL and MZL) and MCL, not eligible to high dose chemotherapy with stem cell transplantation. *Patients and Methods.* The study was a prospective phase II non randomized trial, designed on Simon two-stage Optimal Design. Primary end-point was to obtain an Overall Response Rate (ORR) > 40%. A central histological revision was planned in all the patients at the enrollment. Inclusion criteria were: 18-75 years, relapsed/refractory LL, MZL, MCL after 1-4 lines. Treatment schedule was: one course of 1.6 mg/sqm Bortezomib in combination with standard 375 mg/sqm Rituximab on days 1, 8, 15, 22 followed by two courses of four weekly intravenous bolus of Bortezomib alone; patients with complete (CR), partial remission and stable disease at the intermediate evaluation were planned to be given three further courses with the same schedule. Results. From September 2006 to March 2008, 55 patients were enrolled and six were excluded at central histological revision. Clinical characteristics were: median age 68 (50-74); 16 LL, 8 MZL, 25 MCL; 42 stage III/IV; 33 bone marrow involvement. Thirty-eight patients were at third or fourth relapses, 34 Rituximab pretreated; 21 had refractory disease. ORR was 53% (CR 26.5%); no response was 43% and 4% off therapy. ORRs by clinical subgroup were: LL 37%, MZL 50%, MCL 64%; Rituximab pretreated 62%, Rituximab naïve 33%; relapsed 64% and refractory 38%. With a median follow-up of 26 months, median Overall Survival was not reached and median Progression Free Survival (PFS) was 9.9 months (95% CI: 4.8 - 18.3). Median PFS by histology was: 4.8 (95% CI: 4.1 - 8.9) for LL, 18.3 (95% CI: 5.3 - 29.9) for MCL and 9.9 months (95% CI: 2.4 - not reached) for MZL. Thirty patients completed the treatment and 233 courses were delivered (median: 4.7 courses/patient); 19 patients did not because of no response in 13, adverse events in five, with only one toxic death due to interstitial pneumonia. Of 233 courses performed, hematological toxicity was rare: grade III/IV neutropenia in 5% and thrombocytopenia in < 2% of all courses. Grade III/IV CTC non-hematological toxicities were: neurotoxicity grade III in four patients (all completely recovered) and infections in eight patients (viral reactivation, bacterial pneumonia and micosis). *Conclusions.* Weekly infusion of Bortezomib in combination with Rituximab is effective and safe in relapsed/refractory indolent and MCL, also in Rituximab pretreated patients. Data demonstrated that this schedule is effective mainly in MZL and MCL.

Novel therapeutics, targeted therapies and gene therapy

0382

PGP IS EXPRESSED IN MESENCHYMAL STEM CELLS AND CAN BE IMPLEMENTED FOR THEIR ISOLATION

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Background. Human mesenchymal stem cells (MSCs) are multipotent stem cells that can differentiate *in vitro* and *in vivo* into various cell types. Therefore, MSCs exhibit high potential for therapeutic applications. MSCs are found in various tissues, including bone marrow (BM) and umbilical cord blood (UCB). AIMS. It is commonly accepted that BM- and UCB-derived plastic- adherent cells (mesenchymal-like stem cells) are very heterogeneous, comprising only a small subset of authentic stem cells. These cells are currently identified through a combination of poorly defined physical, phenotypic and functional properties. Thus a more comprehensive view of the MSCs identity and characteristics is urgently needed. METHODS. UCB and BM-derived mononuclear cells were grown in culture for 1 week. The plastic adherent cells were detached by EDTA. Cells were labeled with anti-CD105-APC and with MRK16 antibody against Pgp and secondary rabbit anti- mouse antibody PE. The CD105/Pgp subsets were sorted by Fluorescence-activated cell sorter. These subsets were analyzed for: (1) formation of Colony Forming Unit-Fibroblasts (CFU-Fs); (2) expression of a panel of surface markers of MSCs and lack of expression of hematopoietic markers; and (3) differentiation potential toward osteocytes and adipocytes under specific *in vitro* differentiating conditions. RESULTS. In this study, we demonstrate that a MSCs from human UCB and BM can be identified and isolated based on a single known surface antigen (e.g., CD105) and coexpression of the ABC transporter P-glycoprotein (Pgp). Among the plastic adherent MSCs-like cells, only the CD105+/Pgp+ subset demonstrates all the criteria for MSCs. These include long survival; fibroblast-like (CFU-F) morphology; expression of a panel of MSCs positive markers (CD105, CD44, CD49a, CD73, CD90, CD271) but not expression of the hematopoietic stem cells' markers (CD45, CD34). Moreover, only the CD105+/Pgp+ cells can differentiate into osteogenic and adipogenic cells in the presence of specific supplements. Upon these differentiation pathways, the Pgp is down regulated, suggesting that Pgp is a novel marker for identifying MSCs. Functional blocking of Pgp activity by Pgp-specific inhibitor augment the adipogenic differentiation pathway of the MSCs but has apparently no effect on the osteogenic differentiation pathway. CONCLUSIONS. This study indicates that overexpression of Pgp is characteristics for genuine MSCs and can be utilized to isolate the relatively small subset of multipotent MSCs from the heterogeneous adherent cell populations of BM and UCB.

0383

ENHANCED ADHESION & MIGRATION AND INDUCTION OF PYK2 COMPLEX FOMATION IN NB4 AND K562 CELLS FOLLOWING ATRA AND IMATINIB TREATMENT

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Background/Aims. All-trans-retinoic-acid (ATRA) and imatinib (IM) are targeted therapies used for acute promyelocytic leukemia (APL) and chronic myeloid leukemia (CML), respectively. Despite improved prognosis, ATRA and IM administration has been associated with extramedullary disease (EMD) occurrence. We postulate that, like in the metastatic processes, changes in migration and adherence potential may enable leukemic cells to inhabit extramedullary sites. Focal adhesion complexes linking between extracellular matrix and the cell cytoskeleton are likely to play an important role in these processes. Pyk2 is a tyrosine kinase highly expressed in hematopoietic cells. Proteins such as paxillin, talin, vinculin, etc, interact with Pyk2 creating the "Pyk2 associated signaling complex"at focal adhesion sites. These complexes participate in adhesion and migration processes and may be involved in EMD development. Our objectives are to identify the molecular changes associated with ATRA and IM administration, define their

effect on adhesion and migration ability and to establish the role of these changes in treatment-associated EMD. *Methods.* We studied the effect of ATRA and IM on NB4 and K562 cells by combining adhesion/migration assays, microarray analysis, RT-PCR, Western blots and siRNA experiments. *Results.* 30-40% of ATRA-treated NB4 cells and IM-treated K562 cells adhered to fibronectin as opposed to untreated cells having no adhesion ability. NB4 cells adhered to FN even 5 days after ATRA withdrawal. In addition, we found a 2.4-7.6-fold increase in the migration ability of NB4 and K562 cells following ATRA and IM treatment, respectively as compared to untreated cells. A microarray screen revealed alteration in the expression of many migration/adhesion related genes (pyk2, paxillin, integrin β2 and β7) following ATRA treatment. Following processing of the microarray results and relying on the decision to focus on the "Pyk2 associated signaling complex, we continued our studies on 3 of the most relevant proteins in this complex: Pyk2, paxillin and integrin β2. We found that the mRNA and protein levels of these 3 key proteins are elevated following cellular exposure to ATRA. Moreover, Pyk2, paxillin and integrin β2 were found to be activated in response to ATRA treatment as seen by an increased phosphorylation levels or by the unveiling of activation-specific epitopes. Pyk2 activation is known to lead to the recruitment of paxillin and vinculin to Pyk2 located at focal adhesion sites. We observed an ATRA-dependent increase of Pyk2-paxillin and Pyk2-vinculin complex formation in our cells. In order to prove that Pyk2 is essential for ATRA-induced NB4 cell adhesion/migration, pyk2 expression was knockdown by siRNA. The adhesion and migration ability of si-pyk2 infected NB4 cells was found to be inferior to that of the parental NB4 cells. *Conclusions.* ATRA and IM induce NB4 and K562 cell adhesion and migration accompanied by increased phosphorylation and activation of Pyk2 and of additional focal adhesion proteins in these cells. We also discovered that Pyk2 is one of the key proteins regulating ATRA-induced cell migration and adhesion. Collectively our data suggest a critical role of Pyk2 in adhesion and migration initiated by various targeted therapies and a possible role in EMD development.

0384

CD38-SPECIFIC ANTIBODY DARATUMUMAB SYNERGIZES WITH NOVEL AGENTS LENALIDOMIDE AND/OR BORTEZOMIB TO IMPROVE THE ANTI-MYELOMA EFFECT EVEN IN LENALIDOMIDE/ BORTEZOMIB REFRACTORY PATIENTS

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Background. Multiple myeloma (MM) is a yet incurable malignancy of antibody-producing clonal plasma cells. Recently, significant progress has been made in MM treatment using novel immunomodulating agents such as lenalidomide (LEN) and bortezomib (BORT). Daratumumab (DARA) is a first-in-class human therapeutic CD38-specific antibody with broad-spectrum killing activity. Set out to further improve MM therapy by combining DARA with novel MM therapeutics, we have already shown the significant improvement of *in vitro* MM cell lysis by combining DARA with LEN. *Methods.* In *ex vivo* assays, which allow us to address killing of MM cells in bone marrow aspirates isolated from MM patients, we now explored the impact of combining DARA with LEN+BORT and with two recently introduced triple combination therapies: RDV (LEN+BORT+ Dexamethason) and MPV (Melphalan+ prednisone + BORT). *Results.* Addition of DARA to the combination of LEN and BORT significantly exceeded the effectiveness of the LEN-BORT treatment alone ($P<0.001$). Specifically, we observed a remarkable synergy between DARA and LEN/BORT in samples which responded poorly to LEN+BORT, including those samples obtained from 5 patients refractory to BORT and/or LEN. Furthermore, when combined with RVD and MPV, DARA almost doubled MM cell killing, especially in the low-dose range of the cocktails. *Conclusion.* Our results illustrate that treatment of MM with DARA in combination with novel multidrug therapies bears great promise.

0385

ELIGLUSTAT, AN INVESTIGATIONAL ORAL THERAPY FOR GAUCHER DISEASE TYPE 1: PHASE 2 RESULTS AFTER 3 YEARS

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Background. Gaucher disease type 1 (GD1) is an inherited lysosomal storage disorder caused by deficient activity of acid β-glucosidase, a key enzyme in the degradation of sphingolipids. In GD1 patients, accumulation of glucosylceramide occurs primarily in tissue macrophages and leads to clinical manifestations, including thrombocytopenia, anemia, hepatosplenomegaly, and bone disease. Eliglustat, a novel ceramide analog that is a potent and specific inhibitor of glucosylceramide synthase, is under development as an oral substrate reduction therapy for GD1. *Aim.* To report long-term efficacy and safety results of eliglustat in GD1 patients. *Methods.* This ongoing, open-label, uncontrolled, multicenter, Phase 2 clinical trial of eliglustat (50 or 100 mg bid, depending on plasma trough levels) enrolled 26 previously untreated adults with GD1. Patients had to have splenomegaly with thrombocytopenia and/or anemia. The main efficacy outcomes included mean changes (± SD) from baseline in hemoglobin and platelet levels, spleen and liver volumes, and bone mineral density (BMD); the percentage of patients achieving therapeutic goals for anemia, thrombocytopenia, splenomegaly, and hepatomegaly (Pastores, *Semin Hematol*. 2004); and descriptive changes in skeletal lesions, infarcts, and femur dark marrow (reflecting marrow infiltration by Gaucher cells). Hematology, organ volumes, and biomarkers were assessed every 3-6 months. MRI, DXA, and X-rays were performed yearly and reviewed centrally. *Results.* Nineteen patients have completed 3 years of treatment, and 7 patients have discontinued the trial. After 3 years, hemoglobin increased by 2.6±1.39 g/dL (11.3±1.63 to 13.8±1.37 g/dL) and platelet count increased by 91±65.9% (70,000±21,700/mm^3 to 126,800±40,500/mm^3). Spleen volume (multiples of normal, MN) decreased by 61±12.2% and liver volume (MN) decreased by 29±15.8%. Most patients met long-term therapeutic goals for hemoglobin (100%), spleen volume (100%), liver volume (89%), and platelets (63%), and all patients met ≥3 therapeutic goals at 3 years. In 15 patients with evaluable DXA results at all timepoints, lumbar spine BMD increased by 0.6±0.69 Z-score and 0.6±0.94 T-score. Femur dark marrow was reduced (56%, 10/18) or stable (44%, 8/18) in18 patients with findings at baseline. No bone crises or reductions in mobility occurred. There were no new lytic lesions, bone infarcts, fractures, or areas of osteonecrosis, and no worsening of pre-existing lytic lesions (8 patients) or bone infarcts (7 patients); 1 patient had worsening osteonecrosis noted retrospectively at baseline. Plasma GL-1 levels normalized, and median chitotriosidase and CCL-18 decreased by 80% and 73%, respectively. Eliglustat was well tolerated. Most adverse events (AEs) were mild (74%) and unrelated (95%) to treatment. The most common AEs were viral infections (6 patients); urinary and upper respiratory tract infections (4 patients each); and headache, increased blood pressure, abdominal pain, and diarrhea (3 patients each). Eight drug-related AEs, all mild, occurred in 6 patients. *Summary/Conclusions.* Eliglustat is a potential promising oral substrate reduction therapy for GD1. Clinically meaningful improvements have been observed in hematologic, visceral, and bone parameters, and most patients have met long-term therapeutic goals by 3 years. Eliglustat has been well tolerated and has led to the initiation of 3 international Phase 3 studies, which are actively enrolling patients.

0386

SIRT1: A NOVEL THERAPEUTIC TARGET IN ACUTE MYELOID LEUKEMIA

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Background. Acute myeloid leukemia (AML) is one of the most prevalent forms of adult leukemia with very few long-term survivors. Novel approaches are urgently needed to improve clinical outcomes for patients. A better understanding of the factors that control AML pathogenesis will reveal new therapeutic targets and may offer an opportunity to improve patient survivorship. The sirtuin deacetylases (SIRTs) are a family of histone deacetylases (HDACs) with important roles in the regulation of genes that are essential for longevity, cell growth, tumor suppression, and apoptosis. Dysregulation of SIRT expression has been reported in several forms of cancer and could contribute to disease progression and drug resistance by increasing the lifespan and survival capacity of malignant cells. We hypothesize that SIRT1 is a critical regulator of the survival of AML cells and can be targeted for therapeutic benefit. *Aims.* (1) To elucidate the role SIRT1 in the growth and survival of AML cells. (2) To investigate the mechanism of action of the small molecule SIRT inhibitor tenovin-6 in preclinical models of AML. *Methods.* We tested our hypothesis in human AML cell lines, primary patient specimens and mouse models of AML. *Results.* We assayed the expression levels of SIRT1 and the related factor SIRT2 in normal mononuclear, a panel of human AML cell lines, and primary blasts patients with AML by quantitative RT-PCR and Western blotting. Our results showed that SIRT1 was expressed at significantly higher levels in all AML cell lines and primary AML blasts as compared with normal controls. In contrast, SIRT2 was expressed at very low levels in the majority of the samples analyzed. Although a number of HDAC inhibitors have been clinically investigated for cancer therapy, none of these drugs have significant inhibitory effects against the Class III HDACs (SIRTs). Therefore, the therapeutic potential of disrupting SIRT activity as an anticancer strategy remains to be rigorously investigated. Tenovin-6 is a novel small molecule inhibitor of SIRT activity. Treatment with tenovin-6 caused a dose-dependent reduction in AML cell viability and clonogenic survival and triggered apoptotic cell death. The tumor suppressor p53 is a SIRT1-regulated gene with a critical role in the cellular response to many classes of anticancer agents and its inactivation contributes to disease progression and drug resistance. Increased SIRT1 activity has been proposed as one mechanism by which cancer cells eliminate p53 function through chromatin silencing. Tenovin-6 treatment caused a dose-dependent accumulation of acetylated p53 in AML cells and increased expression of both p21 and PUMA. Targeted knockdown of PUMA with shRNA revealed that PUMA is a critical regulator of the pro-apoptotic effects of tenovin-6. Administration of tenovin-6 to mice was well-tolerated and led to a significant reduction in disease burden. *Conclusions.* SIRT1 is a very promising novel therapeutic target in AML. Further investigation aimed to elucidate the safety, efficacy, and mechanism of action of tenovin-6 is warranted.

0387

TARGETING PLK1 ACTIVITY WITH THE INVESTIGATIONAL DRUG TAK960 SIGNIFICANTLY PROLONGS SURVIVAL IN PRECLINICAL MODELS OF AML

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Background. Acute myeloid leukemia (AML) primarily affects elderly patients that have a reduced ability to tolerate intensive chemotherapy. Novel targeted agents offer an opportunity to achieve better therapeutic selectivity and improve clinical outcomes for patients with AML. Polo-like kinase-1 (PLK1) is a serine-threonine kinase that functions as an essential regulator of mitosis. Overexpression of PLK1 is a frequent event in cancer and is correlated with a poor prognosis. Its intrinsic overexpression in malignant cells and critical role in cell cycle regulation make PLK1 an attractive target for therapeutic inhibition. TAK960 is a novel small molecule inhibitor of PLK1 that has entered Phase I clinical trials. We hypothesized that inhibition of PLK1 would disrupt cell cycle progression, diminish AML cell viability, induce apoptosis, and anatagonize *in vivo* leukemia pathogenesis. *Aims.* (1) To investigate the preclinical activity of TAK960 in preclinical models of AML. (2) To elucidate the mechanism of action of TAK960. *Methods.* The preclinical activity of TAK960 was investigated in human AML cell lines and disseminated and subcutaneous mouse models of AML. *Results.* Treatment with TAK960 potently disrupted the viability of a panel of 8 human AML cell lines. Acute exposure to TAK960 severely impaired the ability of AML cells to form colonies. Analysis of the effects of TAK960 on cell cycle progression demonstrated that TAK960 caused a substantial accumulation of cells with G2/M DNA content. TAK960 treatment led to the dose-dependent induction of apoptosis. Kinetic analyses revealed that cell cycle disruption occurred prior to the onset of apoptosis. We established a disseminated mouse xenograft model of AML with luciferase-expressing human AML cells to investigate the *in vivo* activity of TAK960. Administration of TAK960 to mice was well-tolerated, diminished the numbers of leukemic blasts in the bone marrow, and significantly prolonged animal survival. Analysis of specimens from mice treated with revealed a pharmacodynamic signature consistent with PLK1 inhibition. The *in vivo* efficacy of TAK960 was also evaluated in the subcutaneous MOLM-13 AML xenograft model. Administration of TAK960 significantly inhibited the growth of MOLM-13 tumors, disrupted cell proliferation and led to the induction of AML cell apoptosis. *Conclusions.* TAK960 is a very promising novel PLK1 inhibitor that has potent activity in preclinical models of AML. Further investigation aimed to elucidate the safety, efficacy, and mechanism of action of TAK960 is warranted.

0388

CARFILZOMIB-DEPENDENT SELECTIVE INHIBITION OF CHYMOTRYPSIN-LIKE ACTIVITY OF THE PROTEASOME LEADS TO IN VITRO AND IN VIVO ANTI-TUMOR EFFECT IN WALDENSTROM MACROGLOBULINEMIA

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Background. Selective inhibition of chymotrypsin-like (CT-L) activity of constitutive-(c20S) and immuno-(i20S) proteasome leads to a significant anti-neoplastic effect in a wide spectrum of hematologic tumors. Preclinical evaluation of new proteasome inhibitors with a more targeted inhibition of clonal cells is needed in order to increase efficacy and improve patient outcome. We evaluated the anti-tumor activity of Carfilzomib, a novel selective, irreversible peptide epoxyketone inhibitor of the CT-L activity of i20S and c20S, in WM.selective chymotrypsin-like (CT-L) proteasome inhibitor in WM, both *in vitro* and *in vivo*. *Aims.* 1) To evaluate the distribution of i20s and c20S in WM primary cells as compared to the related normal cellular counterpart. 2) To evaluate the anti-tumor properties of Carfilzomib in WM, both *in vitro* and *in vivo*. *Methods.* Primary WM cells were obtained from bone marrow (BM) of WM patients (CD19+ microbead selection). WM and IgM secreting low-grade lymphoma cell lines were used. Level of imunoproteasome (i20S) and constitutive proteasome (c20S) subunits were detected by an ELISA-based assay. Cytotoxicity, DNA synthesis were measured by MTT and thymidine uptake, respectively. Cell signaling and apoptotic pathways were determined by Western Blot. Effect of Carfilzomib on paracrine WM cell growth in the BM has been evaluated by looking at adhesion, migration and co-colture of WM cells with primary BM stromal cells (BMSCs). Drug synergism was calculated using CalcuSyn software. *In vivo* studies were perfomed using BCWM.1-GFP+/Luc+ cells injected into SCID mice, treated intra-venously with either Carfilzomib or vehicle. Detection of Carfilzomib-induced apoptosis has been validated *ex vivo* using WM cells isolated from BM of SCID mice treated with either vehicle or Carfilzomib. Measurement of human IgM has been performed on serum obtained from treated mice. *Results.* Primary WM cells which are characterized by higher expression of the i20S subunits as compared to c20S subunits, and they contain a higher i20S content as compared to normal CD19+ B-cells. Carfilzomib inhibited the CT-L activity of both i20S (LMP7) and c20S (β5) in primary WM cells, leading to inhibition of proliferation and induction of cytotoxicity; supported by increased PARP-, caspase-9-, -8 and -3-cleavage, as well as induced activation of c-jun-N-terminal kinase and ER-stress in a dose-dependent manner. Carfilzomib targeted WM cells even in the context of BM milieu, where inhibition of adhesion and migration were observed, together with inhibition of WM growth even in presence of BMSCs. Combination of carfilzomib and bortezomib induced synergistic cytotoxicity in WM

cells, as shown by enhanced PARP-, caspase-9- and -3-cleavage; and synergy in inhibiting the CT-L activity of the i20S and c20S. Antitumor activity of Carfilzomib has been validated *in vivo*, where carfilzomib-treated mice presented with a significant lower number of tumor cells (P<.05); increased percentage of apoptotic WM cells (P<.05); and reduced serum IgM levels (P<.05), as compared to control mice. *Summary.* These findings demonstrate for the first time that Carfilzomib targets WM cells both *in vitro* and *in vivo*, due to its anti-CT-L activity of both i20S and c20S proteasome, providing the framework for testing this compound in this disease.

0389

TREATMENT OF B-CELL MALIGNANCIES WITH EPRATUZUMAB ANTI-CD22-SN-38 CONJUGATES ALONE AND COMBINED WITH VELTUZUMAB ANTI-CD20 ANTIBODY THERAPY

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Background. Many B-cell malignancies are responsive to antibody therapy, but more often, antibody therapy is combined with chemotherapy to optimize response. We developed procedures for coupling antibodies to SN-38, a highly potent topoisomerase I inhibitor that is the active component of the prodrug irinotecan. SN-38-conjugates of humanized antibodies epratuzumab anti-CD22 (Emab-SN-38) and veltuzumab anti-CD20 (Vmab-SN-38) were evaluated separately, and Emab-SN-38 was tested in combination with unconjugated Vmab. *Methods.* Emab and Vmab were conjugated with 6 moles of SN-38. The linker used in the preparation of these conjugates allows the SN-38 to be released slowly, with *in vitro* stability studies in human serum estimating that about 50% of the SN-38 is released within 1 to 1.5 days. With this type of linkage antibodies that are not internalized (i.e., Vmab), as well as internalizing antibodies (Emab), can be effective. *In vitro* and *in vivo* studies were performed to assess the activity of the conjugates against several B-cell lymphoma and leukemia cell lines. *In vivo* studies also examined combination therapy with Emab-SN-38 and unconjugated Vmab. *Results. In vitro* studies in 5 B-cell lymphoma cells lines (Daudi, Raji, Ramos, WSU-FSCCL, Jeko-1) and 4 acute lymphoblastic lymphoma cell lines (697, REH, MN-60, and RS4;11) expressing varying amounts of CD22 and CD20 determined by FACScan analysis, showed an IC50 ranging from 0.5 to 10 nM, confirming potent activity of each conjugate. Potency was not correlated to antigen content. Nude mice bearing SC Ramos human lymphoma had significant, yet similar anti-tumor activity, with both conjugates (0.25 and 0.5 mg of each conjugate given twice weekly for 4 weeks). No toxicity is seen at these dose levels, with mice able to tolerate a cumulative dose of the conjugate as high as 60 mg. The Emab-SN-38 conjugate was examined further, since this provided an opportunity for it to be combined with effective unconjugated Vmab therapy without competing for the same target antigen. Selective targeting of the Emab-SN-38 was demonstrated against a control, non-targeting, IgG-SN-38 conjugate. In the WSU-FSCCL human follicular B-cell lymphoma IV model, with treatment initiated 5 days after implantation, the median survival for untreated animals was 42 d (0/10 surviving at 160 d), increasing to 91 d (2/10 surviving at 160 days) for unconjugated Vmab-treated animals. Emab-SN-38-treated animals had a median survival of 63d (0/10 surviving after 160 d), but when combined with Vmab, the median survival had not been reached at 160 d, with 6/10 surviving. Animals treated with a non-targeting IgG-SN-38 conjugate alone or combined with Vmab had a median survival of 63 d (0/10) and 91 d (2/10). The Emab-SN-38 conjugate combined with Vmab was significantly better than all treatment or control groups ($P \leq 0.05$). Similar enhancements were found in SC Ramos. *Conclusion.* Even at non-toxic dose levels, the Emab-SN-38 conjugate is a potent therapeutic, but responses could be enhanced significantly when combined with anti-CD20 immunotherapy. These data indicate Emab-SN-38 should be evaluated clinically alone and in combination with Vmab therapy.

0390

COMBINED THERAPY WITH BONE MARROW-DERIVED ENDOTHELIAL PROGENITOR CELLS AND SONIC HEDGEHOG IMPROVES ANGIOGENESIS AND MYOGENESIS IN AN EXPERIMENTAL MODEL OF MUSCULAR DYSTROPHY

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Recent work demonstrates that vascular abnormalities are required for, and an essential cause of, muscle damage in Duchenne muscular dystrophy (DMD). Dystrophin is physiologically expressed in endothelial cells (ECs) and dystrophin-lacking ECs have significant impairment of flow-induced dilatation. It has been proposed that organ damage in DMD might be aggravated by such a defective arterial response to flow. These data suggest that increasing the vasculature in DMD may ameliorate the histological and functional phenotypes associated with this disease and indicate that, for an effective therapy of DMD, both the muscle and the vasculature need to be addressed. The bone marrow (BM) is an important source of ECs. In DMD, regenerative mechanisms, including angiogenesis, are constantly activated to contrast muscle degeneration, thus in this disease the contribution of BM-derived ECs to the process of regeneration might be substantial. We have recently demonstrated that Sonic hedgehog (Shh) gene therapy has angiogenic and myogenic potentials in the setting of ischemia. We investigated the hypothesis that combining Shh gene therapy with the administration of BM-derived endothelial progenitor cells (EPCs) increases angiogenesis and myogenesis in an experimental model of DMD. EPCs were obtained from the BM of 8-week-old C57BL/6J mice. We constructed a 4,878-bp plasmid containing the 600bp amino terminal domain coding sequence of human Shh. Unilateral hind-limb ischemia was created in 6 months-old mdx mice, the murine equivalent of DMD in humans. Each group was injected with either 1) PBS, 2) phShh (200 μg/mouse), or 3) phShh (200 μg/mouse) + EPCs (1×106 cells/mouse). Blood flow was measured in ischemic and contralateral hind-limbs by laser Doppler perfusion imaging at days 0, 7, 14, 21, and 28 after ischemia. At day 28, mice were sacrificed and adductor muscles were used to assess muscle weight, capillary density, and number of regenerating myofibers. At day 28 after ischemia, blood perfusion ratio between the ischemic and the contralateral leg was higher in EPCs+Shh-treated mice (0.86±0.07), and in Shh-treated mice (0.82±0.11) compared to control mice (0.70±0.04; p=0.01, p=ns). Capillary density was significantly higher in the in EPCs+Shh-treated muscle (106±31.8), and in Shh-treated muscle (86.4±30.3) compared to controls (68.2±17.7; p=0.0002, p=0.02). There was a significant increase in the muscle weight of mice treated with EPCs+Shh, and Shh versus controls (0.25±0.01, 0.29±0.03 vs 0.20±0.01 grams; p=0.005, p=0.004). The number of regenerating myofibers was significantly higher in the EPCs+Shh-treated muscle (44.6±10.75), and in Shh-treated muscle (52.03±29.38) compared to controls (30.5±8.8; p=0.00006, p=0.004) (Figure 1).

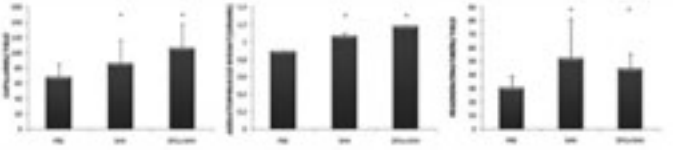

Figure 1.

In summary, Shh gene therapy had significant beneficial effects on blood perfusion ratio, capillary density, muscle weight, and number of regenerating myofibers, compared to PBS. These beneficial effects were further increased by combined therapy with Shh and BM-derived EPCs. Our results show that EPCs implantation combined with transfer of the human Shh gene improves angiogenesis in ischemic muscles of dystrophic mice. Further, this combined therapy enhances muscle mass and increases the number of regenerating myofibers in the mdx muscle. These findings represent a possible tool for future cell and gene therapy applications in DMD disease or other muscular dystrophies.

0391

A TARGETED THERAPY (AVL-292) FOR BRUTON'S TYROSINE KINASE IN B-CELL MALIGNANCIES

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Background. Targeted therapies that suppress B cell receptor (BCR) signaling have emerged as promising agents in the treatment of B cell malignancies. Bruton's tyrosine kinase (Btk) plays a crucial role in promoting B cell proliferation and survival through participation in the BCR signaling pathway and represents a promising new drug target. *Aims.* - Demonstrate the utility of a potent and selective inhibitor of Btk in B cell malignancies. - Investigate effects of clinical candidate, AVL-292, on the survival and BCR activation of primary CLL cells cultured with Nurse-like cells (NLC). - Determine, in a clinical setting, the minimum AVL-292 dose necessary for maximal Btk target site occupancy utilizing a novel translational medicine approach. *Methods.* B lymphocyte cell signaling: Human naïve, primary B cells or cultured Ramos human Burkitt's lymphoma cells were incubated with compound for 1 hour followed by BCR stimulation with -IgM. Antibodies used for immunoblot analysis include P-PLC 2, Btk and P-Btk. B Cell Proliferation: Human B cells were incubated with compound and anti-IgM for 56 h at 37 C and measured for 3H-thymidine incorporation. Btk target occupancy: Cell lysates were incubated with the biotinylated covalent probe for one hour to detect free, unbound Btk. Lysates were then added to a streptavidin-coated plate to capture probe-bound Btk, followed by detection with an anti-Btk antibody. *Results.* AVL-292 is an orally active highly selective small molecule covalent Btk inhibitor that potently silences Btk enzymatic activity (IC50 < 0.5nM) and inhibits primary B cell proliferation and activation (EC50 1-10nM). AVL-292 inhibits proliferation of multiple lymphoma cell lines which are dependent upon BCR signaling. AVL-292 also reduces survival and markers of BCR activation, such as CCL3 and CCL4 cytokine production, of primary CLL cells cultured with NLC. We have developed a covalent probe assay that enables direct measurement of Btk target site occupancy and can be used to correlate target site occupancy with activity of AVL-292 *in vitro* and *in vivo*. Recent completion of an innovative human clinical trial with AVL-292 has delivered important insights into dose-dependent Btk target site occupancy with AVL-292 determined using the covalent probe assay. An AVL-292 dose achieving maximal Btk target site occupancy was identified and the measured rate of Btk protein resynthesis in human subjects supports a once daily dosing schedule. *Summary/Conclusions.* Targeted covalent drug design has enabled the discovery and early clinical development of a potent and highly selective inhibitor of Btk, AVL-292, and supports Btk as an exciting new target for B cell malignancies. These data demonstrate that specific targeting of Btk inhibits growth of B cell lymphoma cell lines as well as the survival of primary CLL cells in the presence of nurse-like cell support. The covalent mechanism of action of AVL-292 can deliver translational pharmacodynamic information early in clinical drug development and has facilitated the rational selection of dose and dose schedule to maximize patient benefit in clinical trials studying B cell malignancies.

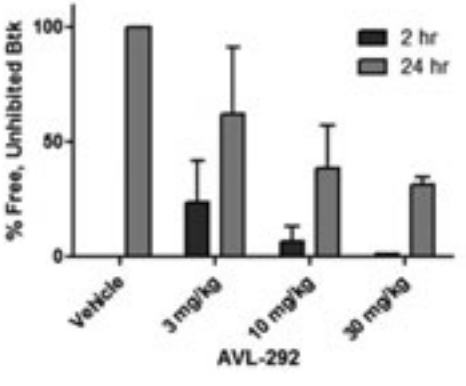

Figure 1. Btk target occupancy *in vivo.*

0392

RAPID TREATMENT OF SYMPTOMS OF TTP IN A BABOON MODEL

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Background. Thrombotic thrombocytopenic purpura (TTP) is characterized by hemolytic anemia and thrombocytopenia, with or without signs and symptoms of ischemic organ dysfunction. TTP has been associated with accumulation of ultra large von Willebrand Factor (ULVWF) due to deficiencies in the VWF cleaving protease ADAMTS13. These ULVWF multimers spontaneously interact with platelets in the microcirculation and promote the formation of platelet thrombi resulting in life-threatening microthrombosis. Recently, we have shown that all the clinical features of TTP can be induced in baboons by infusion of an inhibitory anti-ADAMTS13 antibody (3H9). TTP is currently treated by plasma exchange or plasma infusion. As spontaneous binding of platelets to the ULVWF molecules is responsible for the pathology, inhibiting the binding of platelets to ULVWF might be a good alternative treatment for TTP. *Aim.* To test whether GBR 600, an inhibitory anti-VWF-A1 domain monoclonal antibody previously shown to be safe and effective in preventing arterial thrombosis in a modified Folts model in baboons, will prevent and treat the symptoms of TTP in a baboon TTP model. *Methods.* Eight baboons were given 3H9 at 48 hour intervals to induce TTP for either 5 (n=3, prevention group) or 11 days (n=5, prevention group and control group). In the prevention group, baboons received in parallel a daily injection of GBR 600 (5 days) to see if this could prevent onset of TTP. In the treatment group (n=3), baboons received a daily injection of GBR 600 from the fifth day on to see if this would treat the symptoms of TTP. The baboons in the control group (n=2) did not receive GBR600. Platelet count, haemoglobin concentration and % circulating schistocytes were monitored at regular intervals. *Results.* As previously reported, injection of 3H9 in baboons gradually induced thrombocytopenia reaching platelet counts of less than 30X10^9/l at day 4 in the control and treatment groups. Interestingly however, thrombocytopenia could be prevented when GBR600 was administered starting at day 1 of the 3H9 injections (prevention group). The platelet count in the treatment group showed a steady increase from day 6 and returned to the baseline value in 72 hours after first injection of GBR 600. Haemoglobin concentration did not change significantly in the prevention group and decreased steadily in the control group for the duration of the study. In the treatment group there was a steady decrease in heamoglobin until day 6 after which it stabilized and started increasing from day 8. Prominent schistocytosis was seen in the control and treatment groups. *Summary.* In this study we showed that inhibition of platelet binding to ULVWF can effectively prevent the onset of TTP as well as rapidly invert ongoing symptoms of TTP on the background of fully inhibited ADAMTS13.

0393

THE INVESTIGATIONAL NOVEL MULTI-TARGETED AURORA B KINASE INHIBITOR TAK901 HAS POTENT ACTIVITY IN PRECLINICAL MODELS OF ACUTE MYELOID LEUKEMIA

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Background. The majority of patients diagnosed with acute myeloid leukemia (AML) are more than 60 years of age and their long-term prognosis is dismal. Pre-existing myelodysplasia, multidrug resistance, and co-existing morbidities limit therapeutic options for many patients. Novel approaches are urgently needed to improve clinical outcomes. The Aurora kinases (A, B, and C) are critical regulators of several events during mitosis. The overexpression of Aurora kinases in AML and other forms of cancer contributes to genetic instability, disease progression, and drug resistance. TAK901 is an investigational novel small molecule Aurora B kinase inhibitor with effects against a number of other

kinases with important roles in cancer that is being evaluated in Phase I trials. We hypothesized that simultaneously targeting Aurora B and other oncogenic kinases with TAK901 would disrupt cell cycle kinetics, inhibit proliferation, and induce AML cell death. *Aims.* (1) To determine the preclinical activity of TAK901 in AML. (2) To elucidate the mechanism of action of TAK901 in AML cells. *Methods.* Human AML cell lines and disseminated and subcutaneous mouse models of AML were utilized to investigate the preclinical activity of TAK901. *Results.* TAK901 potently diminished the growth and clonogenic survival of a panel of 8 AML cell lines. Treatment with TAK901 disrupted cell cycle kinetics leading to an accumulation of aneuploid cells, which occurred prior to the onset of apoptosis. A disseminated xenograft mouse model of AML was established using luciferase-expressing MV4-11 cell to investigate the *in vivo* anti-leukemic activity of TAK901. Administration of TAK901 to mice was well-tolerated and led to a highly significant increase in survival that was superior to what was achieved with the standard agent cytarabine. TAK901 induced AML cell apoptosis (active caspase-3) *in vivo* and dramatically diminished the phosphorylation of histone H3 in a manner consistent with Aurora B inhibition. Notably, TAK901 inhibited the infiltration of leukemic cells into the spleen and disrupted homing of AML cells to the bone marrow. The activity of TAK901 was also evaluated in the MOLM-13 subcutaneous AML xenograft model. Administration of TA901 to mice bearing MOLM-13 tumors inhibited AML cell proliferation, induced apoptosis and led to disease regression. *Conclusions.* TAK901 is a novel multi-targeted Aurora B inhibitor that has preclinical activity in AML models and warrants further investigation.

0394

SAFETY AND TOXICITY OF INTRATHECAL LIPOSOMAL CYTARABINE FOR TREATING CENTRAL NERVOUS SYSTEM (CNS) RELAPSE IN PEDIATRIC ACUTE LEUKEMIA: A MULTICENTER RETROSPECTIVE STUDY

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Background. The treatment of Central Nervous System (CNS) relapse in pediatric acute Leukemia (AL) remains a challenging clinical problem. Liposomal Cytarabine (DepoCyte) is a new intrathecal (IT) formulation characterized by the slow-release of free Cytarabine into the cerebrospinal fluid (CSF), resulting in longer drug exposure and a possibly higher leukemic response rate. Severe neurotoxicity has been reported during DepoCyte treatment. *Aims.* We retrospectively evaluated the safety profile of IT DepoCyte in a cohort of 26 pediatric AL patients with CNS relapse. *Methods.* Between May 2005 and August 2010 twenty-six patients (18 males, 8 females; 21 ALL and 5 AML; age at diagnosis 0.6-17 years; age at treatment 0.8-18 years) with CNS relapsing AL were treated with IT DepoCyte at dosages ranging from 20 to 50 mg/dose depending on age, in seven Italian Pediatric Hemato-Oncology Association (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 eleven children. Twenty three out of 26 patients were simultaneously treated with systemic chemotherapy. Concurrent high dose cytarabine or methotrexate was administered to 20 of these patients. DepoCyte was started upon CNS relapse and was administered every 15 days regardless of aplastic phase and underlying chemotherapy until CSF negativity in two consecutive lumbar punctures. Table I summarizes the clinical characteristics of patients. DepoCyte treatment was discontinued when neurotoxicity appeared or in the presence of severe adverse events. Toxicity was evaluated according to the National Cancer Institute Criteria. *Results.* 23 out of 26 patients (88.5%) achieved complete CSF remission and the remaining 3 presented partial remission (CSF negativity with persistence of neuroradiological findings of cerebral localization). The median number of administered doses was 4 (range 2-9), with CSF negativity after a median of three IT administrations. Neurological toxicity > grade 3 was observed in 3 patients (11.5%); one patient experienced posterior reversible encephalopathy syndrome, the second had strabismus and clonus in the lower right limb and the third had partial seizures after CNS hemorrhagic stroke during the aplastic phase. Two of these 3 patients resumed DepoCyte after CNS event resolution without complications. We observed mild headache < grade 2 in 4 other patients. No permanent sequelae were observed. Two patients died of sepsis during treatment and 2 died from transplant-related complications; 8 died of non-CNS disease progression. Fourteen patients are currently alive in complete remission. *Conclusion.* The use of IT liposomal Cytarabine in the Italian experience showed acceptable tolerability and reasonable efficacy in the majority of patients. The frequency of neurological side effects was similar to what has been observed following other types of IT treatment. Despite its potential neurotoxicity, DepoCyte represents 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.

Table 1.

Clinical characteristics of the Patients

Characteristic	No.
Gender:	
Male/Female	18/8
Median age at diagnosis (yrs) (range)	7 (0.6-17)
Median age at DepoCyte treatment (yrs) (range)	10 (0.8-18)
Underlying neurological disease	1
Diagnosis:	
Acute Lymphoblastic Leukemia	21
Acute Myeloid Leukemia	5
N. of CNS relapse at DepoCyte treatment:	
First relapse	12
Second relapse	7
a Third relapse	6
Resistance	1
Tipe of relapse:	
Isolated CNS relapse	21
Combined relapse	5
Median DepoCyte administration	4 (2-9)
Dose Level per patients:	
20 mg	3
25 mg	3
35 mg	14
50 mg	6
CNS response (%):	
Complete response	23 (88.5)
Partial response	3 (11.5)
Neurological toxicity ≥ grade 3 (%)	3 (11)
Posterior reversible encephalopathy syndrome	1
Strabismus and clonie at right inferior limbs	1
Partial seizures after CNS hemorrhagic stroke	1
Toxicity < grade 3:	
Headache grade I/II	4
Irritability	3
Fever	2
Concurrent HD-MTX/ARA-C	20
Outcome:	
Death from sepsis	2
Death from TRM	2
DOD	8
Alive in CR	14
Median follow-up from DepoCyte therapy (mts) (range)	7 (2-37)

CNS: central nervous system; HD-MTX/ARA-C: high dose Methotrexate/ Cytarabine; yrs: years; mts: months; CR: complete remission; TRM: transplant related mortality; DOD: died of disease

0395

PRECLINICAL EVALUATION OF CPX-351 LIPOSOME INJECTION IN A GENETICALLY ENGINEERED MOUSE (GEM) MODEL OF DRUG RESISTANT HUMAN ACUTE MYELOGENOUS LEUKEMIA (AML)

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Background. CPX-351 Liposome Injection is a nano-scale liposomal formulation that contains a synergistic 5:1 molar ratio of cytarabine (Cyt) and daunorubicin (Daun). Previous preclinical studies revealed that CPX-351 provides dramatic efficacy improvements compared to free drugs in a range of syngeneic and xenograft leukemia models.

Clinically, CPX-351 is being developed to replace conventional Cyt plus Daun therapy ("7+3"). A recent Phase II trial in newly diagnosed elderly AML patients treated with CPX-351 demonstrated improved outcomes over conventional "7+3"treatment with the largest improvements occurring in high-risk AML patients including CRs in patients that failed to respond to 7+3 prior to crossing over to CPX-351 treatment. Here we compare the therapeutic effects of CPX-351 and conventional Cyt:anthracycline in a GEM model exhibiting a genetic makeup common in refractory human AML to better understand the basis of the different responses observed between these two treatments. *Aim.* Assess the therapeutic activity of CPX-351 in a GEM leukemia model which reflects the genetics and pathology of refractory human AML. *Methods.* Luciferase expressing AML cells were implanted in C57/BL6 mice, cells were prepared from AML mice induced by MLL/AF9 + Nras oncogene. Mice were monitored for bioluminescence signal every 4 days, starting 10 days after implant. Upon detection of a bioluminescent signal (pelvis, tail, femurs, hepatosplenic infiltration) treatments were initiated with CPX-351 (Q2D x3) or a cocktail of free Cyt (QD x5) and doxorubicin (QD x3). A no-treatment group served as control. Therapeutic activity was monitored by imaging before treatment, after last treatment, and 4 days after last treatment. Histopathological analysis of peripheral blood (May-Grunwald-Giesma) and mouse survival were also monitored. *Results.* Mice inoculated with refractory AML cells were treated once a bioluminescent signal indicative of leukemia cell engraftment was observed. Overall, more pronounced leukemia reduction (reduced bioluminescence) and survival was observed following CPX-351 treatment than was observed with free drug treatment. Images from mice that received no treatment displayed widely disseminated leukemia. Similar images of disseminated disease were observed in the free drug arm; however the extent and intensity required an additional 4 days to develop (8 days from treatment initiation) and only 3 mice survived. In contrast all 5 mice treated with CPX-351 were alive 8 days after treatment initiation and showed reduced bioluminescence than mice treated with free drugs. Peripheral blood smears obtained 3 days after treatment and on the day of euthanasia exhibited reduced leukemia cell burden. The resulting decrease in leukemia led to a statistically significant increase in 50% median survival when comparing the treatment arms. While the treatment-induced increase in lifespan (ILS) for the free drug cocktail was minimal (14%), CPX-351 significantly increased the ILS to 57%. *Conclusions.* CPX-351 was designed to enhance the efficacy of Cyt:Daun therapy by encapsulating both agents within a drug carrier that maintains the synergistic 5:1 molar ratio for extended times. Here preclinical data indicated that CPX-351 treatment of a model with a common genetic translocation for refractory human AML resulted in decreased leukemia burden and improved survival when compared to free drug treatment.

0396

ECULIZUMAB THERAPY FOR ATYPICAL HEMOLYTIC UREMIC SYNDROME IN PEDIATRIC PATIENTS: EFFICACY AND SAFETY OUTCOMES FROM A RETROSPECTIVE STUDY

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Background. Atypical hemolytic uremic syndrome (aHUS) is a rare, life-threatening disease characterized by systemic thrombotic microangiopathy (TMA) due to chronic, uncontrolled terminal complement activation. Systemic TMA manifests as endothelial damage, hemolytic anemia and platelet consumption, leading to multi-organ damage or failure. Many patients receive chronic plasma exchange/infusion therapy, but despite this, still have persistent TMA and poor outcomes - 60% develop end-stage renal disease or die within 1 year of diagnosis. In prospective clinical trials of adult and adolescent aHUS patients, the complement C5 inhibitor eculizumab controlled the TMA process, prevented and/or reversed kidney damage and reduced plasma exchange/infusion requirements. *Aims.* To assess efficacy and safety of eculizumab for treatment of aHUS outside of clinical trials. *Methods.*

Table 1.

Baseline Characteristics*	n=15
Platelet count <150×10⁹/L, n (%)	7 (47)
eGFR <30mL/min/1.73m², n (%)	3 (20)
Serum LDH >ULN, n (%)	10 (67)
Median (range) duration of current aHUS clinical manifestation, days	36 (2, 481)
Median (range) number of PE/PI sessions in week before eculizumab	4 (0, 7)
Identified complement regulatory factor mutation, n (%)	7 (47)
Efficacy Outcomes During Eculizumab Treatment[†]	
Platelet normalization (>150x10⁹/L), n (%)	
All patients	14 (93)
Patients with abnormal platelet count at baseline	6/7 (86)
Hemoglobin improvement ≥20g/L, n (%)	8 (53)
eGFR improvement ≥15mL/min/1.73m², n (%)	8 (53)
Complete TMA response (platelet count/LDH normalization plus ≥25% serum creatinine reduction), n (%)	7 (50)[‡,¶]
TMA event-free status attained (stable platelets, no PE/PI or dialysis), n (%)	12 (80)
Mean (SD) TMA intervention rate (PE/PI or dialysis events/patient/day)	
Pretreatment period	0.67 (0.63)
Treatment period	0.01[¶] (0.02)

eGFR=estimated glomerular filtration rate; LDH=lactate dehydrogenase; ULN=upper limit of normal.
*One patient received eculizumab to prevent TMA in a kidney graft, thus having normal biological parameters at baseline. [†]Intent-to-treat population (n=15). [‡]N=14. [¶]P<0.0001 vs pretreatment period (sign-rank test).

We conducted a retrospective data collection analysis of 30 aHUS patients receiving ≥1 eculizumab dose outside of clinical trials between 2007 and 2009. This report presents efficacy and safety outcomes for the 15 pediatric patients aged <12 years (<2 y [n=5]; 2-4 y [n=3]; 5-11 y [n=7]). *Results.* Baseline data and eculizumab efficacy outcomes for the 15 pediatric patients are presented (Table). Eculizumab efficacy was similar across the 3 age groups. Eculizumab safety in these pediatric patients was similar to eculizumab safety in adult and adolescent patients evaluated in clinical trials. *Conclusions.* In this medical practice setting, results for pediatric aHUS patients are consistent with results from adult and adolescent controlled trials in demonstrating that eculizumab treatment is well tolerated and can control TMA, improve kidney function and reduce need for plasma exchange/infusion, thus showing the promising potential of eculizumab as a new standard of care for aHUS.

0397

FETAL HEMOGLOBIN INDUCTION BY A NEW HISTONE DEACETYLASE INHIBITOR

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Background. Induction of fetal hemoglobin (HbF) remains a promising therapeutic approach for the treatment of β-thalassemia and sickle cell disease, since HbF can substantially ameliorate the clinical symptoms of these genetic disorders. Several pharmacologic agents, such as hydroxyurea, 5-deoxyazacytidine, butyrate and trichostatin A have been shown to induce γ-globin activity. However, the therapeutic use of these products is limited due to their relatively weak and variable efficacy, their cytotoxicity and possible long-term side effects. Thus, more effective agents that can induce higher HbF levels with lower toxicity are needed. One candidate group of substances with high therapeutic potential are the histone deacetylase inhibitors (HDIs), which through decondensation of chromatin structure can lead to reactivation of gene expression. *Aims.* To investigate the effects of a new histone deacetylase inhibitor (HDACi) on erythropoiesis and hemoglobin synthesis. *Methods.* An *in vitro* erythropoiesis model derived from human CD34+ progenitors cells from normal donors was used. HDACi effects on cell growth and viability, on erythroid differentiation and on HbF induction was investigated. *Results.* HDACi reduced cell growth and delay erythroid differentiation in a dose-dependent manner; concentration higher than 50nM significantly decreased the percentage of GPA+ and CD71+ cells and of mature orthochromatic erythroblasts and increased the percentage of immature proerythroblasts. Lower concentration (1-50nM) didn't affect cells proliferation and maturation (table 1). HDACi positively affected hemoglobin production, increasing the γ/γ+β globin gene ratio and the HbF percentage. The increase observed with concen-

Table 1.

	Cell growth (10^6)	GPA+ Cells (%)	CD71+ Cells (%)	% Proerythroblasts	% Orthochromatic e.	% HbF/HbT	γ/γ+β ratio	BCL11A
Control	3,2	76,2	73,3	13	42	2,8	0,5	1
1nM	2,5	88,8	70,7	14	45	2,7	0,5	0,8
10nM	2,8	57,3	85,8	21	35	5,4	0,6	0,7
50nM	1,7	33,4	57,3	36	6	8,8	0,8	0,3
150nM	0,8	28,7	52,7	88	0		1	0,25

tration higher than 50nM could be related to the inhibitory effect on erythroid differentiation, so to the presence of an higher percentage of immature erythroblasts physiologically expressing higher levels of HbF. On the contrary, the increase observed with HDACi 10nM seem to be due to a specific effect of the drug on γ-gene transcription, probably mediated by a decrease in the expression of BCL11A, a known repressor of γ-globin gene. The HDACi 10nM efficacy in inducing HbF was similar to that of butyrate and hydroxyurea, already used in clinical trials. *Conclusions.* HDACi 10nM may improve erythropoiesis and increase the ratio of fetal to adult haemoglobin, although total Hb doesn't increase. Its HbF inducing activity compare favourably with those of known HbF inducer but, unlike these, without affecting cell viability and differentiation. These findings support the evaluation of HDACi as new candidate molecule for hemoglobinophaties treatment.

0398

KNOCK-DOWN OF MLL/AF4 AND AML1/MTG8 AFFECTS PROLIFERATION AND DIFFERENTIATION OF AML AND ALL CELLS AND IMPAIRS CLONOGENICITY

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Background. MLL/AF4 and AML1/MTG8 are two fusion genes most frequently found in infant acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML), respectively. We have previously shown that transient siRNA mediated knock-down of MLL/AF4 and AML/MTG8 impairs proliferation and clonogenicity *in vitro* and causes a significant increase in median survival in a xeno-transplantation model. *Aims.* We aim to investigate the role of MLL/AF4 and AML1/MTG8 in leukaemic maintenance and progression of established decease *in vivo*. We used a constitutive and an inducible lentiviral shRNA expression system to determine the effects of knock-down of MLL/AF4 in the t(4;11)-positive SEM cell line and of AML1/MTG8 in the t(8;21)-positive human leukaemic cell line Kasumi-1. *Methods.* shRNA cassettes specifically targeting the MLL/AF4 and the AML1/MTG8 fusion were either cloned into the pHR'SINcPPT-SEW GFP expressing lentiviral transfer vector where the shRNA is constitutively expressed or into the pTRIPZ vector where shRNA expression and RFP expression are both induced by doxocycline. SEM and Kasumi-1 cells were transduced with lentiviral particles produced in 293T cells and the transduction efficiency was determined by quantifying GFP or RFP positive cells, respectively, using flow cytometry. Knock-down and expression of known targets of the fusion genes were verified at both the RNA and protein level by qPCR and western blotting respectively. Cell growth was monitored by cell counts and colony formation assays including replating experiments were preformed. Immunodeficient NSG mice were transplanted by intrafemoral injection followed by monitoring of disease progression using bioluminescence. *Results.* More than 95% of SEM and kasumi-1 cells were transduced with pHR'shMLL/AF4 and pHR'shAML1/MTG8 3 days after transduction but decreased to 90% after 30 days in culture. We confirmed that kasumi-1 cells transduced with pHR'shAML1/MTG8 had decreased expression levels of AML1/MTG8 at protein and RNA levels associated with decreased expression of CD34, increased levels of IGFBP7 and an impaired clonogenicity. SEM cells transduced with pHR'shMLL/AF4 showed decreased expression of MLL/AF4 with concomitant decreased expression of HOXA7. The inducible shRNA expression systems showed similar results for both cell lines; however the transduction efficiencies were considerably lower compared to the constitutively expressed shRNA experiments. Currently, we are examining the *in vivo* consequences of fusion gene knockdown in our murine xeno-transplantation model. *Conclusions.* shRNA knock-down of MLL/AF4 and AML1/MTG8 with either the constitutive or the inducible systems led to decreased proliferation and impaired clonogenicity and affected genes associated with differentiation. However the effects were delayed compared to transient siRNA knock-down. Further optimisation of the inducible shRNA system is warranted before *in vivo* experiments can be initiated. frida.ponthan@ncl.ac.uk

0399

TARGETING NAD+ SALVAGE PATHWAY IS A NOVEL THERAPEUTIC STRATEGY IN MULTIPLE MYELOMA

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Background. Nicotinamide adenine dinucleotide (NAD+) is a coenzyme crucially involved in several cellular functions. During the neoplastic transformation, nicotinamide phosphoribosyltransferase (NAMPTase), a key enzyme involved in NAD+ biosynthesis, performs an important role since becomes upregulated to compensate for increased metabolic demands. Therefore, drugs capable to inhibit this enzyme, such as APO866, are under investigation for their potential as anticancer therapeutics. In this study we aimed to investigate the role of NAD+ in Multiple Myeloma (MM) by the activity and mechanism of action of the selective Nampt (the rate-limiting enzyme in the NAD+salvage pathway) inhibitor APO866. *Methods.* a panel of 14 MM cell lines, both sensitive as well as resistant to conventional chemotherapy, were used. Additionally 5 samples from peripheral blood of healthy donors were collected. 5x104 cells/well were plated in 96 well plates and treated with increasing concentrations of APO866 (range 10-9-10-6 M). Viability was assessed at 96 h from the beginning of the treatment by propidium iodide exclusion using a FACS CantoII. In selected cases, Annexin-V/propidium iodide staining was done to detect early- vs. late-apoptotic leukemia cells in response to treatment. Proliferation assays were performed using thymidine incorporation. Intracellular NAD+ content was measured using a biochemical assay; the characterization of cell death was performed by pan-caspase inhibitor (zVAD-fmk), the caspase 9 and 8 specific inhibitors (to value apoptosis) and chemical modulators such as wortmannin, LY294002, 3methyladenine and bafilomycin A1 (to assess autophagy). *Results.* We found maximal cytotoxicity of APO866 against MM cell lines, with an IC50 values ranging from 3-30nM at 96h. Remarkably, in healthy leukocytes, APO866 was poorly active and failed to show any cytotoxic effect, indicating an increased reliance on these enzymes'activity by MM cells. Tritiated thymidine uptake assay confirmed the antiproliferative effects of APO866 in MM. Also the intracellular NAD+ levels lowered in the treatment with APO866 at 24 and 48 hours. A strongly expression of Nampt was revealed by western-blot analysis in all the cell lines analyzed. The AnnexinV/PI analysis confirmed APO866's ability to induce apoptosis in a dose- and time-dependent fashion. Interestingly Nampt inhibitor showed anti-myeloma activity even in the presence of interleukin-6 and insulin-like growth factor-1, confirming its ability to overcome the proliferative advantage conferred by this cytokines. Mechanistic studies, showed that APO866 cell death occurred in the absence of caspase activation. Furthermore, autophagy inhibitors reduced APO866 cytotoxic activity. *Conclusion.* our preliminary data show the efficacy of Nampt inhibitor in MM cell lines,at nanomolar concentrations. Ongoing mechanistic and *in vivo* studies will delineate the role of NAD+ and in MM and better define Nampt inhibitors for clinical development in MM.

0400

PLUMBAGIN ENHANCES TRAIL-INDUCED APOPTOSIS OF HUMAN LEUKEMIC KASUMI-1 CELLS THROUGH UP-REGULATION OF DR5 EXPRESSION, ACTIVATION OF CASPASE-8 AND INHIBITION OF CFLIP

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*Background.*Although the patients with t (8; 21) acute myeloid leukemia (AML) have a favorable prognosis when compared with other non-t (15; 17) AML patients, only approximately 50% patients with this relatively favorable subtype are still alive at 5 years and refractory/relapse is still a tough problem in clinical. So the approach of finding new agents and/or methods is important. *Aims.*To investigate the effect of plumbagin alone,TRAIL alone and combination plumbagin with tumor necrosis factor"related apoptosis inducing ligand (TRAIL) induced apoptosis on leukemic Kasumi-1 cells and its mechenisms. *Methods.* Kasumi 1 cells were treated with plumbagin alone, rsTRAIL alone at different concentration, rsTRAIL combining with plumbagin. Cell proliferation was analyzed by CCK-8 assay.

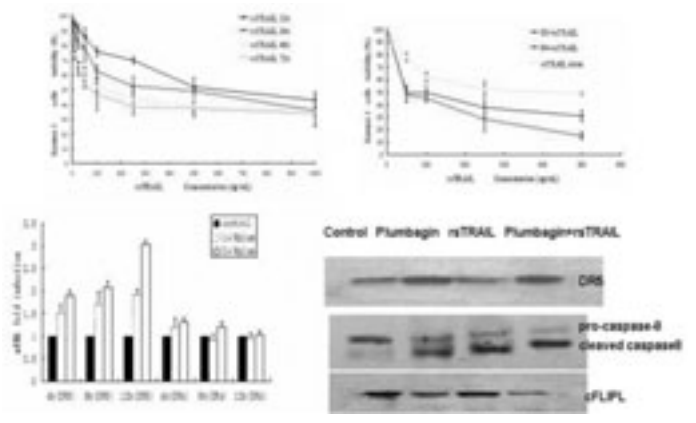

Figure 1.

Apoptosis was studied independently through Annexin /PI double staining by flow cytometry and TUNEL staining The expression of DR4 and DR5 at mRNA lever was detected by real-time PCR. The expression of signal transduction proteins, such as DR5, caspase-3, caspase-8, caspasep-9, Bid, Bax and c-FLIP was detected by Western blotting. 4. *Results.*Both rsTRAIL and plumbagin could induce the apoptosis of Kasumi-1, and rsTRAIL-induced apopotosis of Kasumi-1 could be enhanced by plumbagin. The ratios of annexin V positive Kasumi-1 cells were 27.7±2.9 %, 25.6±3.1 % and (52.1±3.3 % in 100 ng/m1 rsTRAIL groups, 2μmol/Lol/L plumbagin and the combination of the two agents group respectively, and the combination group was significantly higher than the group of rsTRAIL or plumbagin alone. TUNEL assay demonstrated that the number of apoptotic cells in groups of plumbagin combining with rsTRAIL were higher than the groups of rsTRAIL or plumbagin alone. Plumbagin could up-regulate the expression of DR5 at mRNA levels in Kasumi-1 cells by real-time PCR assay, and up-regulation of DR5, activation of caspase-8 and down-regulation of c-FLIP at protein level could be detected in plumbagin-alone and the combination with rsTRAIL groups. *Conclusions.*Plumbagin can enhance TRAIL induced apoptosis of Kasumi-1 cells, and the mechanism is involving in the upregulation of DR5, activation of caspase-8 and the degradation of c-FLIP.

0401

IRON CHELATING AGENTS HAVE ANTI-LEUKEMIC EFFECT ON SECONDARY IRON OVERLOADED LEUKEMIA MOUSE MODEL

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Background. The patients with hematologic diseases receive multiple transfusions due to disease characteristics or treatments. In addition to the removal of iron, iron chelating agents (ICA) have several biological effects, including immunosuppression and changes in cell metabolism. *Aims.* We investigated the anti-tumor activity of ICA in an animal leukemia model with secondary iron overload (SIO). *Methods.* We used 5 week-old male BDF1 ($H2^{b/d}$) mice, and leukemia or lymphoma cell lines (A20, L1210, EL4) of B and T lymphoblast origin. The cell viability of cell lines was assessed in 48 hour culture under the various ICA concentrations with or without ferric chloride by CCK8 method. Also, we performed apoptosis analysis using flow cytometry. All mice were injected subcutaneously with L1210 cells in the right flank area, and measured for tumor mass and weight 3 times a week. All mice except control group received iron dextran (10 mg/day) intraperitoneally for 20 days for SIO from day 7 of tumor injection. Deferrioxamine (DFO) was injected intraperitoneally in dose of 40 mg/kg/day for 6 days, and deferasirox (DFX) orally administered at 20 mg/kg/day for 15 days from day 28 of tumor inoculation, respectively. We assessed tumor iron content (TIC), liver iron content (LIC), tumor size, and survival. *Results.* The viability of A20 and L1210 decreased more than that of EL4 under the ICA. The viability of L1210 under DFX decreased more than that under DFO in therapeutic concentrations ($P<0.01$). The percentage of apoptosis was dependent on the concentration of DFO and DFX, although there was more apoptosis in DFX treated group than in DFO group ($P<0.01$). The expressions of Fas on L1210 did not change according to ICA concentration. Sizes of tumor mass between groups were not different until ICA administration ($P>0.05$). However, tumor grew rapidly in untreated groups (control and SIO) but slowly in ICA treated groups (DFO and DFX) ($P<0.01$). LIC and TIC in SIO were higher than in ICA treated groups. Survival was higher in SIO+DFX ($P<0.01$) but shorter in SIO+DFO ($P<0.01$). *Conclusion.* ICA may have concentration-dependent anti-tumor activity. In SIO with tumor, DFX may result in survival benefit and decreased iron content. We concluded that ICA might be a candidate for anti-tumor treatment along with chemotherapy in leukemia patients who have received multiple transfusions.

0402

A DRUG REPROFILING STRATEGY IDENTIFIES THE ANTI-HELMINTIC NICLOSAMIDE AND VALPROATE (VAN) AS AN EFFECTIVE NOVEL ANTI-MYELOMA COMBINATION THERAPY THAT ALSO REDUCES FREE LIGHT CHAIN PRODUCTION

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Background and Aims. Our *in vitro* and *in vivo* studies have shown that rational drug redeployment (drug reprofiling) can be used successfully to develop novel therapies for haematological tumours and bring significant clinical benefit. In this study, we have investigated a similar drug redeployment approach for multiple myeloma (MM). MM is a malignancy of differentiated B cells characterised by >10% neoplastic plasma cells in the bone marrow, monoclonal immunoglobulin (whole and/or free light chain;FLC) in serum/urine, lytic bone lesions and fractures, anaemia and immunodeficiency. Many patients also develop renal impairment (RI), predominantly caused by elevated nephrotoxic FLC secreted by the malignant plasma cell clone. Despite advancements in therapy and improvement in survival times, particularly for younger patients, MM remains incurable. Relapse with resistant disease is a major cause of death. Furthermore, current intensive treatments are associated with significant toxicity and are not suitable for many MM patients especially the high proportion that are >70yrs. Hence novel therapies with low associated toxicities are urgently needed. The aim of this work was to identify drug combinations for MM using a screen of clinically available safe drugs. *Methods.* A panel of 100 off-patent oral drugs with low toxicity profiles, at drug concentrations consistent with peak serum concentrations achievable for routine clinical indications, were screened against MM cell lines. Cell number was assessed using Alamar Blue assays. Actions of drugs were investigated using flow cytometry assays for apoptosis (annexin V/PI, Caspatag), reactive oxygen species, cell cycle, and mitochondrial membrane depolarisation. Immunoblotting and/or immunofluorescence were used to measure LC3-II levels, and levels/localisation of components of NF-kB pathway. FLC protein was measured using luminex and flow cytometry and mRNA levels using quantitative real-time PCR. *Results.* The screen identified several drugs with anti-MM activity including valproate (already under investigation in myeloma) and niclosamide a broad spectrum anti-helminthic. The combination of niclosamide and valproate (VaN) exhibited greater anti- MM activity against cell lines and primary MM cells than either agent alone and, at least *in vitro*, out-performed the current anti-myeloma chemotherapy combinations of cyclophosphamide/thalidomide/dexamethasone (CTD) or melphalan/prednisolone (MP). Importantly, normal donor haemopoietic progenitor cells were significantly less sensitive to the treatments. Niclosamide treatment of myeloma cells was associated with the generation of mitochondrial superoxide (Mitosox) which was enhanced with the addition of valproate. Treatment with niclosamide was accompanied by rapid depolarisation of mitochondrial membranes as measured by TMRE and JC-1 staining. Cell death was associated with markers of apoptosis, annexin-V positivity and caspase activation. Individually, niclosamide induced markers of autophagy and valproate induced a G1 cell cycle arrest. Furthermore, sub-lethal doses of niclosamide reduced FLC protein secretion from MM cell lines, and some primary MMs. In some, but not all cases, reduced FLC protein secretion was associated with reduction in FLC mRNA transcription most likely through inhibition of NF-kB activity. *Summary.* This study has identified a potent novel combination of off-patent drugs with low associated toxicities which have anti-myeloma activity. Niclosamide, by inhibiting production of FLC, has further potential in light chain mediated diseases including primary amyloidosis.

0403

INHIBITION OF MTOR WITH EVEROLIMUS (RAD001) AND SILENCING BY VASCULAR ENDOTHELIAL CELL GROWTH FACTOR SPECIFIC SIRNA INDUCES ADDITIVE ANTITUMOR ACTIVITY IN MULTIPLE MYELOMA CELLS

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Background. Angiogenesis plays an important role in the pathogenesis and progression in multiple myeloma (MM). MM cells secrete vascular endothelial growth factor (VEGF), which further promotes proliferation of the tumor cells. Several RNA interference (RNAi) methodologies are rapidly being established and hold promise to specifically inhibit gene expression in mammals. RNAi is the sequence-specific, posttranscription gene silencing methods initiated by double-stranded RNAs, which are homologous to the gene being suppressed. Mammalian target of rapamycin (mTOR) is an essential part of tumour growth being capable of integrating proliferative, antiapoptotic and angiogenic signalling by connecting VEGF, hypoxia-inducible factor 1 (HIF-1) and HER family receptors. Several reports have demonstrated the anti-tumor activity of everolimus (RAD001), an mTOR inhibitor, in a variety of cancers, including various leukemias and lymphomas. *Aims.* Therefore, we evaluated the anti-myeloma effect of VEGF siRNA silencing in MM cells and whether it can be augmented by the additional application of everolimus. *Methods and Results.* After transfection with VEGF siRNA we observed a reduction of VEGF expression in all studied cell lines: OPM-2, RPMI-8226, INA-6, Jurkat, Raji and Karpas-299, as well as in cells of MM- and lymphoma patients. Next, using the MM cell line OPM-2 we studied the time courses of VEGF siRNA transfection in order to investigate the knock-down efficiency of VEGF expression. The efficiency of VEGF siRNA transfection in treated OPM-2 cells showed a reduction of 75.5% VEGF protein levels after 24 h compared to the untreated OPM-2 cells ($p<0.001$). Further, VEGF siRNA both significantly induced apoptosis and inhibited proliferation in OPM-2 cells ($p<0.001$), RPMI-8226 ($p<0.001$), and in INA-6 ($p<0.01$) versus controls. To assess whether everolimus treatment of OPM-2 and RPMI-8226 affects the viability of these cells, cells were treated with various doses (1-20 nM) of everolimus for 24 hours, harvested, and analyzed for cell viability by MTT assay. Everolimus significantly decreased the viability of OPM-2 cells (IC50 = 1.9 nM) and of RPMI-8226 cells (IC50 = 2.1 nM), respectively. Everolimus and siRNA both together might to reduce the VEGF gene expression up to 33% ($p<0.001$) compare to siRNA VEGF (61%) alone or everolimus alone (39%), that demonstrated additive effects of everolimus and siRNA. *Conclusions.* These findings suggest that mTOR inhibition and silencing by VEGF specific siRNA may be associated with an additive antitumor activity and might be a suitable target for new therapeutic strategies using RNA interference in MM.

0404

DRUG REPROFILING IN SUB-SAHARAN AFRICA; IDENTIFICATION OF ZINC AND COLCHICINE (ZAC) AS POTENTIAL AFFORDABLE NON-TOXIC THERAPY FOR ENDEMIC BURKITT'S LYMPHOMA

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Background. Burkitt's lymphoma (BL) is a high-grade B-cell Non-Hodgkin's Lymphoma (NHL) with one of the fastest doubling time amongst human tumours. Occurring most frequently in children in areas with holoendemic and hyperendemic malaria, endemic BL (eBL) accounts for ~50% of all childhood malignancies in sub-Saharan Africa. Although intensive chemotherapy is highly effective against BL with potential cure rates of ≥85% the financial implications of high-intensity regimens including the cost of drugs, transfusions, and intensive supportive medical care preclude their use in many developing countries for the majority of patients. Low-dose chemotherapy combinations based on cyclophosphamide costing <US$50 per patient have managed to achieve ~50% 1year disease free survival in eBL (95% chance of a cure). However there is a desperate need for effective, affordable, non-myeloablative therapies. *Aim.* The aim of this study was to identify novel treatments for BL by screening off-patent, non-toxic, non-myeloablative, oral drugs for anti-BL activity. *Methods.* A panel of 100 off-patent drugs at concentrations consistent with peak serum concentrations achievable during their routine clinical indications were screened against 8 BL cell lines. Cell number was assessed using Alamar Blue assay. Actions of selected drugs were investigated using flow cytometry based assays for apoptosis (annexin V/PI, Caspatag), reactive oxygen species (ROS) and cell cycle. Normal donor bone marrow samples were treated and viability assessed using immunophenotyping and flow cytometry. *Results.* The drug screen performed using peak serum drug concentrations identified colchicine and zinc as having potent anti-BL activity against 8 BL cell lines. Colchicine, a microtubule destabilising agent, induced potent G2/M cell cycle arrest by 48hrs to a similar extent as vincristine, a commonly used microtubule destabilising chemotherapeutic which has significant associated toxicities. Colchicine treatment was then followed by rapid cell death with markers of apoptosis including annexin V positivity and caspase activation. Zinc treatment also induced a rapid cell death, within 24hrs for most BL cell lines. Dose titrations of zinc and colchicine demonstrated that each agent had potency at concentrations easily achievable using routine clinical doses. Cross-titration experiments highlighted additive effects of the combination (zinc and colchicine; ZaC) in some of the BL cell lines tested. Importantly, neither agent, either alone or in combination, impacted upon the survival of normal donor CD34+ve haemopoietic progenitors. *Summary.* This study has identified that a novel combination of zinc and colchicine (ZaC) has potent anti-BL activity. Importantly, neither agent affected the survival of haemopoietic progenitor cells indicating that ZaC therapy is unlikely to be myeloablative. This is supported by an absence of toxicities associated with long-term clinical use of each agent e.g. months/years treatment of paediatric Familial Mediterranean Fever demonstrate colchicine to be safe in children. Zinc supplementation is known to be safe and interestingly, the vast majority of BL patients will be zinc-deficient as a result of malnutrition. Individually, each agent was potent at killing BL cell lines and additive actions of the combination were observed in some of the lines. These data indicate that ZaC is an effective, affordable, therapeutic option for endemic BL.

0405

PRALATREXATE SELECTIVELY INDUCES APOPTOSIS AND SYNERGIZES WITH BEXAROTENE THROUGH UP-REGULATION OF P53/BAX/PUMA IN CUTANEOUS T-CELL LYMPHOMA CELLS

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Background. Pralatrexate (PDX), a targeted antifolate, was designed for preferential uptake and accumulation in tumor cells based on its high affinity for reduced folate carrier-1 and efficient polyglutamation by folypolyglutamyl synthetase. PDX is approved by the United States FDA for relapsed or refractory peripheral T-cell lymphoma, and has demonstrated activity in a Phase I study in cutaneous T-cell lymphoma (CTCL). Bexarotene (BEX), approved for treatment of CTCL, is an RXR-selective retinoid, and thus of interest to study in combination with PDX. *Aims.* In this study, we investigated the therapeutic mechanisms of PDX and whether combination with BEX has synergistic anti-tumor effects in CTCL. *Methods.* Cell viability was examined by MTS assay and apoptosis by FACS analysis. Expression of apoptosis-associated proteins was analyzed by Western blotting. *Results.* PDX (1-5 nM for 24 and 48 hrs) decreased cell viability and induced apoptosis in a time- and dose-dependent manner in four CTCL cell lines (MJ, Hut78, HH, and HH/VOR). PDX (1-5 nM for 48 hrs) also caused significantly more apoptosis of $CD4^+$ T cells from six Sézary syndrome (SS) patients with high percentages (74-96%) of circulating $CD4^+CD26^-$ T cells compared to $CD4^+$ T cells from three healthy donors ($p < 0.05$). Moreover, PDX at low dose (2 nM) combined with BEX (10 μM) induced up to 12-fold increase in apoptosis compared to either drug alone in all four CTCL cell lines and in SS patients' $CD4^+$ T cells. Additionally, PDX combined with BEX synergistically increased the tumor suppressor p53, and the p53-regulated pro-apoptosis proteins, Bax, and PUMA. *Conclusions.* Our results show that PDX at low nanomolar concentrations selectively induces apoptosis in CTCL cell lines and SS patients' $CD4^+$ T cells, and PDX in combination with BEX exerts a synergistic pro-apoptosis effect through up-regulation of p53/Bax/PUMA in CTCL. These findings support the ongoing phase I clinical trial of PDX/BEX and provide the rationale for future studies of this combination in CTCL patients.

0406

DNA-DEPENDENT PROTEIN KINASE AS A PROMISING MOLECULAR TARGET FOR THE TREATMENT OF ADULT T-CELL LEUKEMIA-LYMPHOMA

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The DNA repair system is a promising target for sensitization and overcoming drug resistance on cancer treatment. Since the cells that genetically too unstable will die, a treatment that blocks a particular DNA repair system can induce apoptotic cell death on cancer cells but not on normal cells. We recently found that high expression of catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) was observed in adult T-cell leukemia-lymphoma (ATL) cells. In addition, a new chemotherapeutic drug NK314 possessing inhibitory activity for both topoisomerase II and DNA-PK potently inhibited the growth of various ATL cell lines. According to the results, we designed combination treatment with various DNA-PK inhibitors and chemotherapeutic agents. NU7026, a DNA-PK inhibitor, enhanced the anti-cancer activity of etoposide. Such enhancement of cell growth inhibition by DNA-PK inhibitors was not observed in DNA-PK deficient cancer cell line, M059J cells. These results suggested that DNA-PK is a promising target molecule for ATL. We also identified that hnRNP B1, a RNA binding protein, directly bound with DNA-PK complex and inhibited DNA-PK activity in lymphoid malignant cells. Since hnRNP B1 is overexpressed in a population of ATL cells, hnRNP B1 can be a prediction marker for response to combination therapy with DNA-PK inhibitors and chemoradiotherapy.

Red blood cells and iron - Biology

0407

ENUCLEATION OF HUMAN ERYTHROBLASTS IS AN ACTOMYOSIN-DEPENDENT PROCESS FOLLOWING NUCLEAR POLARIZATION

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Backgrounds. During erythropoiesis, stem cells undergo lineage specific commitment and generate erythroid progenitor cells through cellular division events including nuclear (mitosis) and cytoplasmic (cytokinesis) division. In terminally differentiated erythroblasts, the centrally located nucleus becomes eccentrically located in the cytoplasm via a process known as polarization, and is expelled via a process termed enucleation, becoming reticulocytes and subsequently mature erythrocytes. Enucleation of erythroblasts is thought to occur through a process similar to cytokinesis. However, little is known in regards to how the process of enucleation differs from conventional cytokinetic processes as well as to the precise role of non-muscle myosin II in enucleation. *Aim.* The aim of this study is to investigate how the process of enucleation differs from conventional cytokinetic processes, and to elucidate the role of cytoskeletal modifiers during each step of enucleation in human erythroblasts. *Methods.* We investigated the role of cytoskeletal modifiers during conventional cell division of early-stage erythroid cells such as colony-forming unit-erythroid (CFU-E), and compared their roles with that during each step of enucleation in human erythroblasts. For this purpose, highly purified human CD34+ cells were induced to differentiate to the level of CFU-E, and to the level of terminally differentiated erythroblasts that undergo enucleation. Since the efficacy of inhibitors for cell division often varies depending on the species of the cell observed, the immortality of the cells and their redundancy in the cells themselves, efficient inhibitors of cell division in human CFU-E were selected, and then examined the effects of these inhibitors on nuclear polarization and enucleation events. *Results.* We selected blebbistatin, an inhibitor of non-muscle myosin II ATPase, cytokalasin D, an inhibitor of actin polymerization, NSC23766, an inhibitor of Rac 1 GTPases, Y27632, an inhibitor of ROCK, colchicine and vinblastine, inhibitors for microtubules, and monastrol, an inhibitor of the mitotic kinesin Eg5, as efficient inhibitors for CFU-E cell division. When these inhibitors were applied to terminally differentiated erythroblasts, blebbistatin, cytochalasin D increased the number of cells with a polarized nucleus accompanied with a complete block of enucleation. NSC23766 and Y23766 appeared to increase cells with nuclei positioned in the center of the cytoplasm, and caused an immediate and complete inhibition of enucleation. Colchicine, vinblastin and monastrol did not increase cells with a polarized nucleus and did not inhibit enucleation. The degree of the increase in reticulocytes during the initial 24 h was similar to that of cells with a polarized nucleus. *Conclusion.* This study shows that the inhibition of non-muscle myosin II ATPase and ROCK completely blocked enucleation of human erythroblasts, demonstrating for the first time that non-muscle myosin II is required for human erythroblast enucleation. We also suggest that tubulin, kinesin, Rac GTPases and ROCK may be involved in nuclear polarization that is required just prior to enucleation. It is anticipated that these advances will enable the definition of defects in enucleation of erythroblasts in inherited and acquired red cell disorders, and in bone marrow failure syndromes.

0408

GENE EXPRESSION PROFILE ANALYSIS IN HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN (BRAZILIAN TYPE): IDENTIFICATION OF TARGET GENES THAT COULD BE RELATED TO HEMOGLOBIN SWITCHING

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Hereditary Persistence of Fetal Hemoglobin (HPFH) is a heterogeneous and benign group of genetic disorders characterized by an abnormal switching from fetal to adult hemoglobin, resulting in increased fetal hemoglobin (HbF) levels at the adult stage The reactivation of HbF

is an important therapeutic option in patients with hemoglobin disorders, since it inhibits the polymerization of sickle hemoglobin. The non deletional Brazilian type HPFH (BHPFH), a C[ARROWRIGHT]G mutation at -195 position of the A gamma globin gene, was first identified in 1990, but the underlying mechanism of upregulation of fetal hemoglobin is still unclear. In order to elucidate how this point mutation leads to an altered gene expression pattern, the aim of this study was to identify genes that are differentially expressed in reticulocytes of BHPFH subjects and of control subjects using Suppression Subtractive Hybridization Library (SSH). Real Time PCR (RT-PCR) was used for posterior validation. Total mRNAs extracted from reticulocytes isolated from peripheral blood samples were used for the construction of the SSH, a PCR-based method for cDNA subtraction libraries. Genes appearing only in one of the libraries were considered as overexpressed. A total of 57 overexpressed genes were identified in the normal library, while 59 genes were overexpressed in the BHPFH cDNA library. Differentially expressed genes included transcription factors (KLF1, NFIA, FOXP1), transcription coregulators (MKRN1, NCOA4), genes involved in GTPase regulation (HOOK3, RASA1, DOCK8) and erythroblast differentiation (FOXO3a) genes. Genes involved in chromatin organization (MIER1, PPP1R10, WHSC1, MORF4L1) were mostly upregulated in control subjects. Six genes (KLF, FOXO3a, HOOK3, MKRN1, MIER1, PPM1a) were chosen for individual analysis by RT-PCR. Lower expression of KLF1, HOOK3, MIER1 genes and higher expression of FOXO3a, MKRN1 and PPM1a were found in BHPFH subjects, when compared to control subjects. Interestingly, KLF1, a gene encoding a transcription factor that regulates the developmental gamma-to-beta globin switch, was highly expressed in control subjects, and a DNA-protein array confirmed increased activity of the KLF1 protein in erythroid cells of controls subjects compared to BHPFH. These results suggest that, in BHPFH, the maintenance of fetal hemoglobin levels is associated with low KLF1 activity. Our data also suggest for the first time that other genes such as MKRN1, FOXO3a, PPM1A and HOOK3 may play a role in globin gene regulation, gamma gene expression and augmentation of HbF levels, representing novel pathways involved in erythroid cells control of hemoglobin switching to be studied.

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0409

HYDROXYUREA REDUCES THE HYPERCOAGULABLE STATE OF PATIENTS WITH SICKLE CELL ANEMIA

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Background. Nearly every element of hemostasis is altered towards the pro-coagulant state in sickle cell anemia (SCA), and thus, an increased rate of thrombotic complications is observed. A major therapeutic approach in these patients is hydroxyurea, which is capable of reducing vaso-occlusive complications and the hemolytic anemia. *Aims.* To evaluate the effect of hydroxyurea in the hypercoagulable state of patients with SCA; the expression of tissue factor (TF), the physiological initiator of coagulation, and the plasma thrombin-antithrombin complex (TAT), a marker of coagulation activation. *Methods.* This study was conducted on a cohort of 34 SCA adult patients (genotype SS) followed at a Sickle Cell Disease University Clinic, and 22 healthy controls. The SCA patients were all in steady state and 21 of them were on hydroxyurea therapy. Informed consent was obtained from all patients included in the study. We studied the mRNA expression of *TF* in total leukocytes through quantitative PCR (qPCR) and TF protein expression in monocytes by flow cytometry. Plasma TAT levels were measured by ELISA (Enzygnost TAT micro, Siemens). Statistical analyses were done using Mann-Whitney U test and Spearman correlation test. *Results.* Patients with SCA had higher levels of leukocyte *TF* relative gene expression in comparison to healthy controls (4.8 vs 1.3; p=0,0006). Hydroxyurea was effective in significantly reducing this expression, among patients with SCA, the *TF* mRNA levels were significantly lower in patients on hydroxyurea therapy in comparison to those not on hydroxyurea (2.7 vs 8.4; p=0.0083). These results were confirmed by the flow cytometry experiments. Levels of fetal hemoglobin showed a strong negative correlation with the relative quantification of *TF* mRNA (r=-0.5339; p=0.010). *TF* expression also correlated with some hemolysis markers such as hemoglobin concentration (r=-0.6744; p=0.0002) and hematocrit (r=-0.5739;p=0.0034), but surprisingly not with lactate dehydrogenase, reticulocyte count and bilirubin. There was a significant positive correlation between *TF* expression and leukocyte, monocyte and neutrophil counts. (r=0.41, p=0.045; r= 0.54, p=0.006 and r=0.46, p=0.021, respectively). Levels of TAT (ug/L) were significantly higher in SCA patients in comparison to controls (12.1 vs 0.7; p<0.0001), and therapy with hydroxyurea was also able to significantly reduce these levels (9.1 vs 17.0; p=0.0160), further demonstrating the inhibitory effect of hydroxyurea on coagulation activation. *Conclusions.* We demonstrated that hydroxyurea is capable of inhibiting *TF* gene expression in leukocytes of patients with SCA and also of reducing plasma levels of TAT, a marker of coagulation activation. These results clearly indicate that therapy with hydroxyurea modulates the hypercoagulability state encountered in SCA.

0410

KLF1 GENE MUTATIONS ARE ASSOCIATED WITH HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN AND DECREASED KLF1 AND BCL11A GENE EXPRESSION LEVELS

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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 mutations within the KLF1 gene, leading to high levels of HbF in adults. Elevated HbF levels may ameliorate the clinical phenotype of beta-thalassemia. *Aim.* The aim of this study was to investigate the possible correlation between mutations within the KLF1 gene and its expression levels in Serbian patients with high levels of HbF, who were shown to be negative for mutations in the γ-globin genes. *Methods.* Mutation screening in the KLF1, HBB, HBG1 and HBG2 genes was done using PCR and direct resequencing. KLF1 and BCL11A gene expression levels were quantified by RQ-PCR method, using SYBR Green chemistry. GAPDH gene served as an internal control. Relative quantification analysis was performed using comparative Ct method (2-ddCt), where ddCt = dCt (sample) - dCt (healthy (median)). *Results.* Three adult patients presented with high levels of HbF (11-17.7%) 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 the presence of two mutations, namely p.S102P and p.F182L, within exon 2 of the KLF1 gene. One patient (HbF=12%), homozygous for the p.S102P mutation was also shown to be heterozygous for p.F182L mutation. The other two patients (HbF11% and HbF17.7%) were heterozygous for p. S102P mutation only. However, in healthy Serbian controls none of these mutations were detected. RQ-PCR analysis in the patient, homozygous for p.S102P mutation and heterozygous for p.F182L mutation, showed that KLF1 gene expression levels were more than 60% lower than in healthy controls, while BCL11A gene expression levels in this patient were 45% lower compared to healthy controls. *Conclusion.* Our study showed the presence of mutations within the KLF1 gene in patients with elevated HbF levels. We propose that these mutations, in single or compound heterozygosity, influence KLF1 and BCL11A gene expression, which, in turn, affects transcription of the γ-globin genes and, hence, HbF production.

0411

VARIANTS IN GENETIC MODIFIERS OF BETA-THALASSEMIA CAN HELP TO PREDICT CLINICAL SEVERITY

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Background. Patients with beta-thalassemia display large variability in disease severity and are usually classified into thalassemia major

(TM) or intermedia (TI) according to clinical criteria. The major determinant of the severity is the degree of beta-globin chain deficit resulting from the nature of the beta-thalassemia mutations. Other genetic modifiers, affecting the degree of alpha and non alpha-globin chain imbalance, also impact the phenotypic severity: an associated alpha-thalassemia minimizes the excess of alpha-globin chains and tends to produce a less severe condition. An increased residual level of HbF in adult life is also a major amelioration determinant. Three major HbF Quantitative Trait Loci (QTL) have been identified so far. The so-called -158 C>T XmnI SNP, is located in the fetal Ggamma-globin gene promoter. The two others are located in the BCL11A and in the HBSB1L-cMYB inter-region. Some particular tag-SNPs in these regions are associated with high HbF levels in healthy adults and in Thalassemia patients. *Aims.* In this study, we investigated the effect that these SNPs might exert in combination with beta and alpha-thalassemia genotypes on beta-thalassemia severity. *Method.* A cohort of 101 affected patients, included in the French National Registry for Thalassemia, were classified into TM (n=69) or TI (n=32) according to clinical data. All patients were genotyped for (i) beta-thalassemia mutations, (ii) the XmnI SNP, (iii) the -3.7 kb alpha-thal deletion, (iv) the tag-SNP rs 11886868 in BCL11A exon 2 and (v) the tag-SNP rs9399137 in the HBSB1L-cMYB inter-region. Univariate and multivariate analyses were performed to study the risk of TI associated with the presence of favourable alleles. *Results.* As expected, univariate analysis showed that beta-thalassemia mutations and XmnI -158 C>T SNP have the strongest effect on severity. Multivariate analysis performed with the 5 modifiers indicated that presence or absence of these favourable alleles could predict the type of Thalassemia in 83.8% of the cases (major type: 92.2%; intermedia type: 69%). The predictions made from the beta-thalassemia mutations and the XmnI SNP alone were significantly improved by the adjustment with the 3 other modifiers, moving from 75.2% to 83.8% (p<0.001). In order to test an easy-to-use prediction tool, we calculated a 'score variable' defined as the number of favourable alleles carried by each patient. Following this simple scoring, all patients with score 0 were TM (96% with score 0 or 1) whereas all patients with score 5 or 6 were TI. When considering only the beta0/beta0 patients, the scores ranged between 0 and 5 and became informative for all patients: more than 95% patients with a score between 0 and 2 were TM; more than 95% patients with a score between 3 and 5 were TI. *Conclusion.* In this study, we showed that predictions based on genetic modifiers can predict the major or intermedia type of beta-thalassemia, even in cohorts of patients with various beta-globin genotypes (up to 30 different mutations in our series). If further validated, this prediction tool of severity may have implications for genetic counselling but also for decisions regarding therapeutic options such as HSC transplantation.

0412

TELOMERASE ACTIVITY IS USEFUL FOR THE SCREENING OF CRYPTIC AND LATE ONSET DYSKERATOSIS CONGENITA AND THE EVALUATION OF THE TREATMENT RESPONSE TO ANABOLIC STEROIDS FOR THEIR BONE MARROW FAILURE

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Background and Aims. We have recently shown that some patients with cryptic and late onset Dyskeratosis Congenita (DKC) among those with acquired aplastic anemia (AA) or myelodysplasia syndrome have short telomeres due to mutation in the components of telomere-associated genes such as *TERC*, *TERT* or *TINF2*. Clinically it is very important to identify cryptic and late onset DKC patients, because these patients will exhibit refractoriness to conventional immunosuppressive therapy (IST) for their bone marrow failure. Several groups, including our own (15th. Congress of European Hematology Association, 2009, Barcelona, Spain), revealed telomere length in peripheral blood was useful in the screening of these patients, but some patients with telomere-associated gene mutation did not have short telomere. The reasons of normal telomere length in these patients might be that aging and accelerated generation were insufficient. Moreover, recent study reported that the androgen, sex steroid hormone, induced hematopoiesis by activating telomerase in hematopoietic cells. In this study, we evaluated telomerase activity in peripheral blood from patients with DKC and AA patients to clarify its usefulness in diagnosis and the evaluation of the treatment response to anabolic steroid. *Methods.* We analyzed telomerase activity in peripheral blood from one DKC, three cryptic DKCs, 15 AA patients, and 30 healthy controls. Telomerase activity of the cellular extract from 2×10^4 peripheral mononuclear cells was assayed using the TRAPeze Telomerase Detection Kit. We analyzed the entire coding region of each telomere-associated genes by direct sequence, and measured the length of telomeres in peripheral blood by Southern blot analysis. *Results.* Telomerase activity of DKC and cryptic DKC patients were significantly lower than healthy controls (16.3 TPG unite vs 86 TPG unite, p=0.011). Telomerase activity did not significantly differ between AA patients and healthy controls (77.3 TPG unite vs 86 TPG unite, p=0.323). However we identified two AA patients who showed obviously lower telomerase activity (33 and 44 TPG unite) than healthy controls. One of these AA patients showed shortened telomere (4.2kb) without mutations of telomere associated genes. These AA patients with lower telomerase activity did not responded to IST, and may have unknown mutations of telomere associated genes. Next, we analyzed the relationship between telomerase activity and the clinical responses to anabolic steroid in one DKC, two cryptic DKCs, and 3 AA patients who were refractory to IST. The anabolic steroid significantly increased telomerase activity in DKC patient (from 15.7 to 54 TPG unite, p=0.002) and tended to increase telomerase activity in cryptic DKC patients (from 21 to 42 TPG unite, p=0.061) and AA patients (from 77 to 93 TPG unite, p=0.052). Clinically, a slight increase of hemoglobin (from 9.8 to 10.9 g/dl) was found in DKC patient, but no obvious increase in hemoglobin was seen in other patients. *Conclusions.* These findings revealed that the assay of telomerase activity is useful for screening cryptic DKC, but that longer follow-up may be necessary to evaluate clinical response to anabolic steroid.

0413

GROWTH DIFFERENTIATION FACTOR 15 (GDF-15) PRODUCTION IN COBALAMIN DEFICIENCY ANEMIA

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Background. Cobalamin (or vitamin B12) is important to the normal production of thymidilate, and its deficiency may prevent normal DNA synthesis in bone marrow precursors causing megaloblastic anemia. Ineffective erythropoiesis is a hallmark of this condition and high levels of lactate dehydrogenase (LDH) with mild unconjugated hyperbilirubinemia due to intramedullary hemolysis have been described. Inherited hemoglobinopathies such as the thalassemia syndromes also present with ineffective erythropoiesis, and recent studies have shown that erythroblasts in the ineffective bone marrow produce large amounts of Growth Differentiation Factor 15 (GDF-15), a potent downregulator of hepcidin, resulting in increased iron uptake and consequent iron overload. *Aims.* To determine GDF-15 plasmatic levels in patients with megaloblastic anemia, and correlate with iron homeostasis and hematological parameters during the normalization of erythropoiesis after cobalamin replenishment. *Methods.* We selected patients with proven cobalamin deficiency anemia, as confirmed by a typical blood smear (macro-ovalocytes, neutrophil hypersegmentation), low serum cobalamin (<200pg/mL) along with normal serum folate levels and characteristic clinical presentation (anemia with or without neurological symptoms). Peripheral blood samples were collected upon informed consent at diagnosis, 7 days after daily 5mg intramuscular cyanocobalamin treatment and after complete normalization of hematological parameters. Ferritin, transferrin saturation and LDH levels were determined by routine laboratory methods. GDF-15 plasmatic levels were determined by ELISA assay. *Results.* Fourteen patients were enrolled with symptomatic cobalamin deficiency between August 2009 and September 2010 (10 male/4 female, age range 28-80 years old). Results are expressed as mean±SEM. Mean cobalamin levels at diagnosis were 81.5±14.1pg/mL. Mean serum ferritin levels and transferrin saturation were in the normal range, 227.2±41.4 ng/mL and 32.9±5.0%, respectively. As expected, mean hemoglobin (Hb) levels increased with treatment (8.75±0.41 g/dL at diagnosis (D0), 10.64±0.54 g/dL after 7 days of treatment (D7) and 13.02±0.45 g/dL after normalization (DN), p<0.0001). Mean corpuscular volume decreased with treatment (114.4±2.5 fL (D0), 110.1±2.6 fL (D7) and 91.4±2.3fL (DN), p=0.0005). GDF-15 levels were 8057±2215pg/mL at D0 (range 1933-23483) and decreased significantly to 1047±164 pg/mL (D7) and 876±205 pg/mL (DN) (p=0.0039) after adequate treatment. There was

significant correlation between GDF-15 levels and Hb levels (r^2=0.34, p<0.0001), but not between GDF-15 and LDH levels (p=0.45). *Conclusions.* Treatment with cyanocobalamin restored normal erythropoiesis while decreasing GDF-15 levels to baseline levels before normalization of hematological parameters. The lack of correlation with LDH levels, unspecifically produced in increased cell proliferation, is consistent with reports that only more immature erythroblasts produce large amounts of GDF-15. While immunity-driven overproduction of GDF-15 has been reported in anemia of chronic disease not primarily affecting iron homeostasis, and erythropoiesis-driven high GDF-15 levels in thalassemia have been linked to iron overload, there was no association between ineffective erythropoiesis and spontaneous iron overload in cobalamin deficiency. This suggests that long periods of time of exposure to increased GDF-15 levels may be needed for iron overload to take place in this setting. The pathophysiological role of the iron regulatory effect of GDF-15 in megaloblastic anemias should be further investigated.

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0414

ALTERATIONS IN CELL TURNOVER AND PRO-APOPTOTIC SERUM FACTORS MAY MODULATE NEUTROPHIL NUMBERS IN SICKLE CELL DISEASE

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Background. Leukocytes are known to exacerbate inflammatory and vaso-occlusive processes in sickle cell disease (SCD) by adhering to the vascular endothelium and participating in inflammatory mechanisms. The relevance of neutrophil death to inflammatory disease pathogenesis is recognized, since alterations in leukocyte apoptotic processes may affect cellular function and inflammatory processes. *Aim.* Since previous data indicate an inhibition of neutrophil apoptotic pathways in SCD, the present study investigated whether alterations in SCD neutrophil (SCDneu) death pathways are the result of a shift in cell turnover and/or whether factors present in SCD serum (ser) influence this process. *Methods.* The maturity of circulating neutrophils from healthy control (CON) and SCD individuals was determined immunophenotypically. Serum factors affecting neutrophil apoptosis (determined by annexin-V binding) were analyzed by culturing (16h, 37°C) control neutrophils (CONneu) with pooled serum (10%v/v) from CON, SCD and SCDHU (SCD patients on hydroxyurea therapy) individuals. *Results.* Immunophenotypic characterization indicated an increase in immature neutrophils in the circulation of SCD individuals (0.22±0.05; 0.6±0.09% CD13-/CD45+ cells; for CONneu and SCDneu respectively, n≥8; P<0.01) although no differences in the presence of mature circulating neutrophils were observed (41.2±4.6; 41.1±3.3% CD13+/CD45+ cells; for CONneu and SCDneu respectively, n≥9; P>0.05). Whilst SCDneu cultured in the presence of CONserum presented delayed apoptosis compared to CONneu (41.6±4.2; 54.0±2.9% AnnexinV binding; respectively; n≥13; P<0.05), unexpectedly, the culture of CONneu with SCDser significantly augmented apoptosis (56.5±5.4%; n=8) compared to CONneu cultured with CONser (44.4±4.2%; P<0.0001; n=8) or SCDHUser (45.1±4.8%; P<0.0001; n=8; compared to SCD; Annexin V binding). Caspase-9 activity was also significantly increased when CONneu were cultured for 16h in SCDser, compared to CONser (0.025±0.003; 0.016±0.002 OD; n≥12; P<0.01, respectively). However, caspase-9 gene expression was not different between the groups (data not shown). Incubation of neutrophils with serums in the presence of the programmed necrosis inhibitor, Necrostatin (10μM), reduced death cell independently of serum type (2.2±0.3; 0.6±0.06% propidium iodide staining; P<0.001 for CONser; 2.9±0.5; 1.1±0.2%; P<0.001 for SCDser; 2.1±0.3; 0.9±0.1%; P<0.01 for SCDHUser without or with Necrostatin, respectively; n=7), although non-necroptotic death of CONneu incubated with SCDser was significantly higher than for CONneu incubated with CONser (1.1±0.2; 0.6±0.06%; PI positive cells; n=7; P<0.01). *Conclusions.* Characterization of circulating neutrophils demonstrated a higher incidence of immature cells in SCD individuals, indicating a shift in cell turnover and/or earlier release of leukocytes from the bone marrow. While SCDneu appear to demonstrate an increase in cell survival, the serum of SCD individuals seems to contain pro-apoptotic factors, as indicated by the stimulation of phosphatidylserine presentation and caspase-9 induction in leukocytes incubated with SCD serum. Necroptosis may contribute slightly to neutrophil cell death, independently of serum type, although apoptotic cell death seems to be increased by SCD serum. Data indicate that alterations in cell turnover or emigration from bone marrow may contribute to elevate leukocyte number and delay cell death in SCD; however cell death may be subject to modulation by a complex balance of both anti- and pro-apoptotic factors contained in the serum of SCD individuals.

0415

EYA3 SILENCING PROMOTES MODIFICATIONS IN THE EXPRESSION PATTERN OF GLOBINS GENES, HBF AND APOPTOSIS LEVELS SUGGESTING ITS PARTICIPATION IN ERYTHROID DIFFERENTIATION

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Background. Erythroid differentiation is a dynamic process in which a pluripotent stem cell undergoes a series of developmental changes that commit it to a specific lineage. These alterations involve changes in gene expression profiles. Previous results using SAGE identified 93 differentially-expressed genes during erythroid development. One of these genes, EYA3, a homologue gene of Eyes Absent 3 in Drosophila, is a transcription cofactor with intrinsic phosphatase activity and its expression was observed to be high at the end of CD34+ differentiation and in human bone marrow. *Aim.* Evaluate effects of EYA3 gene silencing in the K562 (erythroleukemia cell) line after hemin induction, such as modifications of globins gene expression, apoptosis and fetal Hemoglobin (HbF) expression. Furthermore, we evaluated EYA3 gene expression using CD34+ cells and reticulocytes from sickle cell disease (SCD) patients, compared to a control group. *Methods.* Two different cultures from human K562 cells (1x10^{5}cells/mL in DMEM, 10% FBS, penicillin/streptomycin, 5% CO_2, 37°C) were transfected with control or EYA3 knockdown lentivirus (MOI=1.0). After proliferation and selection of positive cells with puromicin (2.0 ug/mL), cells were treated with 30μM hemin and collected after 0, 24, 48, 72 and 96h for gene expression and flow cytometry analysis. CD34+ hematopoietic cells from 4 control individuals were proliferated and differentiated into late stage erythroblasts. EYA3, α, β and γ-globin gene expression was analyzed by qRT-PCR and quantified using the Gnorm program. HbF expression and apoptosis were evaluated by flow cytometry. *Results.* Analysis of α, β and γ-globin gene expression shows that these genes are downregulated in K562 culture cells knockdowned for EYA3 compared with a control culture at 0, 24, 48, 72 and 96h after hemin addition (see Table, ***p<0.0001 and **p<0.001, n=2). HbF expression was downregulated at 48h after hemin addition in silenced cultures (541.6±77.3; 277.3±7.6, for Control and siRNA respectively, p<0.001, n=2). Finally, apoptosis levels were found increased at 24, 48 and 72h after hemin addition (C24h: 8.76±0.075, siRNA24h: 14±0.04, C48h: 8.47±0.21, siRNA48h: 11.07±0.24, C72h: 8.62±0.3, siRNA72h: 10.94±0.58, p<0.0001, except for C72h vs siRNA72h, p<0.05, n=2) in EYA3 knockdowned culture compared to levels obtained in control K562 culture. Evaluation of EYA3-gene expression using CD34+ primary culture showed that the expression of this gene increases during erythroid differentiation (day 7: 0.98±0.03; day 10: 1.37±0.5; day 13:

Table 1.

		0h	24h	48h	72h	96h
α-globin	Control	0.97 ±0.03	1.02 ±0.06	1.33 ±0.08	0.9 ±0.2	1.6 ±0.1
	siRNA	0.077 ±0.005 ***	0.084 ±0.005 ***	0.23 ±0.03 ***	0.44 ±0.02 ***	0.36 ±0.02 ***
β-globin	Control	0.9 ±0.01	1.83 ±0.2	1.4 ±0.04	1.4 ±0.08	1,7 ±0.03
	siRNA	0.17 ±0.1 **	0.07 ±0.007 ***	0.06 ±0.001 ***	0.09 ±0.01 ***	0.14 ±0.03 ***
γ-globin	Control	1.03 ±0.03	1.53 ±0.2	1.4 ±0.02	1.7 ±0.03	1,7 ±0.03
	siRNA	0.2 ±0.008 ***	0.23 ±0.001 ***	0.33 ±0.05 ***	0.43 ±0.02 ***	0.43 ±0.08 ***

1.94±0.41), although this increase is not statically significant. Conversely, analysis of EYA3 expression using reticulocytes shows that this gene is downregulated in SCD patients (0.3±0.06) compared to the control group (1.18±0.17, p<0.05, n=8). However, no statistical difference for SCD patients on HU therapy was observed (data not shown). *Conclusions.* These results show modifications in the expression pattern of globins genes and HbF as well as modifications of apoptosis levels in K562 EYA3 knockdowned culture cells. Additionally, EYA3 gene expression in primary culture seems to be increased during erythroid differentiation and also modified in reticulocytes of SCD patients, suggesting that EYA3 may participate in the erythroid differentiation process.

0416

RELEVANCE OF SERUM LEVELS AND TISSUE EXPRESSIONS OF FIBROSIS MARKERS IN THE EVOLUTION AND REVERSAL OF LIVER FIBROSIS IN PATIENTS WITH BETA THALASSEMIA MAJOR TREATED WITH DEFERASIROX

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Background. Iron overload in beta thalassaemia major (TM) enhances oxidative stress within the liver which is associated with the development of fibrosis that may progress to cirrhosis. The interactions between various resident hepatic cell populations and immune cells that lead to the establishment of fibrosis are complex and little information is available with respect to the possible alterations in serum levels and tissue expressions of fibrosis markers and contribution to the liver fibrosis in TM. *Methods.* This study was run in Turkey following the completion of the ICL670A0107E extension. TM patients who completed core phase and continued with deferasirox or switched to deferasirox during 4 year extension were included after consenting for participation in this locally run study. Liver biopsy specimens and simultaneously collected frozen serum samples of those patients was used. Serum concentrations of tenascin, collagen IV, tissue inhibitors of metalloproteinase (TIMP-1), and matrix metalloproteinase (MMP-1) levels were measured with enzyme-linked immunoassay kits. Liver iron concentrations (LIC) were measured by AAS. Fibrosis stage and inflammation grade were assessed in a blinded fashion by a single pathologist according to the Ishak (score 0-6, grade 0-18) system and iron stained and staged according to the Sciott (0-4x3). Paraffin sections from formalin fixed material were immunostained with antibodies against alfa-SMA, Collagen-4, TIMP-1 and MMP-1. The intensity of immunostaining in entire representative slides was semiquantitatively graded from 0 to 4. *Results.* A total of 198 liver biopsy specimens and serum samples from 66 patients who received deferasirox (n=41) since the core study and switched to deferasirox (n=25) after 1 year and completed 4 year extension study were included. LIC was correlated with liver fibrosis and inflammation (p=0.000). LIC significantly decreased from baseline (21.2±1.6) at 1sty (14.6±1.2) and the EOS (9.4 ±1.0) (p<0.001) while mean fibrosis score did not differ significantly. However, fibrosis scores decreased significantly by decrease in LIC (p=0.018). Decrease in hepatocyte iron rather than kuppfer or portal iron was related with decrease in fibrosis during Deferasirox therapy up to 5 years (p=0.012). Although, there was a highly significant correlation between LIC and iron deposition in hepatocytes, kuppfer cells and portal field (p=0.000), fibrosis was only correlated with hepatocytes and portal iron. Serum collagen4 and TIMP-1 were correlated with LIC (p=0.0002, p=0.004) but not with the stage of fibrosis. However, portal expression of collagen4 and TIMP-1 were correlated with fibrosis stage (p=0.009, p=0.02). Portal aSMA showed a significant correlation with fibrosis (p=0.001) and changes in portal aSMA was correlated with changes of fibrosis from baseline at 5 years (p=0.04). During treatment all serum fibrosis markers decreased significantly compared to baseline (p=0.000). However, only decrease in TIMP-1 was significantly correlated with decrease in portal iron at tissue level (p=0.04). *Conclusions.* This study first revealed the role of HSC activation leading TIMP-1 upregulation and consequently collagen deposition in the progress of fibrosis in patients with TM. Deferasirox chelation can effectively reduce liver iron burden and help to resolution of fibrosis by blocking HSC activation.

0417

TIMP-1 BALANCE EFFECT ON ELEVATED MMP-9 IN PATIENTS WITH THALASSEMIA MAJOR AND OSTEOPOROSIS

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Background. Tissue inhibitors of metalloproteinases (TIMPs) are endogenous inhibitors of metalloproteinase activities that primarily modulate matrix metalloproteinase (MMP) activity and suppress extracellular matrix turnover. TIMP-1 binds to the hemopexin domain of proMMP-9, but not other pro-MMPs. TIMP-1 also binds to the catalytic domain of all soluble activated MMPs and inhibits their enzymatic activity. Osteoporosis represents a major cause of morbidity in patients with thalassemia major (TM). *Aims.* The aim of the study was to evaluate the serum levels of TIMP-1 and MMP-9 in patients with TM-related osteoporosis and explore possible correlations with bone remodeling and bone mineral density (BMD). *Methods.* Twenty-two patients with thalassemia-induced osteoporosis (10M/12F; median age 42 years) were studied. Patients were blindly randomized to receive zoledronic acid at a dose of 4 mg, iv, in 15 min infusion, every 6 months (n=16) or to receive placebo every 6 months (n=6) for a period of one year. All patients were under oral calcium (500 mg) administration during the treatment period. TIMP-1 and MMP-9 were measured at baseline and after 12 months of therapy using ELISA methodology (Oncogene Science/ Siemens HealthCare Diagnostics, Cambridge, MA, USA and R&D Systems, Minneapolis, MN, USA, respectively) along with a series of serum bone remodeling indices: i) bone resorption markers [C-telopeptide of type-I collagen (CTX), tartrate-resistant acid phosphatase isoform-5b (TRACP-5b)], ii) bone formation markers [bone-alkaline phosphatase (bALP), osteocalcin, and C-terminal propeptide of collagen type-I (CICP)], and iii) osteoclast regulators [receptor activator of nuclear factor-kappa B ligand (RANKL), osteoprotegerin (OPG), and osteopontin]. BMD of the lumbar spine (L1-L4), femoral neck (FN) and wrist (W) was determined using DXA, before and 12 months after treatment. The above bone markers were also evaluated in 30, age- and gender-matched, healthy controls. *Results.* At baseline, six patients (3M/3F; 27%) had elevated values of TIMP-1 (upper normal limit 459 ng/ml for males and 374 ng/ml for women). Furthermore, TM patients had increased values of MMP-9 (median and range: 628 ng/ml, 289-911 ng/ml versus 312 ng/ml, 113-514 ng/ml; p<0.001), CTX (p<0.001), bALP (p<0.001), CICP (p=0.003), sRANKL (p=0.02), and OPG (p=0.001) compared with controls. TIMP-1 serum levels correlated with OPG (r=0.461, p=0.031), sRANKL/OPG ratio (r=-0.483, p=0.023), bALP (r=0.490, p=0.021) and OPN levels (r=0.533, p=0.013). Patients with elevated values of TIMP-1 had increased L1-L4 z-score (median: -1.65, range: -2.5 to -1.5) compared to patients with normal values (median: -2.65, range: -4.4 to -1.2; p=0.042). MMP-9 correlated with CTX (r=0.476, p=0.03) and TIMP-1 (r=-0.445, p=0.041). Administration of zoledronic acid did not alter serum levels of TIMP-1 or MMP-9, although these patients experienced an increase of BMD in all measured sites. *Summary/Conclusions.* TIMP-1 serum levels are elevated in approximately 25% of patients with TM-related osteoporosis and associated with BMD. This increase may reflect a balance effect on the increased MMP-9 activity present in this condition. Larger studies will reveal if high TIMP-1 protects TM-induced bone loss and reveal MMP-9 as possible target for development of novel drugs against TM-induced osteoporosis.

0418

ASSOCIATION STUDY BETWEEN SNPS IN GENES RELATED TO ADENOSINE SIGNALING AND DISTINCT CLINICAL MANIFESTATIONS IN SICKLE CELL DISEASE

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Background. Recent studies have demonstrated the role of high adenosine levels in priapism episodes in a mouse model of sickle cell disease (SCD). Interestingly, in addition to priapism, altered adenosine signaling (through four distinct receptors; A1, A2a, A2b and A3) has been implicated in several physiopathological processes that are asso-

ciated to distinct clinical features observed in patients with SCD. Moreover, adenosine levels are partially controlled by its conversion into inosine, carried by the enzyme Adenosine Deaminase (ADA). Although SCD is characterized by a single base substitution resulting in a mutated β-globin (HbS), a broad spectrum of clinical manifestations and severity are observed in the patients. This variability reflects the distinct genetic background of these patients. *Aims.* To evaluate the potential association of selected single nucleotide polimorphisms (SNPs), present in adenosine receptors and ADA genes, with clinical manifestations observed in SCD patients. *Methods.* DNA was extracted from peripheral blood samples collected from a total of 230 patients with Sickle Cell Disease (SS and Sβ), being assisted in the Regional Blood Center (HCFMRP-USP). Samples were obtained after informed consent, following a protocol approved by the local ethics committee. Three SNPs were evaluated. The SNPs in the ADORA1 (C/T aleles, rs1685103) and ADORA3 (G/T alleles, rs35511654) genes were evaluated by Real Time PCR using TaqMan probes and primer, the SNP 968 G>T and 1007 C>T in the ADORA2B gene were identified and evaluated by sequencing, the SNP ADA*2 (Asp8Asn; G22A, GeneBank M13792) in the ADA gene was evaluated by restriction fragment length polymorphism (RFLP). The main clinical manifestations evaluated, and the age cutoff for inclusion in the study, were: were acute chest syndrome (ACS), pulmonary hypertension (PH; >10 years old), priapism (>15 years old male patients), bone disorders such as osteopenia and osteoporosis (BD; >13 years old), and stroke (>5 years old). The software GENEPOP 3.4 was used to test for Hardy-Weinberg equilibrium and a Fisher exact test was carried to identify potential associations between polymorphisms and the clinical manifestations considered. Haplotype studies and corresponding statistical analysis (Tukey test) were carried using the softwares Arlequim (v3.1) and SAS (v9.13), respectively. Results. Significant differences were found for the 1007 C>T SNP of the ADORA2B gene, with patients in the group with acute chest syndrome showing an increased frequency of the T allele (p=0.032). For this same SNP a significant higher frequencies where of genotypes C/C and C/T were found among patients with BD (p=0.013). For the SNP 968 G>T, we found a significant higher frequency of patients with the G/G genotype among patients with BD (p=0.0043). Haplotype studies, revealed a significant association with the manifestation of distinct clinical features, including, priapism, stroke and ACS. Summary. Our results indicate that distinct genes related to adenosine signaling may play a role as modifiers of severity in sickle cell disease.

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0419

ASSESSMENT OF OXIDATIVE STRESS IN PATIENTS WITH SICKLE CELL DISEASE

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Background. Continuous Reactive Oxygen Species (ROS) production in individuals with Sickle Cell Disease (SCD) may alter their overall redox status and cause tissue damage. The aim of this study was to evaluate oxidative stress in patients with SCD. *Patients and Methods.* A total of 40 patients with SCD and 25 apparently healthy volunteers (control group) were enrolled in the study. Components of glutathione system (GSHtotal GSSG and GSHreduced, vitamins A, C, and E, and malondialdehyde were determined with reverse-phase HPLC, non-transferrin bound iron (NTBI) was assessed with atomic absorption spectroscopy using graphite furnace, superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) activities were determined spectrophotometrically in red cell lysates, nitric oxide (NO) was detected colorimetrically, while FORT (free oxygen radicals test) and FORD (free oxygen radicals defense) using colorimetric assays. *Results.* NTBI values as well as the lipid peroxidation marker MDA were significantly higher in patients with SCD compared to controls, ($p<0.001$ and $p<0.001$, respectively. Impairment of the glutathione system indicated by reduced GSHtotal levels in patients with SCD compared to controls (748.9 ± 135.9 vs 1125 ± 180.0 μmol/L, respectively, $p<0.001$). Similarly, GSHreduced levels were significantly decreased in patients with SCD compared to controls (517.7 ± 133.3 vs 810.0 ± 245.0 μmol/L, respectively, $p<0.001$), while GSSG levels were lower in patients with SCD compared to controls ($p<0.001$). In terms of overall glutathione system function expressed as percentage of GSSG/GSHtotal, this ratio is significantly increased in patients with SCD compared to controls ($p<0.001$). Furthermore, patients with SCD have significantly higher total NO levels compared to controls, ($p<0.001$). FORT levels were significantly higher patients with SCD compared to controls, ($p<0.001$), while FORD levels were significantly lower in the patients with SCD compared to controls, ($p<0.02$). Red cells antioxidant enzymes GPx and SOD, were significantly higher in patients with SCD compared to controls, ($p<0.001$ and $p<0.05$, respectively), while no significance different was observed for GR ($p>0.6$). The above reported observations consisted with the significantly lower plasma concentrations of the antioxidant Vitamins A, E and C found in patients with SCD compared to controls, ($p<0.01$, $p<0.01$ and $p<0.001$, respectively). *Conclusion.* Since oxidative stress seems to play a major role in SCD, the development of novel therapies founded on free radical biology appears imperative. Hemolysis and oxidative stress are only partially reduced by blood apheresis and hydroxyurea therapy, and in our SCD group seems to play no significant role. Newer therapeutic agents that can target oxidative stress, such as NADPH oxidase inhibitors, NO based therapeutics, anti-inflammatory agents and antioxidants supplementation may constitute valuable means for improved manifestations and outcome.

0420

TUMOR NECROSIS FACTOR POLYMORPHISMS IN PATIENTS WITH AUTOIMMUNE HEMOLYTIC ANAEMIA

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Background. Tumor necrosis factor (TNF) is a multifactorial cytokine that is secreted by monocytes (TNF-α) or lymphocytes (TNF-β). TNF cytokines have numerous immunoregulatory effects as well as potent proinflammatory effects, and they are implicated in many inflammatory and autoimmune diseases, like inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, immune thrombocytopenia, etc. Autoimmune hemolytic anemia (AIHA) is a second most common autoimmune blood disorders. AIHA may occur as primary (idiopathic) or secondary to other lymphoproliferative or immune disease. The etiology of AIHA remains unclear, but both genetic and environmental factors are thought to play role in the development of the disease. *Aims.* The aim of our study was to investigate a possible association of some single nucleotide polymorphisms (SNP) in genes for tumor necrosis factor beta (TNF-β +252 G/A) and tumor necrosis factor alpha (TNF-α -308 G/A) with autoimmune hemolytic anemia. *Methods.* We have analyzed 60 adult patients with AIHA; 30 patients with idiopathic AIHA and 30 patients with secondary AIHA and chronic lymphocytic leukemia (CLL). Controls were 120 healthy individuals and 100 CLL patients without AIHA. DNA was isolated from peripheral blood mononuclear cells with standard phenol-chloroform extraction. Genotyping was performed by using PCR and RFLP methods. The distribution of genotypes and allele frequencies were compared between patients and controls using a chi-squared test or Fisher's exact test. *Results.* Our results demonstrated that the G allele of the TNF-β (+252 G/A) was significantly more frequent among the patients with AIHA (n=60; G/G=14, A/G=23, A/A=23) compared to controls (n=120 G/G=16, A/G=35, A/A=69), p=0.043. This difference was even more significant when only CLL patients were compared between them (CLL with AIHA n=30; G/G=8, A/G=12, A/A=10 versus CLL without AIHA n=100; G/G=10, A/G=31, A/A=59), p=0.018. We found that the A allele of the TNF-α (-308 G/A) was also more common in patients with AIHA (n=60; G/G=33, A/G=22, A/A=5) than in controls (n=120; G/G=95, A/G=23, A/A=2), p=0.002. This difference was most striking when only CLL patients were compared between them (CLL with AIHA n=30; G/G=15, A/G=12, A/A=3 versus CLL without AIHA n=100; G/G=82, A/G=17, A/A=1), p=0.0006. There was no significant difference in genotype distributions between CLL patients without AIHA and healthy control individuals for both genes

(p=0.74 for TNF-β and p=0.83 for TNF-α). *Conclusion.* The obtained data indicate that the G allele of TNF-β (+252) and A allele of TNF-α (-308 G/A), which are both associated with increased TNF production and secretion, are more frequent in patients with AIHA than in controls, especially in the group of CLL patients with AIHA. These results implicate that these two polymorphisms may predispose to the development of autoimmune hemolytic anemia, especially in the group of patients with CLL.

0421

ERYTHROPOIESIS DISTURBANCE IN HEREDITARY SPHEROCYTOSIS CLINICAL OUTCOME

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Hereditary Spherocytosis (HS) is the most common non-immune hemolytic anemia in individuals of northern European ancestry (1/2000). In a previous study, we showed that high levels of erythropoietin (EPO) were not able to induce a proportional reticulocyte production in moderate HS patients that was observed in mild HS (Rocha S, Br J Haematol, 2005, 131, 534-542). In the present study, our aim was to evaluate the relationship between that erythropoietic disturbance and inflammation, in the clinical outcome of HS (mild, moderate and severe HS). We studied 82 unsplenectomized HS Portuguese patients, presenting mild (n=49), moderate (n=27) and severe (n= 6) HS. We evaluated plasma levels of EPO, soluble transferrin receptor (sTfR), iron, transferrin, ferritin, folic acid, vitamin B12, C-reactive protein (CRP), granulocyte-monocyte colony stimulating factor (GM-CSF), tumor necrosis factor (TNF)-α, interferon (IFN)-γ, elastase and lactoferrin; determined reticulocyte count and the total and differential leukocyte counts, and calculated the reticulocyte production index (RPI). Mild HS patients showed a rise in EPO, sTfR, reticulocytes and RPI, reflecting a compensated hemolysis, and positive statistical significant correlations between EPO and sTfR, reticulocytes and RPI, were observed. In moderate and severe HS, in spite of significantly higher EPO, sTfR, reticulocytes and RPI than mild HS, these correlations were not observed. For all patients iron stores, folic acid and vitamin B12 were within normal values or were slightly higher. HS patients presented a low grade inflammation that was particularly enhanced in severe HS, as shown by the highest median levels of GM-CSF, CRP, TNF-α, IFN-γ and elastase and the lowest levels of iron and lactoferrin. Our data show HS as a disease linked to enhanced erythropoiesis that in the more severe forms (moderate and severe) is disturbed. Inflammation may contribute, at least in part, to that disturbance, especially in the severe cases of HS. This study was supported by a PhD grant (SFRH/BD/22442/2005) attributed to S.Rocha by FCT and FSE.

0422

MLPA ANALYSIS OF BETA GLOBIN GENE CLUSTER: DETECTION OF 41 ALTERATIONS (INCLUDING FIVE NOVEL DELETIONS) IN SPANISH POPULATION

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Background. Genetic alterations in the beta globin cluster can lead to a variety of diseases such as High Persistence of Fetal Hemoglobin (HPFH), deltabeta thalassemia (β thal), beta thalassemia (β thal), and more. Sequencing the globin genes is the more suitable method for the identification of point mutations or small deletions. However, large deletions are common cause of disease, due to the elimination of entire genes or regulatory elements dispersed in the beta cluster. There are PCR methods designed to detect nothing but a few of this known deletions. Thus, those screening techniques can not be used in the search

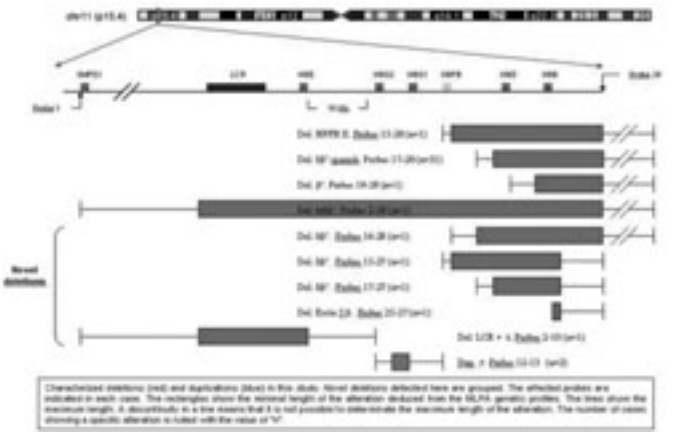

Figure 1.

of novel alterations and the molecular diagnosis becomes difficult in a considerable proportion of the cases. Recently, the development of the Multiplex Ligation-dependent Probe Amplification (MLPA) technique has solved this problem, and now it is possible a quick identification of any copy number variation in the beta globin cluster. *Aim.* This is a descriptive work showing the results of the analysis by MLPA method of cases suspected to carry an alteration in the beta globin cluster. *Methods.* This study included 66 patients with a phenotype of HPFH, βthal or β thal. The existence of point mutations or small deletions/duplications in the β gene was ruled out by sequencing. The MLPA technique is based on the quantitative 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. A deletion is detected when a reduction of the amount of amplified product of several consecutive probes is observed in the genome. Here we used a commercial kit (MLPA kit P102-B1 HBB, MRC-Holland) that contains 28 probes designed to detect copy number changes in the beta cluster, from LCR to 10Kb downstream of β globin gene, spanning more than 80Kb. *Results.* Sixty-three percent of the patients are carriers for an alteration that can satisfactorily explain theirs clinical manifestations. A total of 41 chromosomes contain a deletion or duplication in the βcluster. Ten different genetic alterations have been detected in this study; five of them has not been previously described in scientific publications and are novel. Twenty-five patients have shown a normal genetic profile. The alteration most frequently found is the (β)° Spanish deletion. A total of 31 patients are heterozygote carriers for this deletion. Duplication in the region of gamma genes has been found in two cases. All the other alterations detected here have been found in only one case (detailed view in figure 1). Finally, we are currently designing long range PCRs for the identification of the breakpoints of the novel deletions mentioned above. *Conclusions.* Almost all the deletions detected have been found in only one case. The results support the idea of the existence of a high molecular heterogeneity for the alterations in the β cluster. Within this scenario, MLPA analysis means an improvement of the molecular diagnosis and the genetic counseling applied to this group of diseases.

Stem cell transplantation - Clinical 1

0423

OUTCOME OF SECOND ALLOGENEIC HCT FOLLOWING RELAPSE OF HAEMATOLOGICAL MALIGNANCIES AFTER FIRST ALLOGENEIC HCT: EXPERIENCE FROM THE GETH (GRUPO ESPAÑOL DE TRASPLANTE HEMATOPOYETICO)

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Background. Disease relapse is the most frequent cause of treatment failure following allogeneic haematopoietic cell transplantation (allo-HCT), and carries a very poor prognosis. A second allo-HCT may be the only curative option for the majority of these patients. Patient and transplant factors that may associate with the outcome of a second allo-HCT are required for clinical-decision making in such high-risk patients. *Aims and Methods.* We performed a retrospective analysis of adult patients receiving a second allo-HCT for disease relapse after a prior allo-HCT reported by GETH centres. Our aim is to analyze our experience in this setting, and to identify factors associated with patient outcome. *Results.* We present data on 66 patients who underwent a second allo-HCT for disease relapse in Spain between 1990 and 2010 (median year 2003), with a median follow-up for survivors of 63 months (3-224): median age 38 years (range 14-69); 35 male and 31 female; initial diagnosis AML 25 patients, ALL 13 patients, MDS/MPD 11 patients, CML 11 patients, lymphoprolipherative disorder 6 patients; 23 (35%) cases of myeloablative conditioning. Donors were related in 55 cases. Also, 11 second allo-HCT were performed with new donors different from the donors in the previous allo-HCT. Only 4 cases had T-cell depletion. Median time from the first to the second allo-HCT was 21 months (1-170). The cumulative incidence of non-relapse mortality was 26.2%. The median overall survival (OS) was 315 days (95% CI, 176 - 454), with an OS at 1, 3 and 5 years of 38%, 31% and 31%, respectively. OS survival was significantly better in patients who underwent second allo-HCT with low disease burden (complete response, good partial response, or chronic phase; 61% at 1 year, 51% at 3 years) than in patients in active relapse or progression (22% at 1 year, 17% at 3 years; p<0.001). Patients who relapsed early after the first allo-HCT and required a second allo-HCT <1 year after first HCT also had a poorer outcome (OS 17% at 1 year, 9% at 3 years) than those having the second allo-HCT 1 year or longer after the first one (50% at 1 year, 43 at 3 years; p<0.001). Time to second allo-HCT and disease status also associated with the probability of progression free survival in this series (p<0.001 and p=0.002, respectively). Type of donor (related versus unrelated and new donor versus same donor) and type of conditioning (myeloablative versus non-myeloablative) had no statistically significant association with patient outcome. *Conclusions.* The experience from GETH reported here shows that over 30% of patients who relapse after an allo-HCT can achieve long-term survival of 5 or more years following a second allo-HCT. Also, our data suggest that disease status at HCT and time to relapse between first and second HCT are two significant prognostic factors for survival outcome. The indication of a second allo-HCT should be thoroughly discussed for individual cases with early relapse and active disease. Donor type does not appear to influence the outcome.

0424

PRIOR MALIGNANT COMORBIDITY: RISK FACTOR FOR ALLOGENEIC STEM CELL TRANSPLANTATION?

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Background. Prior malignancies are considered important comorbidities for patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT). In the widely used Charlson's Comorbidity Score and Sorror's Hematopoietic Cell Transplantation Comorbidity Index, prior malignancies are being attributed high risk rankings. *Aims.* We evaluated the impact of prior malignant comorbidities on the outcome of allogeneic HSCT for hematologic diseases in a large, independent patient cohort. *Patients.* From 1995 to 2010, 800 patients received allogeneic HSCTs at our institution. 283 patients had de novo acute myeloid leukemia (AML), 156 myelodysplasia or secondary AML, 114 acute lymphoblastic leukemia, 111 myeloproliferative disorders, 102 lymphomas, 2 solid tumors, and 32 nonmalignant hematological diseases. 340 were females and 460 males, with a median age of 47 years (range: 16 to 72). *Results.* 57 of 800 patients (7%) had suffered from other malignancies before their current hematologic disease. Prior malignancies included breast cancer (n = 19), lymphomas (n = 15), cancers of the uro-genital tract (13), gastro-intestinal cancers (n = 5), malignant melanomas (n= 3), thyroid cancers (n = 2), among others. 2 of 57 patients had two different malignant comorbidities before allogeneic HSCT. Prior malignancies had required systemic (n = 44) or only local treatment (n = 15, including 4 carcinomas-in-situ). At the time of allogeneic HSCT, prior malignancies were in complete (n = 57) or partial (n = 2) remission with a median follow-up of 6.2 years (range, 0.4 to 39.7). 4 of 57 patients (7%) relapsed with their prior malignancies after a median of 11.4 years (range, 3.7 to 29): two patients with late metastases after adjuvant therapy of breast cancer are still receiving hormonal treatment, whereas two patients with locally controlled carcinoma-in-situ of the larynx and malignant melanoma, respectively, died of cancer recurrence. Compared with the entire cohort, patients with prior malignancies were predominantly female, older, and more likely to have received reduced intensity conditioning, but otherwise similar regarding diagnoses, risk status, and remission, as well as donor, gender, and cytomegalovirus match. With 2 years of median follow-up (range: 0 to 12.4), their overall (OS) and disease-free survival (DFS) as well as non-relapse mortality were not different from those of the entire cohort. In multivariate analysis, prior malignancies had no prognostic impact; instead, significant factors for DFS were myeloablative conditioning (p = 0.010; Hazard Ratio, HR, 1.29), hematologic diagnosis (p = 0.002, HR 1.39), and remission status before HSCT (p < 0.001; HR 2.13), whereas for OS only hematologic diagnosis (p < 0.001; HR 1.61) and remission status before HSCT (p < 0.001; HR 2.10) remained significant. *Conclusions.* Patients with prior malignant comorbidities represent a small, but relevant subgroup of allogeneic HSCT candidates, whose transplant outcomes do not appear to be significantly impaired. To refine existing comorbidity indices in that respect, much larger databases are likely required.

0425

INCREASED INCIDENCE OF ACUTE GRAFT-VERSUS-HOST DISEASE IN CHILDREN WITH THALASSEMIA FOLLOWING HLA-IDENTICAL SIBLING BONE MARROW TRANSPLANTS WITH HIGH CD3+ AND CD34+ CELL DOSES

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Background. It is generally accepted that children are at less risk for GVHD than adults, however its incidence is still significant especially when using alternative donors. Few studies have examined the impact of T cells in the graft on acute GVHD (aGVHD) after HLA-identical sibling BMT in adults with controversial results. Pediatric transplant recipients differ from adults regarding cell dose in the harvested bone marrow: the donor who is likely close in age, usually younger, tend to have a rich harvest leading to the infusion of a higher number of nucle-

ated cells. Therefore, we hypothesized that the impact of graft composition on acute GVHD in children undergoing BMT could be different and a threshold dose of cells that affects GVHD can be defined. This is the first study prospectively evaluating the impact of the CD3+ and CD34+ cell doses infused on GVHD in children undergoing HLA-identical sibling BMT for nonmalignant diseases to date. *Aims.* Assessing the impact of graft composition on aGVHD in children underwent HLA-identical BMT from sibling donors. *Methods.* Between 2004 and 2010, 92 patients with median age of 8 years (range, 1.6-17) were given a bone marrow graft for thalassemia. The preparatory regimens for class 1 and class 2 patients (n=49) consisted of BUCY200 ± thiotepa, and for class 3 patients (n=43) of BUCY160 ± thiotepa (preceded by cytoreduction/immunosuppression with hydroxyurea, azathioprine and fludarabine). From June 2006 onwards, all patients were given targeted i.v.Busilvex (Pierre Fabre Medicament, France). As GVHD prophylaxis patients received CSA, a short course of MTX and methylprednisolone. *Results.* The median of 4.6x 108/kg (range 1.3-10.8) total nucleated cells (TNC), 7.2x106/kg (range 0.8-35) CD34+ cells, and 55.3x106/kg (range 3.8-208) of CD3+ cells were infused. There was a weak correlation (Spearman's test) between CD34+ and CD3+ cell doses (ρ=.35, p=.001). Cumulative incidence of grade 2-4 and 3-4 aGVHD was 35% (95% CI: 25-44) and 9% (95% CI 4-16), respectively. In univariate analysis only CD3+ and CD34+ cell doses above or equal to the median were significantly associated with grade 2-4 aGVHD (49% vs 20%; p=.005 and 46% vs 23%; p=.021, respectively). Multivariate analysis confirmed that high CD3+ (HR, 4.6; p=.010) and CD34+ (HR, 4.3; p=.011) cell doses were major risk factors for grade 2-4 aGVHD. We further examined the effect of CD3+ and CD34+ cell doses on aGVHD using quartile cutoff points and found a minimum threshold for CD3+ (4-38x106/kg) and CD34+ (0.8-4x 106/kg) cells above which the incidence of grade 2-4 aGVHD is significantly increased (8% to 38%-54% and 5% to 41%, respectively). Cumulative incidence of extensive cGVHD was 10% (95% CI 5-18). *Conclusion.* This study for the first time demonstrated that high doses of CD3+ and CD34+ cells within the graft are risk factors for grade 2-4 aGVHD in children with thalassemia undergoing HLA-identical BMT, and defined a minimum threshold dose for these cells above which the incidence of aGVHD significantly increases. These data indicate that patients receiving CD3+ and CD34+ cell doses beyond minimum threshold should be given additional immunosuppressive agents.

0426

THE RISK FACTORS FOR EBV VIREMIA EARLY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Objective. To identify the risk factors for Epstein-Barr Virus (EBV) viremia early after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Patients and *Methods.* Between January 2007 and January 2009, total 277 patients after allo-HSCT (haploidentical 116, unrelated 75, matched sibling 86) with continuous monitoring plasma EBV-DNA were studied. Conditioning regimens were BUCY/Flu or CY/Flu TBI mainly. ATG was added in both haploidentical and unrelated donor transplants. Cyclosporine, methotrexate and mycophenolate mofetil were employed for GVHD prophylaxis. Serum EBV status of donor and recipient pre-HSCT was determined by ELISA. The levels of plasma EBV-DNA were monitored with real-time quantitative polymerase chain reaction (RQ-PCR) 1 to 2 times weekly in the first 3 months after allo-HSCT. EBV viremia was diagnosed when plasma EBV-DNA was more than 5 x 102 copies/ml but without symptoms. Acyclovir (10mg/kg or 500mg/m2 iv q8h) was administrated for pre-emptive therapy and immnuo-suppressants were decreased if possible. *Results.* Total 33 patients (11.9%) developed EBV viremia with the median time at day 44 (day 19 to day 84). The incidences of EBV viremia were 15.5%, 20.0%, 0% in haploidentical, unrelated, matched sibling transplant, respectively. There was no significant difference on the incidences of EBV viremia between haploidentical and unrelated transplants (p=0.09), but much less EBV viremia was seen in matched sibling transplant (p=0.001). Twenty of 33 patients with EBV viremia (60.6%) had complete response to the pre-emptive therapy with acyclovir. The median time to reach plasma EBV-DNA negative was 11 days (4 to 56 days). The median duration of pre-emptive therapy was 21 days (14 to 60 days). Both univariate and multivariate analysis indicated that haploidentical and unrelated transplants, acute GVHD were the risk factors for EBV viremia. Two-year overall survival in the patients with EBV viremia was significantly lower than that without EBV viremia (54.2% vs. 72.1%, p=0.006). *Conclusions.* Our large clinical study has demonstrated that it is not necessary to monitor plasma EBV-DNA in matched sibling transplant. The incidences of EBV viremia are similar between haploidentical and unrelated transplants. Majority of patients with EBV viremia benefits from the pre-emptive therapy with acyclovir. Haploidentical and unrelated transplants, acute GVHD are the risk factors for EBV viremia early after allo-HSCT which has negative impact on survival.

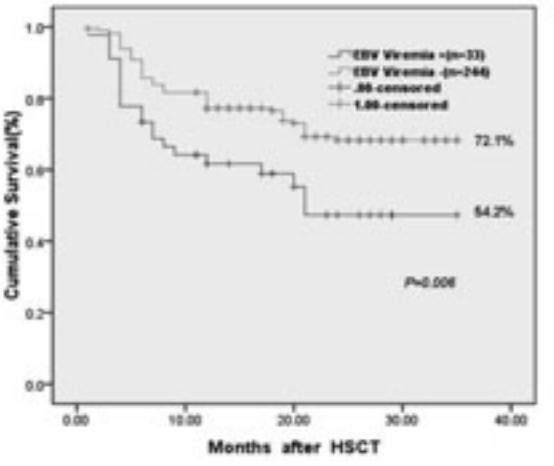

Figure 1.

0427

PROGNOSTIC RELEVANCE OF MINIMAL RESIDUAL DISEASE (MRD) PRIOR TO AUTOLOGOUS TRANSPLANTATION ON LONG-TERM FOLLOW-UP IN ACUTE MYELOID LEUKEMIA (AML). ON BEHALF OF ROME TRANSPLANT NETWORK (RTN)

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Background. autologous stem cell transplantation (ASCT) is currently considered a therapeutic option improving outcome of patients with acute myeloid leukemia (AML). Up to now, it is controversial in which risk patient subgroup it may play a role as post-consolidation therapy. Therefore, risk assessment should be integrated with other parameters to point out this issue. *Aim.* in order to evaluate the prognostic significance of MRD prior to ASCT, its value detected by flow-cytometry (FMC) has been retrospectively analyzed in AML patients followed on a long-term follow-up.. *Patients and Methods.* 89 AML pts with median age of 50 years (range 20-75) undergoing ASCT (June 1995-October 2009) in four Institutions participating to the Rome Transplant Network (RTN), a metropolitan transplant network, have been analyzed. The cytogenetic risk groups were distributed as follows: 46 (63%) intermediate, 12 favourable, 15 adverse; 7 (12%) pts were Flt3-ITD positive. Patients received ASCT in first CR after standard induction- consolidation chemotherapy according to AML EORTC-GIMEMA trials. Bone marrow MRD by FMC was determined prior to ASCT using a cut-off level of 3.5x10-4 leukemic cells. MRD was available in 58 pts, of whom 22 were MRD negative and 36 MRD positive. Fifty-seven out of 89 pts received Bu-Cy conditioning regimen, the others BEAM/BEAM-like regimens. Median CD34+ collected by aphaeresis was 4.57 x106 /Kg (range 0.5-76) and median CD 34+ infused was 4.43 x106 /Kg (range 0.5-26.76). *Results.* at 12 years, overall survival (OS) risk of relapse (RR) and disease free survival (DFS) are, respectively, 54%, 32% and 53% for MDR negative pts, which are significantly better than 20%, 81% and 13% respectively calculated for MRD positive pts. The p-value of DSF (p=0.028), RR (p=0.008), OS (p=0.084) is statistically significant (see Figure 1). The risk assessment at diagnosis has not been considered in these curves. *Comments.* MRD assessment detected by FMC before ASCT has a high prognostic value in term of DFS, RR and OS for pts with CR1 AML and should be considered in the choice

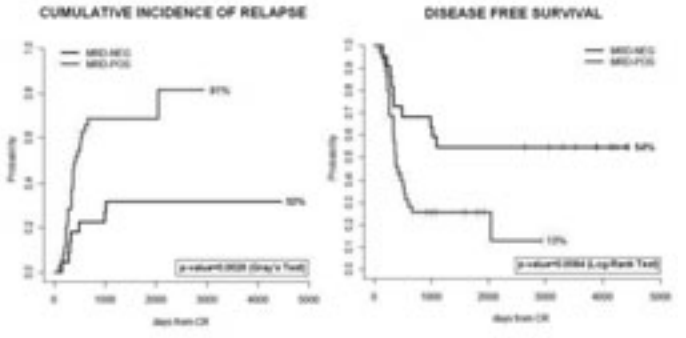

Figure 1.

of the most appropriate transplant approach after consolidation therapy.

0428

A PROSPECTIVE RANDOMIZED TRIAL COMPARING PHLEBOTOMY AND DEFERASIROX FOR THE TREATMENT OF IRON OVERLOAD IN PAEDIATRIC THALASSEMIA MAJOR PATIENTS CURED BY STEM CELL TRANSPLANTATION: 6-MONTH FOLLOW-UP

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Background. Patients with β-thalassaemia major who have undergone curative haematopoietic stem cell transplantation (SCT) are at increased risk of iron overload because of previous transfusion therapy. There are limited data on post-SCT iron removal achieved with phlebotomy or iron chelators. *Aims.* To compare efficacy, safety and convenience of phlebotomy versus deferasirox for the treatment of iron overload in children with thalassaemia major after allogeneic SCT. *Methods.* Study LB03T is an ongoing prospective, 1-year, randomized trial in paediatric patients with thalassaemia major who have undergone allogeneic SCT. Chelation-naïve, hepatitis B- and C-negative patients aged 2-<18 years with iron overload (serum ferritin ≥500ng/mL on ≥2 monthly occasions, and liver iron concentration [LIC] >3mg Fe/g dry weight [dw] determined by MRI) were eligible. Patients were randomized to phlebotomy (6mL/kg blood/2 weeks) or deferasirox (10mg/kg/day starting dose; 5mg/kg/day adjustments up to a dose of 20mg/kg/day according to 3-monthly ferritin trends were allowed). Compliance with phlebotomy was determined by the ratio of phlebotomy performed over planned phlebotomy; compliance with deferasirox was determined by missed tablet counting. *Results.* 28 patients were enrolled and randomized to phlebotomy or deferasirox. Two patients randomized to phlebotomy refused treatment. Baseline parameters for 26 patients were comparable between treatment groups (Table). Mean patient age: 12.6 years; mean follow-up: 6.8 months. For patients with serum ferritin <1000ng/mL at baseline, the efficacy of deferasirox at 10mg/kg/day and phlebotomy was similar (Table); for patients with serum ferritin ≥1000ng/mL, the absolute median change was non-significantly greater with phlebotomy. For TIBC, the increase with deferasirox was significantly greater than with phlebotomy in all groups. The absolute mean decrease in haemoglobin was -0.12 and -0.59g/dL (P=0.027) for the deferasirox and phlebotomy groups, respectively. Two patients reported treatment-related adverse events (AEs) with deferasirox (skin rash [n=1] and mild nausea/flatulence [n=1]) and four reported difficulty with phlebotomy (difficult veins [n=3], distress [n=1]). Compliance was excellent for 11 (91.7%) and 12 (85.7%); good for 1 (8.3%) and 1 (7.1%) and poor in 0 and 1 (7.1%) patients in the deferasirox and phlebotomy groups, respectively. Parents of 13/14 children randomized to phlebotomy voiced their wish for their children to receive deferasirox because of pain, risk of anaemia and longer/more frequent hospital visits (missing school days for the child and work days for parents) associated with phlebotomy. Parents of 1/14 were content with phlebotomy because of concerns over possible AEs with deferasirox. *Summary/Conclusions.* For post-haematopoietic SCT patients with serum ferritin <1000ng/mL, phlebotomy and deferasirox (10 mg/kg/day) were equally effective in ferritin reduction; in patients with serum ferritin ≥1000ng/mL, phlebotomy reduced serum ferritin to a greater extent (difference did not reach significance between treatment groups). This highlights that a starting dose of 10mg/kg/day may not be sufficient to reduce iron loading in such patients, and that earlier and appropriate dose adjustments should be carried out. Deferasirox increased TIBC to a more statistically significant extent than phlebotomy. Deferasirox was well tolerated; the majority of parents with children receiving phlebotomy noted a desire to switch to deferasirox.

Table 1.

Baseline and efficacy assessments

Parameter	Deferasirox (n=12)	Phlebotomy (n=14)	*P*-value
Baseline median SF (range), ng/mL	998.5 (502.5–5884.0)	1422.5 (505.8–2945.0)	0.817
Baseline mean LIC ± SD, mg Fe/g dw	12.5 ± 10.1	10.2 ± 6.8	0.607
Baseline mean TIBC ± SD, µg/dL	236.8 ± 33.0	260.9 ± 39.5	0.129
Baseline mean haemoglobin ± SD, g/dL	12.5 ± 1.5	12.6 ± 1.5	0.938
Absolute median change in SF, ng/mL			
Baseline mean SF <1000	–220.5 (n=6)	–290.0 (n=4)	0.749
Baseline mean SF ≥1000	–239.5 (n=6)	–806.0 (n=10)	0.481
Baseline mean LIC ≤7	–208.0 (n=5)	–649.0 (n=7)	0.144
Baseline mean LIC >7	–233.0 (n=7)	–747.5 (n=7)	0.443
Absolute mean change in TIBC, µg/dL			
Baseline mean SF <1000	130.7 (n=6)	26.2 (n=4)	0.025
Baseline mean SF ≥1000	131.8 (n=6)	24.7 (n=10)	0.002
Baseline mean LIC ≤7	120.9 (n=5)	30.4 (n=7)	0.015
Baseline mean LIC >7	138.6 (n=7)	19.84 (n=7)	0.002

SF, serum ferritin; TIBC, total iron binding capacity

0429

EPIDERMAL LANGERHANS CELLS IN THE CONTEXT OF FULL AND REDUCED INTENSITY CONDITIONING

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The interaction of residing host-derived dendritic cells (DC) and donor T cells is a crucial step in the induction of acute graft versus host disease (GVHD). In particular, epidermal Langerhans cells (LC) of the recipient persist after allogeneic hematopoietic stem cell transplantation (HSCT). Allo-reactive T cells mediate GVHD but also promote the switch from host to donor LC. We investigated on the LC chimerism early after transplantation in patients who had been treated with a reduced intensity conditioning (Fludarabine / Melphalan) combined with *in vivo* T-cell depletion mediated by high-dose alemtuzumab (100mg) prior to HSCT. In addition, we compared the impact of this conditioning regimen on LC-density with that of a 12Gy total body irradiation (TBI)-based full intensity conditioning regimen. Epidermal skin layers were prepared from 6 mm punch biopsies and split for further immunofluorescent staining as well as for the generation of single cell suspensions. CD1a/MHC-class II-positive LC were analyzed and subsequently sorted by flow cytometry. LC frequency among the isolated epidermal cells was analyzed by flow cytometry prior to conditioning, on the day of transplantation as well as on day +20. In addition, LC density was semiquantitatively assessed by confocal laser-microscopy on stained epidermal sheets. Donor chimerism was measured by STR-based assays on LC isolated on day + 20. Despite of a low LC-density on day +20 compared to that before transplantation, LC could be isolated in 51/52 patients. The numbers of isolated cells ranged from 2 to >1000. In samples with >300 isolated cells, these were re-analyzed by flow-cytometry and showed a purity of >90%. Chimerism analyses led to valid results in 39 samples. Of 19 patients who suffered from an

early GVHD after transplantation, 10 patients (53%) had predominantly donor-derived LC as early as day +20 after HSCT. In contrast, of 20 patients without primary GVHD, only 3 (15%) predominantly had donor-derived LC. The 20 patients without GVHD subsequently received prophylactic CD8-depleted donor-lymphocyte infusions (DLI). DLI induced acute GVHD in 11 patients, but we did not find a correlation between day +20 LC chimerism and the incidence of DLI-associated GVHD. The relative LC-density, calculated as the quotient of LC frequency among epidermal cells before and after conditioning was 60% (+/-30%) after Flu/Mel/Alemtuzumab (n=5) and 6% (+/-3.5%) TBI-based conditioning (n=4). These results were confirmed by manual counting of LC in stained epidermal sheets. We have established a sensitive method enabling us to investigate LC chimerism and follow LC chimerism prospectively. Early after SCT following a Flu/Mel/Alemtuzumab conditioning, the majority of patients still have predominantly host-derived LC. However, the predominant donor-LC chimerism in patients with primary GVHD on day +20 supports the hypothesis that persisting LC are relevant targets of allo-reactive T cells in acute GVHD. In addition, first data in a small series of patients point towards an impact of the conditioning regimen on LC density. Further studies will focus on the persistence of host-LC particularly after reduced intensity conditioning and might lead to DC-depleting strategies to further reduce the rate of acute GVHD in this setting.

0430

RESULTS OF THE PHASE I/II CLINICAL TRIAL MESENCHYMAL STEM CELLS EXPANDED IN VITRO WITH HUMAN SERUM FOR THE TREATMENT OF ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE

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Background. Several clinical trials have reported the evaluation of the therapeutic potential of mesenchymal stem cell (MSC) infusion for the treatment of acute graft-versus-host disease (GVHD), but information from the chronic GVHD setting is far more limited. Most clinical trials use MSC generated in medium with fetal calf serum (FCS). However, FCS is an undesirable source of xenogeneic antigens and bears the risk of transmitting animal viral, prion and zoonose contaminations. Platelet (PL) lysate and human serum (HS) have been proposed as an alternative, although only one study has evaluated the efficacy of these approaches in the clinical setting. *Aim.* In the current trial we have evaluated the feasibility and efficacy of the infusion of MSC expanded using HS for the treatment of refractory acute or chronic GVHD. *Methods.* Overall, 28 expansions were started. MSC were expanded from donor's bone marrow. Culture medium was enriched with HS from the MSC donor, and PL was added in cases of slow growth. Ten patients received MSC for the treatment of refractory or relapsed acute GVHD. Two patients received MSC as the second-line, five as the third-line and three as ≥ the fourth-line of treatment. Eight patients received MSC for the treatment of chronic GVHD. Four received MSC as second-line and four as third-or-more-line treatment. None of the patients receiving MSC as a second-line treatment had previously responded. *Results.* In 22 of the expansions, the minimum number of > 1x106 MSC/kg were obtained after a median of 26 days. After the addition of PL only one case did not reached this number of cells so this procedure yielded enough cells in most cases. In ten cases cells were not infused either because of patient's death prior to expansion due to GVHD or due to response to the previous line. Regarding patients treated for aGVHD, one obtained complete remission (CR), six had a partial response (PR), and three patients did not respond. In the subset receiving MSC for the treatment of cGVHD: one obtained CR, three had PR and four did not respond. No adverse events could be directly attributed to the MSC. *Conclusions.* The current study is the first clinical trial evaluating the feasibility and safety of MSC expanded *in vitro* using HS with PL for the treatment of heavily treated patients of both acute and chronic GVHD. In conclusion the present study shows that the use of MSC is safe and feasible in such patients. Moreover in it is the first clinical trial showing promising results in chronic GVHD.

0431

EXTRACORPOREAL PHOTOPHERESIS (ECP) AND RITUXIMAB (RTX) FOR THE TREATMENT OF STEROID REFRACTORY GRAFT VERSUS HOST DISEASE (SR-GVHD)

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Background. Treatment options for SR-GVHD after allogeneic stem cell transplantation (alloSCT) are unsatisfactory. ECP has immunomodulating effect mainly on T cells and has been used for the treatment of SR-GVHD with encouraging results. Rtx targeting B cells showed efficacy in SR-GVHD. We present a retrospective analysis of ECP and Rtx combination targeting two pathogenetic GVHD treads T and B cells used in our center to treat SR-GVHD after alloSCT. *Aims.* To determine the clinical benefit of ECP and Rtx combination in SR-GVHD in terms of clinical response rate (CRR) and site using the National Institutes of Health consensus criteria. To evaluate lymphocyte subpopulations as prognostic markers of response. *Methods.* Patients with acute or chronic GVHD, refractory to steroids, received ECP plus Rtx (off label use) as add-on to steroids ± imumosuppressive agents. 12 ECP cycles (each consisting of two procedures on two consecutive days) were given by weekly schedule followed by monthly ECP for up to 16 cycles. 1 g of Rtx was given after 1st and 3rd and optionally after 9th and 11th ECP cycles. Evaluations were performed after 6, 12, 14 and 16 ECP cycles. *Results.* 10 patients with aGVHD (2 - grade II; 8 - grade III-IV) and 9 with cGVHD (3 - moderate, 6 - severe) received ECP + Rtx. The median observation time was 4.5 months (range: 1-28). The median number of ECP cycles was 14 (range: 5-16). 13 patients are alive (7 patients completed treatment schedule, 6 are ongoing) and 6 died before the treatment had been completed. Clinical response was achieved in 16 patients (84%) with complete remission (CR) in 7 (37%) and partial remission (PR) in 9 (47%). 3 (16%) patients were non responders (NR). 3 (33%) cGVHD patients achieved CR, 5 (56%) achieved PR, one (11%) did not respond. The best response in cGVHD patients was observed in skin and mouth (100% CRR; 83% and 85% CR). CRR in other sites were: gastrointestinal tract (GI) (100% CRR; 43% CR), eyes (33% CRR; 33% CR) and liver (66% CRR; 0% CR). 4 (40%) aGVHD patients achieved CR, 4 (40%) had PR and 2 (20%) were NR. aGVHD response by site was: skin (71% CRR; 57% CR), GI (78% CRR; 44% CR). Treatment was well tolerated with no procedure related serious adverse events. 3 patients experienced hypocalcaemic reactions during ECP, 1 nausea, 1 vomiting and 1 fever were recorded. Immunosuppression was reduced in 10 and discontinued in 1 patient at first evaluation (after 6 ECP cycles) and further reduced in 2 and discontinued in 3 patients. The median survival was 10 months (1.5-19; CI 95%). One year survival was 44%. The causes of death were infections in 5 patients and intracranial hemorrhage in 1 patient. One patient had no GVHD at the time of death, 2 had minimally active GVHD and 3 had active GVHD (all NR). None of the lymphocyte subpopulations (CD3+ CD4+, CD3+ CD8+, NK cells, Treg) significantly predicted response to treatment. *Conclusion.* ECP and Rtx treatment is feasible and effective in SR-GVHD.

0432

IMPACT OF THE NEW ANTI-MYELOMA DRUGS ON OUTCOME OF ALLOGENEIC STEM-CELL TRANSPLANTATION WITH REDUCED-INTENSITY CONDITIONING IN PATIENTS WITH HIGH-RISK MULTIPLE MYELOMA

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Introduction. The increasing use of the novel agents lenalinomide and bortezomib to treat multiple myeloma (MM) has contributed to higher complete remission (CR) rates and longer overall (OS) and event free survival (EFS). We set out to assess the impact of these drugs on the outcome of high-risk MM patients treated with allogeneic stem-cell transplantation (allo-SCT) after reduced-intensity conditioning (RIC) over the last 10 years. *Methods.*This retrospective study compared 45 patients (group1) transplanted in our centre between January 1999 and January 2006 and who had not received either novel agent prior to transplant (as induction or relapse therapy) with 34 patients (group 2) transplanted between January 2006 and june 2010 who received either one or both drugs before allo-SCT. *Results.* The median time between diagnosis and Allo-SCT was 37 months (6-161) and 41 months (9-145)

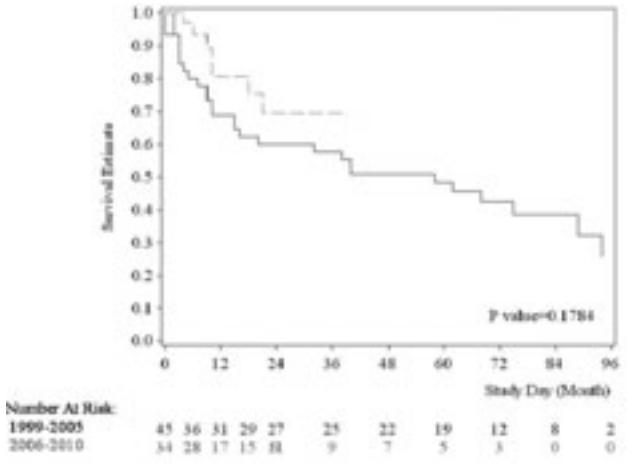

Figure 1. OS.

in the two groups respectively. The median follow-up after transplant was 45 (2-127) and 16 (3-39) months in the first and second group respectively.The cumulative incidence of acute graft versus-host disease (GVHD) was significantly higher before 2006 (47% vs 24%; p=0.0584). The cumulative incidence of chronic GVHD was also different (56% vs 30%; p=0.0241). The estimated probability of non relapse mortality (NRM) at day 100 was 12% in the first group vs 0 % in the second group transplanted after 2006. The one and two years NRM was 18% vs 23% (p =0,537). The overall survival (OS) at two years was 60% vs 70% in the first and second group respectively (p=0.1784). The progression-free survivals (PFS) was significantly different at 2 years, 45% before 2006 compared to 65% after 2006 (p=0,056). The PFS median not reached in the second group compared to 22 months before 2006 (p=0.1811). *Conclusion.* We documented a lower incidence of acute GVHD and NRM associated with a higher CR rate as well as significantly improved survival and relapse incidence since the introduction of novel reduced-intensity preparative regimens and peri- and post-transplantation strategies.. These results suggest that enhancing the graft-versus-myeloma effect is of key importance for managing high-risk MM patients.

0433

EARLY PERIPHERAL BLOOD AND T CELLS CHIMERISM DYNAMICS AFTER SINGLE CORD BLOOD TRANSPLANTATION WITH CO-INFUSION OF CD34+ CELLS FROM A THIRD PARTY DONOR PREDICTS CB GRAFT FAILURE

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Background. Umbilical cord blood (CB) transplant from unrelated donors has been increasingly used as an alternative stem cell source for patients with hematological malignancies lacking HLA-matched adult donors. The co-infusion of mobilized and selected CD34+ cells from a non-HLA-identical donor (dual transplant) has shown to reduce the period of postransplant neutropenia and related early morbidity and mortality of single CB transplantation. The aim of this study was to analyze the predictive value of early postransplant peripheral blood (PB) and T lymphocytes (TL) chimerism analysis after dual transplant regarding CB engraftment or failure. *Patients and Methods.* 15 patients with high risk disease underwent 16 dual transplants between 2004 and 2011. Chimerism analysis was performerd weekly after graft infusion until complete chimerism was achieved by STR-PCR (AmpFlSTR SGM Plus; Applied Biosystems) in PB and TL purified using immunomagnetic technology (CD3+, Miltenyi Biotec). Complete chimerism (CC) was defined as <1% recipient in PB and <5% in leukocyte lineages (95% purity of enriched samples). *Results.* From the 16 transplants, 12 (Figure 1a-l) showed engraftment (>500 TNC) in a median of 16 days (11-28) reaching full CB chimerism in a median of 24 days. Early postransplant PB chimerism analysis showed increasing percentages of CB cells in 8 cases (Figure 1a-h). Only 2 cases showed low (<15%) percentages of CB cells in the first sample (day +14), although both showed an increase in the second determination (day +21). In the remaining 4 cases the proportion of CB cells remained stable or slightly

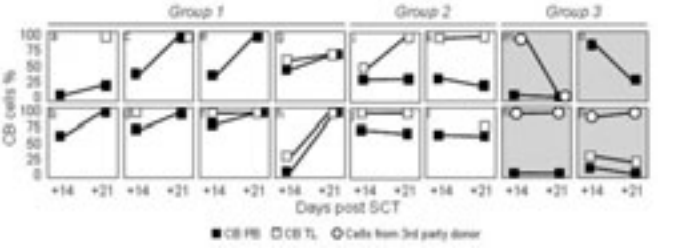

Figure 1.

decreased from day +14 to day +21 (Figure 1i-l), however TL chimerism showed a significant increase or remained near 100% CB cells in both determinations. On the other hand, 4 out of the 16 transplants experienced primary CB graft failure (Figure 1m-p). Three of them showed low percentages of CB cells in PB (<15%) on day +14 with a further decrease in the second sample. The fourth case showed an initial high proportion of PB CB cells with a significant decrease in the following sample (TL chimerism not available). *Conclusions.* Early postransplant chimerism dynamics in PB and TL can predict CB engraftment or failure in dual transplants. Initial low percentages of CB cells in PB without an increase within the first month post-transplant as well as a decrease in the proportion of PB CB cells without an increase in TL, seem to correlate with CB failure. Therefore, an early significant proportion of CB in TL associates with CB engraftment irrespectively of the dynamics of chimerism in PB.

0434

TREATMENT OF STEROID RESISTANT GRADE II TO IV ACUTE GVHD BY INFUSION OF MESENCHYMAL STROMA CELLS EXPANDED WITH HUMAN PLASMA AND PLATELET LYSATE - A PHASE I/II STUDY

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Introduction. For numerous malignant and non-malignant hematological diseases allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative therapy. Despite multiple improvements in the last decade in the field of HSCT, acute graft versus host disease (aGVHD) remains a life-threatening complication and reduces substantially efficacy of HSCT. In particular, the outcome of patients with severe steroid-resistant aGVHD is very poor. Therefore, it remains important to search for new therapeutic strategies for the treatment of aGVHD. *Objective.* Feasibility of the generation and efficacy of mesenchymal stroma cells (MSCs) generated with fetal calf serum (FCS) has been suggested recently. However, FCS is a putative source of prions and virus transmission. Therefore, the feasibility of the generation of MSCs expanded with human plasma and platelet lysate (hPPL) was tested as well as the feasibility and safety of the application of hPPL-MSCs in patients with steroid-refractory aGVHD. *Method.* In an open-label, non-randomized prospective phase I/II study MSCs were extracted from the bone marrow of healthy volunteers, expanded with hPPL, and stored. Patients with steroid-refractory aGVHD grade II to IV were treated with ~2x106/kg hPPL-MSC. Response rate, transplantation-related deaths, and other adverse events were assessed for up to 12 months after the last infusion of the cells. *Results.* Between January 2009 and December 2010, 20 patients were included, 2 patients drop out, and 18 patient were available for further analysis: 5 children and 13 adults. Median age was 32.5 years (range 1.3-65.9). Organs involved in aGVHD were the skin (67%), the gastro-intestinal tract (83%) and the liver (28%). Overall grade was II for 4 (22%), III for 13 (72%), and IV for 1(6%) patients. 1 patient received one infusion, all other patients received two or more infusions. No patient had side-effects during or immediately after infusions of the hPPL-MSC. Median follow-up was 5.5 months (range 0.33-12). Complete overall response was observed in 11 patients (61%) after a median of 65 days (range 10-184 days). The overall survival was significantly better in responders when compared to non-responders (p <0.001). Of the 11 patients who reached a CR, 8 patients relapsed approximately 2 months after reaching CR (median 59 days, range: 1-244). Three children relapsed with clinical signs of an allo-immune-lung, auto-immune-cytopenia or limited cGVHD and all 5 adults relapsed with GVHD of the gut (median 98 days after reaching CR, range: 35-302 days). However, GVHD of the gut was then again sensitive to steroids. Overall, 7 patients died, 4 due to progression of

aGVHD, 1 patient due to abdominal bleeding and 2 due to sepsis. *Conclusion.* Generation and infusion of hPPL-MSCs in steroid-resistant aGVHD grade II- IV is feasible, safe and very effective. In addition, also patients who initially responded to hPPL-MSCs but develop later a relapse of aGVHD during tapering or cessation of immunosuppressive drugs become again sensitive to the treatment with steroids.

0435

ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION WITH RIC CONDITIONING IN PATIENTS WITH HIGH RISK MULTIPLE MYELOMA: COMPARATIVE ANALYSIS OF OUTCOMES BETWEEN UNRELATED AND RELATED DONOR

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The purpose of this study was to assess the results of allogeneic stem cell transplantation (Allo-SCT) after reduced-intensity conditioning (RIC) from an unrelated donor in patients with high-risk multiple myeloma (MM) in a single centre. From January 2007 to January 2010 we transplanted 33 consecutive patients with MM. *Methods.* Thirteen (39%) (Group 1) and 20 patients (61%) (Group 2) had unrelated and related donor respectively. The median age was 48 years (39-63) in the first group and 56 years (40-67) in the second group.Thirty two patients (97%) received one or more autologous transplantation. The disease status at transplantation was [Complete Remission (CR) or VGPR in (15%) vs (40%); Partial remission (PR) in (77%) vs (55%); progression or refractory disease in (8%) vs (5%)] in the first and second group respectively (p=0.1770). stem cell source was peripheral blood steam cells (PBSC) in all patients in the related donor group and in 10 patients (77%) in the second group, the other 2 patients (15%) received marrow and one patient (8%) received cord blood cells. Twenty five patients (Group 1: N=4 (31%); Group 2: N=4 (20%) were treated with a RIC based on Fludarabine (30mg/m²/d x 5); Busulfan (4 mg/kg/d p.o. or 3.2 mg/kg/d IV over 2 to 3 days) and rabbit ATG (2.5 mg/kg/d x 2). *Results.*The median follow-up was 17 months (4-39). None of our patient experienced a graft rejection. The cumulative incidence of grade II-III acute graft versus-host disease (GVHD) was higher (38%) for the unrelated donor vs (15%) for the related donor (p= 0.12). The cumulative incidence of chronic GVHD was no different between the first and second group (31% vs 30% respectively).The estimated probability of non-relapse mortality (NRM) at day 100 was 0% in the two groups. At two-year the NRM probabilities was lower in the unrelated group 14% vs 24% in the related group (p =0,477). Also at 2 years, patients receiving unrelated transplantation had superior overall and progression-free survivals, 83% and 65% respectively compared to patients with related donor transplantation, 67% and 36% (p= 0.241). The incidence of acute GVHD, OS, PFS and NRM were not significantly different between the two groups. *Conclusion.* in patients with high risk multiple myeloma; RIC Allo-SCT with unrelated donor, is feasible and effective treatment with low non-relapse mortality, high complete remission rates and prolonged disease-free survival. This procedure seems to be comparable to those of HLA-identical siblings.

Table 1.

Characteristics	Related Donor 20 Patients (61%)	Unrelated Donor 13 patients (39%)	P value
Auto-SCT	20 (100)	12 (92)	0.393
0	0	1 (8)	
1	12 (60)	7 (54)	
2	7 (35)	5 (38)	
3	1 (5)	0	
Allograft in 1st line	4 (20)	4 (31)	
> 1st	16 (80)	9 (69)	0.6806
Cytogenetics at diagnosis			
Normal	2 (10)	1 (8)	
Del (13) Del (17) t(4; 14)	10 (50)	6 (46)	
NA	8 (40)	6 (46)	
Disease status			
CR or VGPR	8 (40)	2 (15)	0.2455
PR or SD	11 (55)	10 (77)	
Progression or refractory	1 (5)	1 (8)	
Cells type			
PBSC	20 (100)	10 (77)	0.06242
MB		2 (15)	
CORD		1 (8)	
CD34 + / kg	5.82(1.7-9.1)	5.4 (0.17-8.8)	
CD3+ / kg	389 (130-745)	189 (3-321)	
Conditioning regimen			
FBu2/2	10 (50)	7 (54)	
FBu4/2	2 (10)	5 (38)	
FTBI	7 (35)	0	0.08382
FCYTBI	0	1 (8)	
FdBu2x2	1	0	
GVHD prophylaxis			
CSA	13 (65)	7 (54)	0.7171
CSA + MMF	7 (35)	6 (46)	
Acute GVHD	3 (15)	5 (38)	
grade (II - III)	2 (10)	5	0.2134
grade (IV)	1 (5)	0	
Chronic GVHD	6 (30)	4 (31)	1
Limited	2 (10)	1 (8)	
Extensive	4 (20)	3 (23)	
Relapse post Allo-SCT	5 (25)	2 (15)	0.6756
status at the last follow-up			
alive	15 (75)	12 (92)	0.3644
Death	5 (25)	1 (8)	
Cause of Death			
TRM	4 (20)	1 (8)	0.6253
Disease	1 (5)	0	
Disease Status at the last follow-up			
CR / VGPR	8 (40)	7 (54)	0.4928
PR / SD	5 (25)	4 (31)	

0436

EFFICACY AND LONG-TERM OUTCOME OF INTERVENTION FOR PURE RED CELL APLASIA (PRCA) FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FROM MAJOR ABO-INCOMPATIBLE DONORS

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Background. No standard of care for PRCA following allogeneic hematopoietic stem cell transplantation (HSCT) from major ABO-incompatible donors has been established. *Aims.* The primary objective of the present study was to learn the efficacy and long-term outcome of intervention for post-transplant PRCA. *Methods.* The present study was conducted as a retrospective observational study and approved by the IRB and the Japan Society for Hematopoietic Cell Transplantation. The patient cohort was selected from the registry database of the Transplant Registry Unified Management Program (TRUMP) that covered both adult and pediatric transplantation using all kinds of graft sources in Japan. One-hundred forty-five recipients who achieved engraftment with delayed recovery of erythropoiesis and survived >6 months after transplant without disease relapse were selected from 2,846 records of major ABO-incompatible allogeneic transplantation during 2003 to 2007. The questionnaires were sent to the transplant centers and the response rate was 68.3% (99/145 recipients). Forty-eight recipients were identified as having PRCA, and then the detailed transplant data of 46 out of those 48 recipients could be collected. *Results.* The grafts were bone marrow and blood stem cells in 33 and 13 patients, respectively. No patient with PRCA was reported after cord blood transplantation. Donors were related in 24 patients, and unrelated in 22. Incompatible hemagglutinins were anti-A in 28 patients, anti-B in 12, and both in 6. Treatment of PRCA except for transfusion was performed in 22 patients (intervention group) but not in other 24 patients (non-intervention group). Response to the primary treatment was observed in 2 out of 8 patients who had been rapidly tapered calcineurin inhibitors (2CR), and 6 out of 12 patients receiving corticosteroid (5CR, 1PR). None of the patients receiving rituximab (n=1) or erythropoietin (n=1) responded. Secondary therapy including rituximab, additional immunosuppressants, or DLI was given in 8 patients with 50% response rate. Overall response rate of intervention was 54.5%. Four out of 10 patients who did not show any responses to intervention spontaneously became transfusion-independent. Days from the diagnosis of PRCA to recover reticulocytes >1% and cumulative doses of RBC transfusion during the period were not significantly different between the two groups. Incompatible hemagglutinin titers at diagnosis of PRCA were not different, either. Strikingly, the Kaplan-Meyer estimate of the survival demonstrated the inferior survival of the intervention group (log-rank 0.001). Eleven and 2 deaths were observed in the intervention and the non-intervention groups, respectively. Infections accounted for the death of 7 patients in the intervention group. Univariate analysis indentified the 5 variables influencing the OS, and

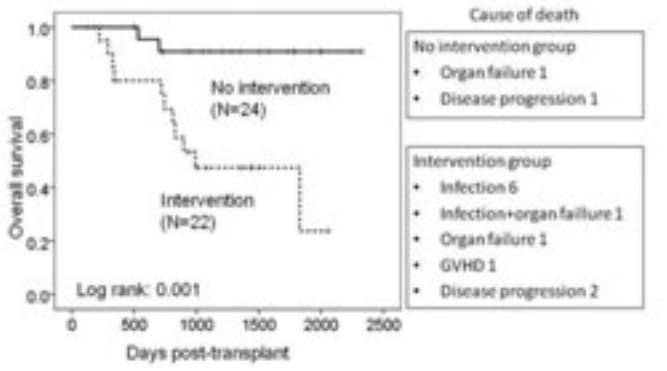

Figure 1. Overall survival of post-transplant PRCA.

Cox regression analysis revealed that the intervention for PRCA remained as the only factor negatively affecting OS (p=0.040). Matched pair analysis using the non-PRCA cohort as a control showed the marginal probability of difference (log-rank 0.114), possibly because of the limited number of patients. *Summary/Conclusion.* The present study suggests that the intervention for PRCA may adversely affect the outcome of the patients. Prospective cohort study or randomized controlled trial should be encouraged to explore the role for intervention of PRCA following major ABO-incompatible HSCT.

0437

REDUCED INTENSITY ALLOGENEIC TRANSPLANTATION IN YOUNG PATIENTS WITH VERY HIGH RISK ACUTE LYMPHOBLASTIC LEUKEMIA (VHR ALL)

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Backround. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) with reduced intensity conditioning regimens (RIC) has become a well-established approach in adult patients with hematologic malignancies. The role of this type conditioning in pediatric cancer has yet to be defined. The aim: to compare efficacy of reduced intensity conditioning (RIC) and myeloablative conditioning (MAC) for allo-HSCT in children and adolescents suffering from VHR ALL. *Patients and Methods.* 98 ALL patients (pts) from 1 till 21 y.o. (mediana 12 y.o.) were underwent allo-HSCT between 12/2000 and 12/2010. Indication for allo-HSCT in children and adolescents was VHR ALL: late responder on chemotherapy (induction failure), poor-risk cytogenetics (t(9:22), t(4:11)) and MRD(>10 -2), infants with 11q23 rearrangement, short first remission, primary resistance or resistant relapse. RIC allo-HSCT performed in 28 pts (RIC- group): 16 pts in I or II complete remission (CR), 12 pts were in resistant relapse. Indication for RIC allo-HSCT was poor performance status, organ dysfunction due to previous therapy or infection complication at the moment of allo-HSCT. MAC was used in 70 pts (MAC-group): 42 pts were in I and II CR at the moment of HSCT, 28 pts were III and IV CR or in resistant relapse. RIC consisted of Flu 150 mg/m2/d + Mel (140 mg/m2/d)±ATG or Flu 150 mg/m2/d + Bu 8 mg/kg±ATG; MAC consisted of Bu 16 mg/kg (or Treo 36-48 mg/m2) +Cy 120 mg/kg ±ATG. Allo-HSCT from matched related donor was performed in 27 pts (RIC, n=6), from matched unrelated donor - in 71 pts (RIC, n=22). *Results.* (tab 1,2): In RIC-group granulocyte's engraftment >=0,5x109/l was on D+18 (in ranges D+13-31). For pts in I or II CR at RIC allo-HSCT 9 of 16 are alive. Overall 7-years survival (OS) and disease-free survival (DFS) were 50% and 46%, corresp. Five pts died within 100 days: infection (1), aGVHD (4). Relapse occurred in 3 pts (19%), 2 relapsed pts achieved CR after chemotherapy+DLI in 1st ptn and immunosuppression withdrawal in 2nd ptn, one ptn died due to relapse in 5 months. CR was achieved in 5 of 12 pts after alloHSCT at resistant relapse. But 1-year OS was 0%. Patients died from infection (3), aGVHD (1), and disease progression (7). MAC-group: granulocyte's engraftment >=0,5x109/l was on D+21 (in ranges D+10-49). Eighteen of 42 pts are in CR after MAC allo-HSCT in I or II CR. Overall 7-years survival (OS) and DFS were 51% and 44%, corresp. 11 pts relapsed after MAC allo-HSCT (28%), but 2 of them achieved CR after chetmotherapy+DLI. Ten pts died from relapse, aGVHD-5; infection -2; transplant related toxicity -2, non-engraftment - 1. Five from 28 pts after MAC alloHSCT in relapse either III or IV CR are in CR (1-104 months; mediana 44 months). Other pts died - relapse (13), infection (7), aGVHD (3). *Conclusion.* RIC allo-HSCT of VHR ALL in CR pts ≤ 21 yo is effective and comparable with MAC allo-HSCT. These results make new approaches for pts in VHR ALL in CR, indicate sensitivity to immunoadoptive therapy and produce the base for clinical trials.

Table 1. Table 2.

Outcomes after allo-HSCT

Complication after allo-HSCT

Condition regimen	Status at the moment of allo-HSCT (n)	Relapse n(%)	TRM n(%)	7-OS	7-EFS
RIC	I or II CR 16	3(19)	5(30)	50%	46%
	Relapse 12	6(50)	4(36)	0	0
MAC	I or II CR 42	11(26)	10(24)	51%	44%
	Relapse 28	13(46)	10(50)	14%	14%

	RIC (%)	MAC (%)
Acute GVHD grade 1-4	55	53
Chronic GVHD	78	54
infection	62	84
non-hematological toxicity	31	54
hemorrhagic	13	75

0438

RESULTS OF A MULTICENTRIC EXPERIENCE OF PLERIXAFOR USE IN HEMATOPOIETIC STEM CELLS MOBILISATION

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Background. Plerixafor the major inhibitor of the α chemokine CXCL12 receptor has been largely used in the context of hematopoietic stem and progenitor cells mobilisation (HSPC) since its approval in 2009 in Europe. In France, its main indication remains for poor mobilizers patients with lymphoma and or myeloma. *Aims and Methods.* We retrospectively reviewed the data collection of 65 patients assigned to autologous stem cells transplantation (ASCT) and who underwent their apheresis at the French Blood Institute between 2008 and 2010. All patients who received Granulocyte-colony stimulating factor (G-CSF: 10 micrograms/kg/dose subcutaneously daily x 4 days) during mobilisation attempt + Plerixafor (0,24 mg/kg/dose subcutaneously daily, after at least the fourth dose of G-CSF) were evaluated. Additional doses of G-CSF and Plerixafor for subsequent apheresis sessions were administered using a COBE Spectra separator. *Results.* Baseline characteristics of the patients were: median age = 57 yrs (range, 12-67); sex ratio M/F =1,3; haematological malignancies = 58; non-haematological malignancies = 7; 3 patients received high dose cyclophosphamide during mobilisation setting; median lines of chemotherapy = 2 (range, 1-6). Plerixafor was well tolerated and no severe side effects reported. Patients had a median of 2 mobilisation attempts (range, 0-4). Sixty three percent of patients achieved the aim of ≥ 2x106 CD34+ cells/kg collection after a median number of apheresis sessions of 2 (range, 1-3). The median level of CD34+ cells in the peripheral blood (pCD34+) at day 1 of apheresis after Plerixafor injection was 19/µl (range, 0-145), 7,3 hours (range, 4,3-11,5) prior to apheresis. The median yield of CD34+ cells was 1,8x106/kg (0,1-16,5) after the first session of apheresis (Table 1). Interestingly, Plerixafor could lead 58 % of patients

Table 1.

Characteristics of patients and collection

No of Patients	65
Diagnosis (Myeloma/non Hodgkin Lymphoma/Hodgkin Lymphoma/Chronic Lymphocytic Lymphoma and Waldenström/ Non-hematologic Malignancies)	15/32/6/5/7
Median Weight (range) kg	70 (16-125)
Median sessions of apheresis (range)	2 (1-3)
Median Doses of G-CSF (range) µg/kg	10 (6-16.8)
Median Total blood volume processed at first apheresis (range) Liters	10,4 (4,4-20,5)
Median CD34+cells x10^6/kg collected at day 1 of apheresis (range)	1.8 (0.1-16.5)
Median CD34+cells x10^6/kg collected at day 2 of apheresis (range)	1.1 (0.3-4.6)
Median Total CD34+cells x10^6/kg collected (range)	2.66 (0.7-5.7)

to a pCD34+ level ≥ 20 /µl and collection of ≥ 3x106 CD34+cells/kg during only 1 session of apheresis. Moreover, the median level of pCD34+ available for 21 patients among the 65 before and after injection of Plerixafor was 4/µl (range, 0-16) and 12,5/µl (range, 0-97) respectively after a 3,2-median fold expansion (range, 0-16,2). At the day of analysis, twenty patients had already undergone ASCT with successful engraftment. *Summary.* Mobilisation with G-CSF + Plerixafor is an excellent strategy in the context of heavily pre-treated patients with haematological and extra haematological malignancies, can lead to early collection of HSPC and requires close collaboration between clinicians and physicians collection facilities. Further leg of this analysis will evaluate the data of patients harvested with Plerixafor + chemotherapy during neutrophil recovery and engrafment data after ASCT.

0439

THE PRESENCE OF CIRCULATING ANTI-PR1 AND ANTI-SURV SPECIFIC CD8+ LYMPHOCYTES POST ALLOGENEIC STEM CELL TRANSPLANTION IS ASSOCIATED TO MORE FAVORABLE OUTCOME

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Background and Aims. Allogeneic stem cell transplantation (allo-SCT) is a potentially curative treatment option for patients with hematological disorders. Alloreactive donor-derived T lymphocytes exert a benefitial graft-verus-leukemia (GVL) effect through the recognition of leukemia-restricted (or preferentially expressed) antigens as Wilms tumor protein (WT1), survivin (SURV) or proteinase (PR1). Current research in transplant immunology focouses in enhacing GVL while preventing the deleterious graft-versus-host disease (GVHD) that could be achieved by manipulating donor-derived antigen-specific T-populations. In this study we sought the presence of peripheral blood leukemia-associated antigen-specific CD8+ T-lymphocytes and the impact on outcome post-allo-SCT. *Methods.* 54 consecutive HLA*0201 patients undergoing conventional myeloablative (n=32) or non-myeloablative (n=22) allo-SCT as treatment of hematological disorders were included. Donor was an HLA-identical sibling in 46 cases (80.5%) and unrelated in 8 cases (14.5%). Stem cell source included mobilized peripheral blood (n=22), bone marrow (n=25) and umbilical cord blood (n=7). Twenty-nine patients received rabitt antithymocyte globulin at 6-8mg/kg. Wiht a median follow up was 27.5 months (range 2-88) four patients had relapsed 9-14 months after allo-SCT. We used four color multiparametric flow cytometry in a FACSCanto II adquiring at least 5 x105 viable (Propidium Iodide low) lymphoid gated events, stained with MnAbs: CD8-FITC and CD3PE/APC. To identify leukemia-antigen specific CD8 lymphocytes we used APC or PE conjugated class I HLA*0201 pentamers (Proimmune) against the following nonapeptides: Proteinase 1:VLQELNVTV; WT1:RMFPNAPYL and SURV:ELTLGEFLKL. Fuctional assesment of CD8+ was perfomed by intraccellular IFN- detection using 1x106 PBMCs stimulated with or without SURV, PR1 and WT1 synthetic peptides (10µg). After one hour in culture 10µg/ml breferdin A was added. As negative controls we used PE/APC labelled HLA-A-0201 negative control pentamer and as positive control we used CMVpp65/HLA-A0201. *Results.* Donor-derived CD8+ lymphocytes against PR1, WT1 and SURV were detected in periheral blood samples in 56.4%, 47.5% y 37.1% of recruited patients respectively. Median percentage of anti-PR1 was 0.051% (range:0.001-0.367% over CD3+CD8+ events), 0.03% for WT1(range:0.001-0.457%) and 0.020% for SURV(range:0.001-0.250). Detection of leukemia-antigen specific CD8+ lymphocytes was not significantly associated with clinical variables such as conditioning regimen (conventional or non-myeloablative), age donor, alloSCT source. The presence of anti-PR1 specific CD8+ lymphocytes was significantly more frequent in patients grafted with an HLA-identical sibling donor (P<0.01), and in patients not receving ATG (P=0.05). In addiction, the presence of circulating anti-SURV specific CD8+ lymphocytes was more frequently found in patients developing GVHD post-allo-SCT(P=0.043). Detection of circulating anti-PR1 and anti-SURV specific CD8+ lymphotes was associated with less mortality rate (P<0.01 and P<0.01 respectively). In the univariate analysis we found that overall survival and event free survival was significatly better in patients with anti-PR1 and anti-SURV specific circulating CD8+ lymphocytes (P<0.01 and P=0.024, respectively). *Conclusions.* Multiparametric flow cytometry is a useful tool to detect and quatify rare donor-derived CD8+ lymphocytes specific for leukemia-associated antigens as PR1, WT1 or SURV. The presence of anti-PR1 and anti-SURV specific CD8+ lymphocytes in peripheral blood is associated with a better survival and this finding could be related to an increased immunosurveillance against residual tumor cells in allo-SCT.

0440

SEQUENTIAL STRATEGY OF REDUCED-INTENSITY ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION (IDA-FLAG-MELPHALAN) FOR PATIENTS WITH PRIMARY REFRACTORY OR RELAPSED ACUTE MYELOID LEUKEMIA

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Background. The prognosis of patients with acute myeloid leukemia (AML) failing to frontline therapy remains poor, with allogeneic hematopoietic stem-cell transplantation (alloHSCT) arising as the only option with long-term curative potential. Nonetheless, only a minority of such patients ultimately undergo alloHSCT after salvage therapy. Sequential strategies are aimed to integrate a cytoreductive phase followed by reduced intensity conditioning in a sole procedure, in order to increase the proportion of patients who might benefit from the alloreactive graft-versus-leukemia effect. *Aim.* To compare the outcome of a series of patients with primary refractory (Ref) or relapsed (Rel) AML treated according to a sequential protocol with a group of patients receiving conventional salvage chemotherapy in a single institution. *Patients and Methods.* In 2005 we designed a sequential protocol based on a cytoreductive phase with the IDA-FLAG regimen (days -11 to -7) followed by melphalan (70 mg/m2 x days -3 & -2) for patients with Rel or Ref AML not considered candidates to a myeloablative alloHSCT, due to age >50 and/or previous transplantation. The outcome of these patients (SEQ arm) was compared with a cohort of Ref/Rel AML patients from our center consecutively treated since 1998 with a standard approach (CONV arm), consisting of 1-2 cycles of IDA-FLAG followed by alloHSCT in responding patients. *Results.* Overall, 22 patients (age: 52, 29-65; 50% male) included in the SEQ arm were compared to 51 patients (age: 48, 22-68; 53% male) treated according to CONV strategy. Within SEQ arm, a higher proportion of patients were diagnosed of secondary AML (36 vs. 8%, p=0.005), received more lines of previous therapy (≥2: 45 vs. 6%, p<0.001), and had previously undergone HSCT (41 vs. 4%, p<0.001), whereas the CONV arm contained a higher proportion of Ref AML (59 vs. 27%, p=0.013). The SEQ strategy was followed by a high immediate antileukemic response (complete response at day +30, 95 vs. 45% after CONV arm, p<.001). Of note, only 38% of patients in CONV arm finally received an alloHSCT. Among patients achieving CR after salvage therapy, cumulative incidence of relapse at 2-yr was 31% (95% CI: 15-67%) and 45% (95% CI:29-72%) in the SEQ and CONV arms, respectively. Moreover,

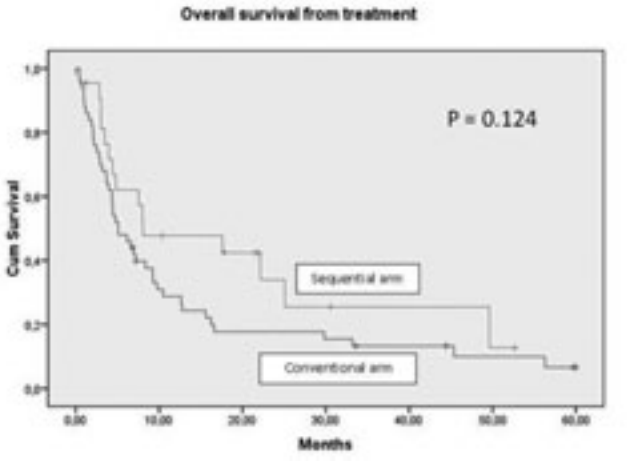

Figure 1.

non-relapse mortality (NRM) at 2-yr was 41% (95% CI: 24-71%) and 31% (95% CI:17-58%) after SEQ and CONV strategy, respectively. As a result, overall survival at 2-year after salvage therapy was 34±12% and 18±6% for patients treated according to SEQ strategy and CONV arm, respectively, without achieving statistical significance (p=0.12, see figure). *Conclusions.* Despite a remarkable antileukemic effect, the herein described sequential strategy did not result into a survival benefit over a standard salvage strategy, probably due to high non-relapse mortality. Further strategies aimed to diminish toxicity and relapse after allogeneic are warranted to improve the overall results of sequential strategies for the management of refractory and relapsed AML.

0441

HIGH DOSE THERAPY AND AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN RELAPSED/PRIMARY REFRACTORY HODGKIN LYMPHOMA PATIENTS: OUTCOME AND PROGNOSTIC FACTORS. EXPERIENCE FROM TWO GREEK CENTERS

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Background. High dose therapy and autologous hematopoietic stem cell transplantation (HDT/ASCT) is considered the standard of care for patients with relapsed or primary refractory Hodgkin lymphoma (HL). *Aims.* To analyze the prognostic factors and outcome of HDT/ASCT in 142 patients with primary refractory/relapsed HL from two transplantation centers. *Methods.* 142 patients from the Department of Hematology and the 2nd Department of Propaedeutic Internal Medicine of the University of Athens were studied. Progression free survival (PFS) was calculated from ASCT to relapse/progression or death and overall survival (OS) from ASCT to death from any cause. *Results.* Median age at HDT/ASCT was 30 years (17-66) and 56% were male. At diagnosis 8%, 46%, 22% and 24% of the patients had clinical stage I, II, III and IV respectively, 43% had B symptoms, 31% bulky disease, 86% were initially treated with ABVD, while 39% received additional radiotherapy. At relapse/progression 14%, 43%, 5% and 38% of the patients had clinical stage I, II, III and IV respectively, 19% B symptoms, 10% bulky disease and 41% extranodal disease. Forty-three % of them were treated with HDT/ASCT for 1st relapse, 11% for multiple relapses and 46% for primary refractory disease. After the last salvage chemotherapy and just prior to HDT/ASCT, 33% were in complete remission, 44% in partial remission and 23% were chemorefractory. At a median follow-up of 45 months (2-173), 5-year PFS was 50%, while OS at 5 and 10 years was 81% and 72% respectively. Chemoresistance before HDT/ASCT ($p<0.0001$), bulky disease ($p<0.02$) and B symptoms at relapse ($p<0.002$) proved to be poor prognostic factors for PFS. Thus, 5 year PFS was 56% for chemosensitive patients vs 21% for chemorefractory ones, respectively. Moreover, patients who were transplanted due to multiple relapses had a better outcome compared to others ($p<0.05$). Chemoresistance ($p<0.0001$), bulky disease ($p<0.0001$) and B symptoms at relapse ($p<0.0003$) were found statistically significant for OS, as well. In addition, age ≥ 45 years proved an unfavorable prognostic parameter for OS ($p<0.01$). Multivariate analysis documented the independent prognostic value of chemosensitivity and B symptoms for PFS and OS. Age was also an independent prognostic factor for OS. *Conclusions.* HDT/ASCT may cure half of the patients with relapsed or primary refractory HL. Chemorefractory patients and those with bulky disease and B symptoms at relapse or progression have a dismal outcome.

0442

AUTOLOGOUS STEM CELL TRANSPLANTATION AS A CONSOLIDATION TREATMENT FOR MANTLE CELL LYMPHOMA

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Background. Mantle cell lymphoma (MCL) is an aggressive subtype of B-cell non-Hodgkin lymphoma with poor prognosis and a reported median overall survival (OS) of 3 to 6 years. In an attempt to improve the prognosis of these patients, several therapeutic strategies have been tested, before and after introduction of monoclonal antibodies but there is no consensus about the choice first line therapy. However, a consistent number of studies show that high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) increases relapse free survival (RFS) of MCL patients. *Aim.* To analyse retrospectively the role of ASCT (with BEAM conditioning) as consolidation for newly diagnosed MCL patients. *Materials and Methods.* We analysed 23 patients with confirmed diagnosis of MCL that have been submitted to ASCT between 1999 and 2010 in our institution. We included patients treated with different induction chemotherapy regimens. We collected data related to sex, age, stage of the disease, response to induction chemotherapy, evaluation at day +100 after transplant and RFS and OS were calculated. *Results.* Male sex was predominant (82.6%), median age was 58 years (42-67). All patients were in advanced stage disease: 91.3% in stage IV and 78.3% with leukemic expression. Eight patients were treated upfront with CHOP+/-R (x6) and 13 with Hiper-C-VAD+/-R (x4). One patient was treated with R-FC and another with FCM followed by 6x R-CHOP. Fifteen patients (65.2%) attained complete remission (CR) after the induction courses in which are included 12 of 13 patients treated with Hiper-C-VAD; eight patients only achieved partial response after induction but one of these obtained a CR after R-ESHAP. In total, 16 patients (69.6%) were in CR at the time of transplant. CR rate at day +100 after ASCT increased to 86.9%. The median RFS was 58 months and the median OS was 79 months. With a median time of follow-up of 3 years and 7 months, there are 71.4% patients alive. Of these patients, 2 have relapsed so far. There was not any transplant related mortality (TRM) in this series. *Conclusions.* Our retrospective analysis suggests that ASCT as a consolidation therapy in MCL patients is a safe, effective and well tolerated therapeutic option, in patients under 65 years. In our study, median OS and RFS of patients that were submitted to ASCT was not reached yet. Until the last follow-up, only 1 patient treated with R-Hyper-CVAD followed by ASCT relapsed. These results emphasize a role for Hyper-C-VAD induction followed by ASCT as frontline management of MCL patients.

Stem cell transplantation - Experimental & clinical

0443

PALONOSETRON + APREPITANT VERSUS GRANISETRON FOR PREVENTION OF NAUSEA AND VOMITING IN PATIENTS RECEIVING HIGH DOSE CONDITIONING CHEMOTHERAPY REGIMENS PRIOR TO STEM CELL TRANSPLANTATION (HSCT)

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Background. Nausea and vomiting (CINV) in patients receiving high-dose, multiday conditioning regimens prior to stem cell transplantation is particularly common (55-100% of patients) and troublesome, especially in the delayed phase (beginning 24 hours after the start of conditioning). Newer antiemetics (palonosetron and aprepitant) appear to significantly reduce acute and delayed CINV as compared to the classic setrons; however, few studies have prospectively evaluated the efficacy of these drugs in this challenging setting. *Aims.* To assess whether palonosetron (0.25 mg iv every 48 h) administered in combination with aprepitant (125 mg on day 1 followed by 80 mg each of the remaining days) during the conditioning period (duration 5-6 days) improved control of CINV compared with daily granisetron (3 mg iv). *Methods.* This was a prospective, multicenter, randomized, stratified (by conditioning regimen), double-blind study. Patients received either BEAM (78%), BUCY (17%), or CBV/Cy-TBI (5%) as the conditioning regimen prior to HSCT. The primary efficacy endpoint was complete response (defined as no emesis and no use of rescue medication); secondary endpoints included evaluation of emesis and nausea throughout the conditioning period. Adverse events were also assessed. *Results.* Sixty consenting patients were included in the study (n = 31 palonosetron + aprepitant; n = 29 granisetron). The mean age was 39.5 ± 20 years; 50% were women; 53% were being treated for non-Hodgkin lymphoma, 27% for E. Hodgkin's and 12% for AML. 94% of HSCT were autologous. There were no between-group differences in variables that could potentially influence emesis (sex, previous chemotherapy or CINV, and regular intake of alcohol). Significantly more patients in the palonosetron + aprepitant group versus the granisetron group had a complete response during the acute (0-24h; 92.3% vs 67.9%, respectively), delayed (24-120h; 61.5% vs 28.6%) and overall (0-120h; 61.5% vs 28.6%) periods. In addition, palonosetron + aprepitant significantly reduced the proportion of patients with emesis during the acute, delayed and overall periods and showed a trend toward a reduction in percent of patients with significant nausea during the delayed period (see Table). There were no significant differences between the groups in adverse events or in the times of graft infection / severe infections. *Conclusion.* The combination of palonosetron + aprepitant was well tolerated with superior protection from nausea and vomiting compared with granisetron in patients receiving multiday highly emetogenic conditioning chemotherapy regimens prior to HSCT.

Table 1.

Efficacy Endpoints (Palonosetron + Aprepitant vs Granisetron): % of Patients						
	Acute (0-24h)		Delayed (24-120h)		Overall (0-120h)	
	P + A	G	P + A	G	P + A	G
Complete Response	92.3%*	67.9%	61.5%*	28.6%	61.5%*	28.6%
Vomiting	7.7%*	32.1%	38.5%*	71.4%	38.5%*	75.0%
Nausea						
- Significant (>25mm)	15.4%	14.3%	42.3%**	64.3%	46.2%	64.3%
- Any (>5 mm)	23.1%	35.7%	69.2%	71.4%	69.2%	71.4%

* P < 0.05, ** p < 0.1 (trend for significance)

0444

PROGNOSTIC IMPACT OF PRE-TRANSPLANT SERUM HEPCIDIN LEVELS ON CLINICAL OUTCOMES AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Iron overload is an adverse prognostic factor in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-SCT). Hepcidin, a peptide hormone produced by the liver, plays a central role in the regulation of iron homeostasis. We previously reported that elevated pre-transplant serum hepcidin levels were significantly associated with a higher incidence of early bacterial infection independent of ferritin levels (Kanda *et al.*, BBMT 2009). *Aim.* To investigate the association between pre-transplant serum hepcidin levels and clinical outcomes of allo-SCT. *Methods.* We retrospectively studied 100 patients (age, 17-64 years; median age, 48.5 years) who underwent their first allo-SCT for hematologic malignancies at our institution between July 2006 and December 2010. Written informed consent was obtained from all patients. The primary diseases were myeloid (n = 66) and lymphoid malignancies (n = 34). Serum hepcidin-25 levels prior to the administration of conditioning regimen were measured using a liquid chromatography-tandem mass spectrometry-based assay system. The primary endpoint was overall survival after allo-SCT, and the secondary endpoints were the cumulative incidence of acute and chronic graft-versus-host disease (GVHD), transplant-related mortality (TRM), and relapse. Factors evaluated in the analysis included the recipient's age, sex, diagnosis, disease status, source of stem cells, conditioning regimen, GVHD prophylaxis, serum hepcidin levels, ferritin levels, and C-reactive protein (CRP) levels. *Results.* The median hepcidin level of the patients was 29.3 ng/mL (range, 0.4-371 ng/mL; normal level, 22.2 ± 12.3 ng/mL). There was a weak correlation between hepcidin and ferritin levels (r = 0.217, $P = 0.030$). These patients were divided into 2 groups: the low-hepcidin group (<30 ng/mL, n = 50) and the high-hepcidin group (≥30 ng/mL, n = 50). Ferritin levels were higher in the high-hepcidin group than in the low-hepcidin group ($P < 0.001$). Patients in the high-hepcidin group had a significantly inferior overall survival at 2 years than those in the low-hepcidin group (49% vs. 69%, $P = 0.044$) (Figure), although the association was not significant in the multivariate analysis. Older age, high ferritin levels, and high CRP levels were significantly associated with lower overall survival rate in the multivariate analysis. A trend toward a higher incidence of grade 3-4 acute GVHD was observed in the high-hepcidin group (16% vs. 4%, $P = 0.078$). The incidences of chronic GVHD, TRM, and relapse were not different between the 2 groups. *Conclusions.* In the present study, elevated pre-transplant hepcidin levels were associated

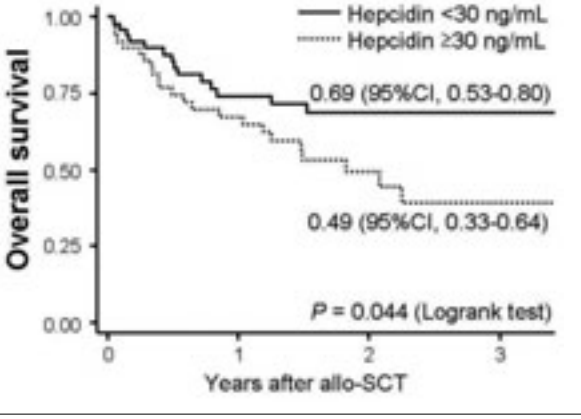

Figure 1.

with an inferior overall survival in the univariate analysis, but not in the multivariate analysis. Our data confirmed that elevated ferritin and CRP levels were strong adverse prognostic factors for survival, as many other studies have shown. In addition, we observed a tendency toward a higher incidence of grade 3-4 acute GVHD in the high-hepcidin group. Larger studies are necessary to elucidate the association between hepcidin levels and acute GVHD.

0445

EXTRACORPOREAL PHOTOPHERESIS FOR STEROID REFRACTORY OR DEPENDENT ACUTE GVHD IN PEDIATRIC PATIENTS

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Background. Acute graft-versus-host disease (aGVHD) is a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Glucocorticoids are the standard treatment for aGVHD. Patients who fail to respond to this first-line therapy have a poor prognosis, with high transplant-related mortality due to GVHD itself and to it's treatment complications such as opportunistic infections. Extracorporeal photopheresis (ECP) has been shown to be effective for patients with GVHD not responsive to conventional therapeutic approaches. We report our experience on ECP treatment in pediatric patients affected by aGVHD. *Aims.* To evaluate the efficacy of extracorporeal photopheresis for the treatment of steroid-refractory or steroid-dependent aGVHD in pediatric patients. *Methods.* From 1997 to 2009, 49 children (32 males, 17 females) with steroid-refractory or dependent aGVHD have been treated with ECP. Patients underwent HSCT for ALL (n=25), AML (n=15), NHL (n=4), CML (n=3) or other non-malignant diseases (n=2). The median age at ECP was 8.7 years (range: 0.95-18.7) and the median body weight was 29 Kg (range: 7-98). The stem cell sources were unrelated donors (n=32), siblings (n=11), cord blood (n=5) or haploidentical donor (n=1). The overall clinical stage of aGVHD was grade II (n= 24), grade III (n=13) and grade IV (n= 12). Cutaneous GVHD was diagnosed in 44 children, liver and gastro-intestinal tract involvement in 11 and in 43 children respectively. ECP was started after a median interval from HSCT of 42.5 days (range: 13-91) and after a median time from aGVHD onset of 28.5 days (range: 4-91). A Hickman-Broviac double-lumen central venous line was used in all patients. The median duration of treatment was 4.6 months (range: 0.5-10.2), with a median number of 10 cycles (range: 3-45). ECP was performed using the on-line technique (n=17) or using off- line technique (n=32). *Results.* 34 patients (69.4%) survived while 15 died (30.6%), due to relapse of the underlying disease (n=9), GVHD (n=3), CMV-related interstitial pneumonia (n=2) or encephalopathy (n=1). Among the 34 patients who survived, a complete, partial, or no response to ECP was seen in 79%, 0%, and 21% of patients with aGVHD II respectively, in 69%, 15% and 16% of patients with aGVHD III, and in 50%, 33% and 17% of those with GVHD IV respectively. A complete response of aGVHD manifestations of skin, gut and liver was observed in 75%, 74%, 91% of patients respectively. *Conclusions.* Our results confirm the efficacy of ECP in the treatment of steroid resistant or dependent aGVHD in pediatric patients. Moreover, for all skin, gut and liver, a good response to ECP was found.

0446

SIBLING CORD BLOOD TRANSPLANTATION FOR DIAMOND-BLACKFAN ANEMIA: THE EFFECT OF MINOR HISTOCOMPATIBILITY ANTIGENS ON GVHD

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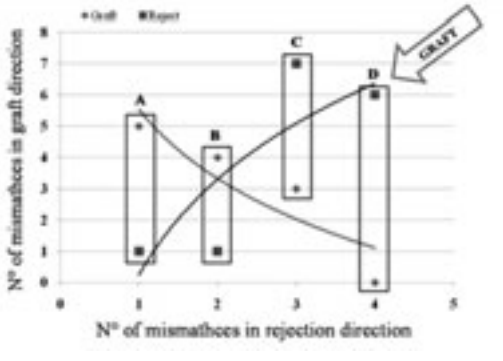
Diamond-Blackfan anemia (DBA) is a clinically and genetically heterogeneous condition characterized by pro-apoptotic hematopoiesis, congenital anomalies and predisposition to cancer. Cord blood (CB) stem cell transplantation is becoming an effective cure for DBA patients, especially if HLA-identical sibling donors are available. In transplantation setting, in addition to Major Histocompatibility Complex antigens (HLA), minor Histocompatibility Antigens (mHAgs) may affect the outcome in terms of engraftment, rejection and Graft versus Host Disease (GvHD). In HLA-identical sibling transplantation, mHAgs mismatches can stimulate T-cell activation, leading to specific alloreactivity against donor's or recipient's antigens, consequently supporting rejection or GvHD, respectively. We considered four DBA patients and their HLA-identical (HLA-A, -B, -DRB1) CB sibling donors to investigate the role of 12 polymorphic mHAg: HA-1, HA-2, HA-3, HA-8, HB-1, ACC-1, ACC-2, CD31 (codons 125, 563 and 670), CD62L (codons 206 and 213), PANE-1, UGT2B17, SP110 (mHA Minitray kit, University Clinic Heidelberg, Germany), and HY. The patients' characteristics and the cell dose of CB grafts are shown in the table. All patients reached full chimerism, and no-one experienced graft failure. At present, they are all alive and disease-free. Considering the two directions of mHAg mismatches (GvHD: donor versus recipient; rejection: recipient versus donor), we investigated the mHAgs-mismatching-grade in all patients with respect to GvHD, neutrophil and platelet recovery times. The only patient (D) who experienced aGvHD showed no mHAg mismatches in GvHD direction, while the other ones, with no signs of aGvHD, showed at least three mismatches in GvHD direction (see the graph). Moreover, going from patient A to patient D, the mHAg-mismatching-grade increases in rejection direction, as well as platelet recovery time augments (21 to 44 days), whereas the mHAg-mismatching-grade in GvHD direction decreases. Despite this kind of sample (homogeneous for HLA-identity, non-malignant condition, conditioning regimen and GvHD prophylaxis) could be suitable to investigate the mHAg effect on CB transplantation, its size is restricted. However, we put forward a few hypotheses. Data regarding patient D seem to be in contradiction with the classical histocompatibility assumption, which predicts GvHD according to HLA disparities in GvHD direction. Moreover, patient D received Treosulfan-based conditioning regimen, which is reported to be less toxic than Busulfan. At most, Treosulfan may help to overcome the mHAg-mismatching-grade in rejection direction, as it is associated to excellent engraftment results. Therefore, trying to explain the occurrence of aGvHD in patient D, the involvement of an autologous-GvHD syndrome can be reasonably hypothesized. Recent studies in human and animal models highlighted that an autoimmune syndrome can occur after bone marrow transplantation (BMT) between identical twins, or even after autologous BMT, and this syndrome seems to be pathologically identical to the GvHD after allogeneic BMT. Autologous-GvHD is a special autoaggression syndrome which may cause the exacerbation of GvHD. Two major factors may induct an autologous-GvHD: the failure to re-establish peripheral self-tolerance and the disruption of thymic-de-

Table 1.

Characteristics of DBA patients and cell dose of cord blood grafts				
Patient	A	B	C	D
Age at transplant (years)	2.02	9.03	11.03	8
Body weight (kg)	12	36.5	32	25
Conditioning regimen	BU+TT+FLU	BU+TT+FLU	BU+TT+FLU	TT+TREO+FLU
TBI	NO	NO	NO	NO
GvHD prophylaxis	Cs-A	Cs-A	Cs-A	Cs-A
NC infused (x10e7/Kg)	10.00	2.8	3	3.2
Neutrophils recovery day	11	15	15	14
Platelet recovery day	21	25	40	44
aGvHD (II-IV)	NO	NO	NO	YES, grade III
aGvHD day				22



pendent immune reconstitution. Further investigations are needed to confirm the role of mHAgs on GvHD, first of all by enrolling in the study an increased number of patients.

0447

EFFICACY OF MOBILIZATION WITH PLERIXAFOR IN PATIENTS FAILING A PREVIOUS MOBILIZATION ATTEMPT AND AS FIRST-LINE MOBILIZING THERAPY IN PATIENTS AFFECTED BY MULTIPLE MYELOMA

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Peripheral blood stem cells (PBSC) represent a well established viable alternative to bone marrow as a source of hematopoietic stem and progenitor cells for autologous transplantation, a standard approach for treatment of different hematological malignancies. A dose of 2.0x10E6 CD34+cells/kg is generally considered sufficient to allow a successful engraftment after high-dose chemotherapy. Nonetheless a high fraction of patients (pts) ranging from 11 to 53% will fail mobilization of stem cells and won't be able to benefit from autologous stem cell transplantation (ASCT). Plerixafor (AMD 3100) a bicyclam antagonist of the SDF-1 alpha/CXCR4 complex, has been previously reported to improve PBSC collection in pts undergoing PBSC mobilization. From April 2009 to February 2010, a total of 13 patients affected by hematological malignancies (5 Hodgkin Lymphoma, 5 Non-Hodgking Lymphoma, 3 Multiple Myeloma) who had already failed a previous mobilizing attempt, underwent stimulation with plerixafor at a standard dose after receiving G-CSF for 4 days in order to mobilize PBSC; other 6 patients who had never been mobilized before, received the same stimulation therapy following immunomodulatory drugs containing induction chemotherapy for multiple myeloma (MM). Pts characteristics were the following: 15 were female, 4 were male, median age was 53 years (27-70); median number of previous lines of therapy was 2 (1-7) and 4 pts had received radiotherapy. Overall plerixafor administration was safe and no serious adverse events were reported. The median number of circulating CD34+ cells/μl following plerixafor was 22 (11-138). All 19 patients were able to collect the minimum required dose for ASCT in a median number of procedures of 1 (1-3); median numbers of CD34+ cells collected was 2.5x10E6/kg; notably the 6 patients affected by MM, stimulated with plerixafor upfront were able to collect in a single procedure the target CD34+ dose to be used for a tandem transplant. At the time of the analysis, 13 of the 19 pts had already undergone ASCT: 13/13 engrafted with a median time to ANC≥500/ul of 12 days and to a PLT≥20000 of 16 days. We conclude that mobilization with plerixafor is safe and effective being able to rescue patients who failed a previous mobilizing attempt and likely being an effective mobilization strategy for pts with MM who need to collect a greater number of CD34+ cells/kg for a tandem transplant.

0448

MYELOABLATIVE CONDITIONING FOLLOWED BY UNMANIPULATED HAPLO-MISMATCHED MARROW FOR ADVANCED HEMATOLOGIC MALIGNANCIES

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Background. Haplomismatched marrow has been grafted successfully following a non myeloablative conditioning (NMA) and high dose cyclophosphamide (HDCY) post-transplant. HDCY is capable of preventing acute graft versus host disease (GvHD), despite the HLA mismatched haplotype (Luznick *et al*, BBMT 2008) *Aim of the study.* In this study we tested whether HDCY could also prevent aGvHD in a setting of myeloablative conditioning regimen. *Methods.* Patients were prepared with Fludarabine 50 mg/m^2/day x3, i.v. Busulfan 3.2 mg/kg/day x3; Thiotepa 5 mg/kg/dayx2. HDCY was given at 50 mg/kg day+3, day+5 Cyclosporin and mycophenolate were given from day -1. One patient was prepared with FLU-TBI. Marrow was harvested from haplo-mismatched family members according to standard procedures. *Results.* We have grafted 16 patients with leukemia beyond second remission: the diagnosis was AML (n=4), ALL (n=2), CML (n=2), lymphoma (n=3), myelofibrosis (n=1), myelodisplasia (n=3). Three patients were receiving a second allogeneic transplant. The median marrow cell dose given was 4.2x10^8/kg (range 2.7-7.8). Median day to 0.5x10^9/L neutrophils was day+20 (13-30). Two patients died of hemorrhage before day 7. Of the 14 evaluable patients all engrafted with 100% donor chimerism by day +30. Hematologic recovery was complete in all patients. GvHD was scored as grade I in 7 patients and grade II in one patient. No patients developed grade III-IV GvHD: CMV and EBV infections were not a problem in this initial series of patients. Transplant related mortality was seen in 4 patients (25%). 3 patients died of leukemia relapse. 11/16 patients survive (68%) 30-280 days post transplant. *Conclusions.* This initial series of patients suggests that (a) haplo mismatched unmanipulated marrow can be grafted after myeloablative conditioning; (b) hematologic recovery is reliable and complete (c) transplant mortality acceptable for a group of advanced patients and (d) most importantly transplants can be organized in useful time for advanced leukemia.

0449

HIGH BASELINE BAALC EXPRESSION AT DIAGNOSIS PREDICTS POOR POST TRANSPLANT OUTCOME IN PATIENTS WITH CORE BINDING FACTOR POSITIVE ACUTE MYELOID LEUKEMIA

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Background. High BAALC transcripts are associated with unfavorable outcome in newly diagnosed acute myeloid leukemia (AML) patients. *Aims.* We analyzed BAALC gene expression in patients with core binding factor (CBF) positive AML undergoing hematopoietic stem cell transplantation (HSCT) in order to assess whether this prognostic impact parallels with the post-transplantation outcome. *Methods.* Among fifty three consecutive adult patients diagnosed as CBF-AML between January 2008 and June 2010 in Catholic Blood and Marrow Transplantation Center, quantification of BAALC transcripts by real-time quantitative PCR in diagnostic bone marrow (BM) as well as assessments of FLT3, NPM1 and c-kit mutation status were performed in 41 patients. We retrospectively analyzed the treatment outcome on 31 CBF-AML patients who completed induction chemotherapy and received stem cell transplantation. *Results.* The median age at diagnosis was 45 years (range, 16-61). The median number of BAALC transcripts measured on diagnostic BM of available 41 samples were 62.293 (range, 9.587 - 497.4). All patients (n=31) achieved complete remission (CR) after 1 cycle of induction chemotherapy and remained so by the time of transplantation. The median BAALC transcript level after achieving CR reduced to 2.171(range, 0.251-31.604). At the time of transplantation after 1 or 2 courses of consolidation chemotherapy, the median transcript level was 1.1705(range, 0.291-34.174). Six patients (19%) had complex karyotype and c-kit mutation was detected in 11 patients while NPM in two patients. FLT 3 was negative in all patients. Thirteen patients (42%) received autologous stem cell transplantation while the remaining 18 patients underwent allogeneic HSCT; myeloablative and reduced intensity conditioning in 14 and 4 patients, respectively. The median follow-up duration of survivors was 12.25 months (range, 1.54-23.13). The estimated 1-year overall survival and disease-free survival of all patients were 68.7% (±10) and 65.9 % (±10), respectively. The 1-year cumulative incidence of treatment related mortality was 15.34% (±7.3) while that of relapse was 10.55% (±5.8). When the baseline BAALC transcript levels were dichotomized into high and low levels, as high level being upper third quartile, the higher level group had significant poor results in 1-year cumulative incidence of relapse.(4.3 % vs 37.5%, p-value=0.012). *Conclusions.* CBF-AML patients expressing high BAALC RNA levels at diagnosis had significantly higher risk of relapse after undergoing transplantation.

0450

ALLOGENEIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH MULTIPLE MYELOMA AT RELAPSE: A SINGLE CENTRE EXPERIENCE

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Background. The survival of patients with multiple myeloma (MM) has considerably improved over the past decade. Although allogeneic stem cell transplantion (allo-HSCT) may provide a curative option, its place in the therapeutic armamentarium has been very controversial.

Aims. We here report the clinical outcomes of patients with MM allografted at the Geneva University Hospital between 1988 and 2010. *Methods.* 23 patients (15 males and 7 females) were included in this retrospective analysis. Their median age at transplantation was 48 (range, 24-57) years and the median interval from diagnosis to allo-HSCT was 18 (range, 5-78) months. Sixteen (70%) patients had IgG, 3 (13%) IgD, 1 (4%) IgA and 3 (13%) light chains MM. Almost all patients (91%) were Salmon-Durie stage III and 12 patients (52%) were ISS stage III. Cytogenetics data were not available for this analysis. Most of the patients (n=18, 80%) were allografted at relapse: 11 (48%) at 1st, 4 (17%) at 2nd and 3 (13%) at 3rd relapse. The median number of chemotherapy lines prior to allo-HSCT was 2 (range, 1-4). Thirteen (56%) patients had received novel drugs and 14 (60%) had undergone an autologous transplantation prior to allo-HSCT. *Results.* Almost all patients (n=20, 87%) were transplanted with an HLA identical sibling donor. Twelve (52%) patients received a myeloablative (MAC) and 11 (48%) a reduced intensity (RIC) conditioning regimen. T-cell depletion of the graft was performed in 15 (65%) cases. At time of allo-HSCT, 9 (40%) patients were in CR/VGPR and 13 (60%) in PR/SD (1 missing). The median follow-up was 48 (range, 9-128) months. After allo-HSCT, 20 (92%) patients reached CR/VGPR. The incidence of acute grade>2 and chronic GvHD were 30% (SE + /- 20%) and 57% (SE +/- 22%), respectively. The incidence of transplant-related mortality (TRM) was 23% (SE +/- 20%). At 5 years, the progression free (PFS) and overall survival (OS) were 34% (SE +/-21%) and 59% (SE +/- 23%), respectively. We found no impact of chronic GVHD, conditioning regimen intensity, T-cell depetion and the number of prior treatment lines on PFS and OS. *Conclusion.* Although few patients were included in this study to allow a reliable estimation of clinical outcomes, our series suggest that high-risk patients may substantially benefit from allo-HSCT at relapse. Still, chronic GVHD and transplant related mortaliy remain the major limitations that likely compromise the allo-HSCT risk-benefit balance

0451

PLASMA HUMAN HERPESVIRUS 6 (HHV-6) DNA LOADS AND INTERLEUKIN-6 CONCENTRATION AS FACTORS IN THE DEVELOPMENT OF HHV-6 ENCEPHALITIS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Reactivation of human herpesvirus 6 (HHV-6) is associated with the development of encephalitis after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Recently, a surprisingly high incidence of HHV-6 encephalitis (5-12%) has been reported from many Japanese transplant units. Little is known about the pathogenesis of HHV-6 encephalitis. Several reports have shown that most HHV-6 encephalitis develops after an episode of pre-engraftment immune reaction or engraftment syndrome. Such findings suggest hypercytokinemia may play a role in the progression to encephalitis. *Aims.* To determine the roles of both HHV-6 and cytokines in the pathogenesis of HHV-6 encephalitis, we analyzed the kinetics of HHV-6 DNA and 17 kinds of cytokines and chemokines after allo-HSCT. *Methods.* This retrospective analysis involved 100 consecutive patients who received allo-HSCT in Oita University Hospital and Oita Prefectural Hospital. Among these, 6 patients (6%) developed HHV-6 encephalitis. HHV-6 DNA levels and concentrations of cytokines and chemokines in weekly collected plasma were evaluated until 70 days and 28 days after HSCT, respectively. HHV-6 DNA in plasma was quantified using real-time PCR and cytokine concentrations were quantified using ELISA. Measured cytokines were IL-1beta, L-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, interferon-gamma, MCP-1, MIP-1beta, and TNF-alpha. All study protocols were approved by the ethics committee of the Faculty of Medicine at Oita University, and written informed consent was obtained from each patient. *Results.* According to Kaplan-Meier analysis, the cumulative rate of positive results for plasma HHV-6 DNA by day 70 was 62.7%. Conversion of HHV-6 DNA concentrated around 3-4 weeks after HSCT. Median (range) peak HHV-6 DNA levels in 6 patients with HHV-6 encephalitis and 94 patients without HHV-6 encephalitis were 104,397 copies/ml (20,647-208,614 copies/ml) and 382 copies/ml (0-417,829 copies/ml), respectively (P = 0.0004). None of the 74 patients whose peak HHV-6 DNA was <10,000 copies/ml developed HHV-6 encephalitis, while 6 of 26 patients (23%) with peak HHV-6 DNA ≥10,000 copies/ml developed HHV-6 encephalitis. Examination of plasma cytokines concentrations showed that peak concentrations of IL-6, IL-8, IL-10, IL-13, MCP-1, and MIP1beta were significantly higher in patients who developed HHV-6 encephalitis than in patients who did not. In particular, IL-6 concentration was very high in patients who developed HHV-6 encephalitis (median, 1215 pg/ml *versus* 70.1 pg/ml, P = 0.0007). None of the 68 patients whose peak IL-6 concentration was less than double the median (155 pg/ml) developed HHV-6 encephalitis, while 6 of 32 patients (19%) with peak IL-6 concentration more than double the median developed HHV-6 encephalitis. Among the 6 patients who developed HHV-6 encephalitis, central nervous system dysfunction developed concomitant to peak HHV-6 DNA in each patient. Plasma IL-6 concentration peaked 1 week before the development of HHV-6 encephalitis in 5 patients and at the time of developing HHV-6 encephalitis in 1 patient. *Discussion.* Increased IL-6 before the development of HHV-6 encephalitis suggests the involvement of increased IL-6 production in the pathogenesis of HHV-6 encephalitis. Control of inflammatory conditions before engraftment may prevent the development of HHV-6 encephalitis.

0452

AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) FOR RELAPSED OR REFRACTORY HODGKIN'S LYMPHOMA (HL): A SINGLE-CENTER EXPERIENCE

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Background. Relapsing patients with HL are usually treated with intensive chemotherapy and ASCT since mid 1990s. Pretransplant cytoreductive chemotherapy regimens and supportive care changed during this period but it is not known whether this affects outcome. *Aims.* Analyze outcome of relapsing / refractory HL patients treated with ASCT in a single center between 1994 and 2010. Identify variables affecting outcome. *Methods.* Retrospective study performed by chart review. All patients received supportive care standard at the time of transplantation; pretransplant cytoreductive therapy varied, but all were conditioned using BEAM or BEAC. Areas not in CR prior to transplantation were irradiated after hematological recovery. *Results.* During this period 87 patients, 52 men and 35 women, 15-55 years old (median 30) with relapsed / refractory HL were autografted at our institution. Thirty-one were refractory, 26 in early relapse, 25 in late relapse and 5 had multiple relapses prior to transplantation. Sixty patients had nodular sclerosis, 22 mixed cellularity, 2 lymphocyte predominant HL and in 3 the type was unknown. With a median follow-up of 34 months 3-year and 5- year overall survival (OS) of the entire cohort are 78% and 66% and event-free survival (EFS) 66% and 60% respectively (Fig). HL type, response to last previous treatment (refractory vs. early relapse vs. late relapse vs. multiple relapses), pretransplant therapy (miniBEAM vs. DHAP vs. high-dose ifosfamide and mi-

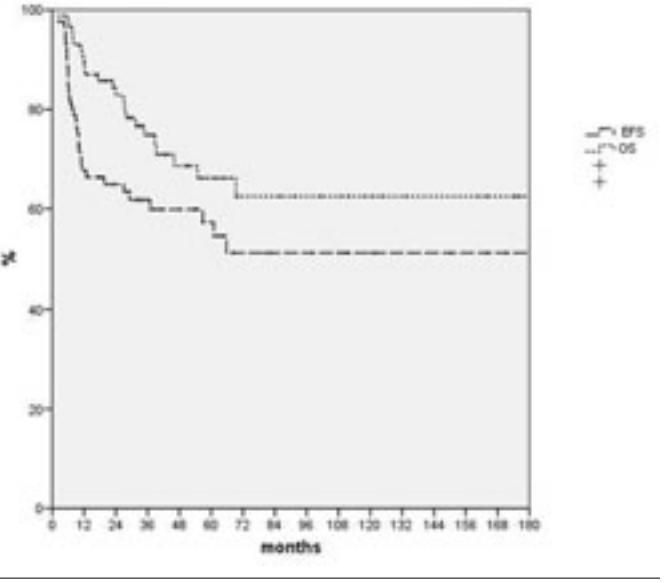

Figure 1.

toxantrone) and date of transplantation did not affect outcome. Older age at transplantation was a negative prognostic factor for OS but not EFS. The only statistically significant prognostic factor that we were able to identify was response to pretransplant cytoreductive therapy. In the group transplanted in CR, 3-year OS was 87% and EFS 85%, in PR 80% and 63%, in stable disease 52% and 20% and in progressive disease 25% and 25% respectively. This difference is highly statistically significant (p<0,001, log-rank test). *Conclusions.* Outcome of autografted HL patients has not changed significantly in the last 15 years. Sixty percent of these patients remain long-term free of their cancer. In our experience, response to pretransplant cytoreductive chemotherapy is a more important prognostic factor than response to last previous treatment.

0453

GASTROINTESTINAL SYMPTOMS FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION: MYCOPHENOLATE MOFETIL OR GRAFT-VERSUS-HOST-DISEASE?

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Background. Gastrointestinal (GI) complications occur frequently after allogeneic stem cell transplantation (alloSCT). Difficulties in confirming a diagnosis could emerge when mycophenolate mofetil (MMF) is used as part of the GVHD prophylaxis regimen, as it may cause gastrointestinal toxicity mimicking graft-versus-host-disease (GVHD). Currently, dose reduction or even discontinuation of MMF is the only way to clarify the origin of gastrointestinal symptoms, though this could be dangerous if GVHD is the cause of symptoms. Furthermore, there may be an increased risk of graft rejection associated with premature discontinuation of MMF. *Aim.* The aim of this study is finding markers that enable to differentiate between gastrointestinal symptoms as caused either by MMF or by GVHD. This would prevent unnecessary discontinuation of MMF in patients that suffer from GI GVHD and would better guide physicians in establishing which patients may require MMF discontinuation. *Methods.* All stored gastric and colonic biopsy specimens at the department of clinical pathology, that were taken between 2004 and 2009, of patients who suffered from GI symptoms and had received MMF after allogeneic SCT were reviewed. Additionally, clinical data were retrieved by review of medical records. Biopsies were scored for apoptosis, crypt cell destruction, inflammation of the lamina propria and denudation of the mucosal wall. Together these parameters constituted the histological grade of gastrointestinal damage. Clinical features contained clinical grade of GVHD, temporal relations between start of MMF and occurrence of symptoms, the effect of interruption of MMF and the final clinical diagnosis. *Results.* Biopsies of 54 patients were available. In 16 patients (29,6%) the gastrointestinal symptoms were assigned to MMF, in 34 patients (63%) to GVHD and in four patients (7,4%) to infection. Based on a 7-level scale scoring epithelial apoptosis (no apoptosis=0, none-minor=1, minor=2, minor-intermediate=3, intermediate=4, minor-severe=5, intermediate-severe=6 and severe=7) the mean score (SD) in the MMF group was 3,31 (1,78) and in the GVHD group 4,47 (1,88). This difference was statistically significant (p=0,047). No other significant differences were revealed by review of the histological specimens. *Summary/conclusions.* In almost 30% of patients who had histological evidence of gastrointestinal damage, the symptoms were designated as MMF-induced. Histological differentiation between gastrointestinal toxicity due to either MMF or GVHD may be supported by scoring the grade of epithelial apoptosis, which could therefore be important in deciding whether to stop MMF or not.

0454

BK-RELATED HAEMORRHAGIC CYSTITIS IN ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANT: A SINGLE CENTER EXPERIENCE

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Background. Haemorrhagic cystitis (HC) is considered a primary manifestation of BK virus (BKV) infection in patients undergoing allogeneic haematopoietic stem cell transplantation (allo-SCT). Roughly 50-100 % of all allo-SCT recipients develop BK viruria, while only 5-40 % progress to HC, which is associated with significant morbidity and mortality. *Aims.* To evaluate the incidence, associated risk factors and clinical impact of HC in a single centre series during a three-year period (2008-2010). *Methods.* All consecutive Allo-SCT performed at our unit during the period of study were evaluated prospectively for HC. All patients received the same graft-versus-host-disease (GVHD) prophylaxis. BK viruria and BK virus load in plasma were systematically recorded, along with a set of clinical variables: age, sex, underlying diagnosis, type of donor, source of stem cells, conditioning regimen, presence of symptoms, grade of GVHD, HIV status and absolute neutrophil count. Samples of urine and plasma were tested for BKV by PCR. Kaplan-Meier method was used for survival analysis, and log-tank test for differences in overall survival (OS). Statistical analysis were performed with SPSS v 15.0 package. *Results.* 37 patients had an allo-SCT during the period of study, four of them were HIV positive. The median age was 41 (17-63), 24 men (64.9 %) and 13 women (35.1 %). 13 patients (35.1 %) developed HC between 4 and 115 days after allo-SCT: 10 of 24 men (41.6 %) and 3 of 13 women (23 %); only one of the 13 HC patients was HIV positive; 3 of 5 (60%) had grade ≥3 and 10 of 32 (31.2%) ≤ 2 GVHD; 3 of 14 (21.4 %) and 10 of 23 (43.5 %) had a related o unrelated donor, respectively. 4 patients had only dysuria while 20 patients had no genitourinary symptons. A significative BK viruria presented in 10/20 (50 %) in asymptomatic group, 3 of 4 (75 %) in de group of dysuria and 12 of 13 (92.3 %) in the HC group. Median survival (see figure 1) was 12.3 months (2.7-21.8) and 8.2 (1.9-14.5) in the non-HC and HC groups, respectively (p=0.113). *Conclusions.* HC is a serious and frequent event in the Allo-SCT setting. We confirm a tendency to HC in patients with unrelated donors and severe GVHD. Every effort should be made to minimize this complication in order to improve transplant-related mortality.

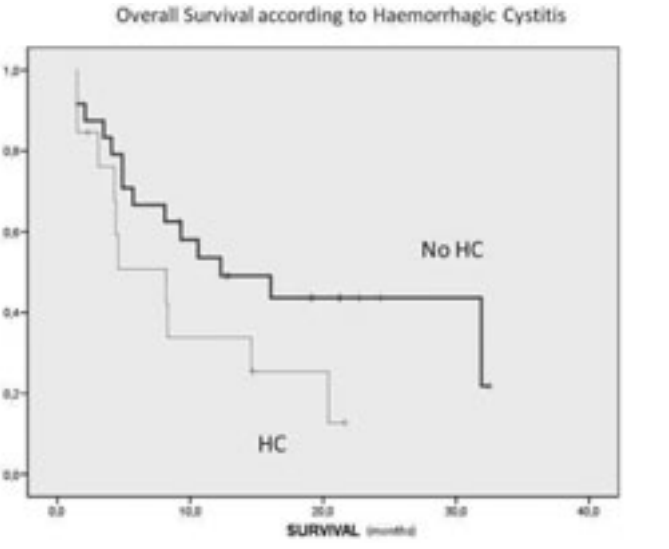

Figure 1.

0455

IMPACT OF INFLAMMATORY CYTOKINE GENE POLYMORPHISMS ON DEVELOPING ACUTE GRAFT VERSUS HOST DISEASE IN CHILDREN RECEIVING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Acute Graft Versus Host Disease (aGVHD) is one of the major causes of morbidity and mortality in the first 100 days after Allogeneic Hematopoietic Stem Cell Transplantation (HSCT). It can occur, despite aggressive immunosuppressive prophylaxis, even when the donor is a "perfectly"matched (HLA-identical) sibling, suggesting that the risk of developing this complication does not only depend on HLA matching. Many recent studies have reported the association between inflammatory cytokine gene polymorphisms and the occurrence of aGVHD in transplanted adults, but few data are available about the pediatric population. *Aims.* The aim of this study is to analyze the association between a wide panel of inflammatory gene polymorphisms and the occurrence of aGVHD in a setting of pediatric HSCT from HLA-

Table 1.

Characteristics of Patients and Donors		Number 136	Percent 100.0
Patient characteristics			
Sex	Male	94	69.1
	Female	42	30.9
Median age (range)		9 (1-18)	
GVHD Prophylaxis	MTX+CsA+ATG	81	59.6
	CsA	44	32.3
	Ex-vivo TCD	11	8.1
Underlying disease	ALL	53	39.0
	AML	24	17.6
	SAA	11	8.1
	NBL	10	7.4
	ß-Thalassemia	8	5.9
	NHL	6	4.4
	ES	6	4.4
	RMS	6	4.4
	HL	3	2.2
	MDS	2	1.5
	JMML	2	1.5
	HLH	1	0.7
	WT	1	0.7
	Blackfan-Diamond anemia	1	0.7
	Sickle cell disease	1	0.7
	Thrombocytopenia	1	0.7
Donor characteristics			
Sex	Male	61	45
	Female	75	55
Median age (range)		26 (0-53)	
Tissue compatibility	MFD	44	32.3
	MUD	49	36.0
	MMD	43	31.6
Cell type	Bone marrow	96	70.6
	Peripheral stem cells	32	23.6
	Cord blood	6	4.4
	Bone marrow + peripheral stem cells	1	0.7
	Bone marrow + cord blood	1	0.7

GVHD: graft-versus-host disease; MTX: methotrexate; CsA: cyclosporine A; ATG: anti-thymocyte globulin; TCD: T-cell depletion; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; SAA: severe aplastic anemia; NBL: neuroblastoma; NHL: non-hodgkin lymphoma; ES: Ewing sarcoma; RMS: rhabdomyosarcoma; HL: hodgkin lymphoma; MDS: myelodisplastic syndrome; JMML: juvenile myelomonocytic leukemia; HLH: hemophagocytic lymphohistiocytosis; WT: Wilms tumour; MFD: matched family donor; MUD: matched unrelated donor; MMD: mismatched donor.

matched family and unrelated donors (MFD and MUD). *Methods.* The study population consisted of 136 children who underwent HSCT at the Pediatric Hematology-Oncology of the Bologna University between 1995 and 2010 and their respective donors. Patient (pts) and donors characteristics are resumed in Table 1. Genotypes of 38 single nucleotide polymorphisms (SNPs) in 19 immunoregulatory genes known to be related to GVHD onset risk (ESR1, FAS, FCGR2A, IL1A, IL1B, IL2, IL6, IL10, IL10RB, IL18, MBL2, MTHFR, NOD2, TGFB1, TGFBR2, TLR4, TNF, TNFRSF1B and VDR) were determined by the Sequenom MassARRAY system. Results were checked for Hardy Weinberg equilibrium and filtered to exclude SNPs with minor allele frequency < 0.15. Association with aGVHD was analysed in a case-control study format by Chi-square statistics, with OR ±95%CI estimation, using SNPator package (http://www.snpator.org). *Results.* aGVHD (grade I-IV) was observed in 17/44 (38%) pts and 53/92 (57%) receiving a MFD and a MUD respectively. In the whole cohort of pts we found statistically significant associations between recipient IL10 592A and 819T single nucleotide polymorphisms (SNPs) (P=0.02, OR 2.1, CI: 1.10-4.08) and donor TNF +488C (P=0.03, OR 1.86, CI: 1.03-3.37) with grade I-IV aGVHD. The same association between recipient -592A and -819T polymorphisms of the IL10 gene was confirmed even in the sole MFD cohort (P=0.04, OR 2.94, CI: 1.01-8.57). In this latter cohort also donor -889C SNP of IL1A was associated with aGVHD grade I-IV (P=0.04, OR 2.76, CI: 1.03-7.40). Regarding the MUD cohort, a statistically significant association with grade I-IV aGVHD was observed for donor IL1B 3954T and donor and recipient TNF+488C and -857C (P=0.01, OR 3.03, CI: 1.25-7.36; P=0.003, OR 3.29, CI: 1.45-7.49; P=0.01, OR 2.87, CI: 1.23-6.66, respectively). *Conclusions.* Polymorphisms in genes of clear importance in the regulation of the inflammatory response such as TNF, IL1A, IL1B and IL10 play a crucial role in the risk of developing aGVHD in the pediatric population. In particular in the setting of HSCT from MFD, where a perfect high resolution HLA-matching is warranty, IL10 gene SNPs in the recipient can predispose to the risk of developing aGVHD.

0456

CXCL10 CONTRIBUTES TO THE PATHOGENESIS OF CHRONIC SKIN GVHD BY RECRUITING CXCR3+ T CELLS

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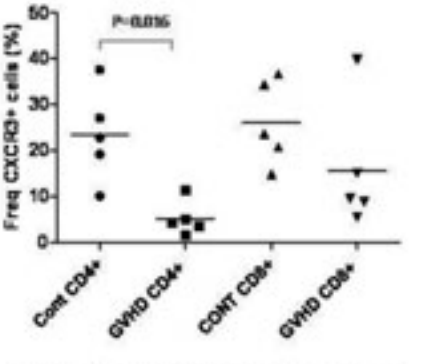
Background. Chronic Graft versus host disease (cGVHD) remains a major cause of morbidity and mortality following allogeneic stem cell transplantation (SCT), affects up to 60% of patients who survive 100 days post transplant and significantly impacts upon quality of life. Novel therapies for the treatment of cGVHD are required. The chemokine-receptor axis represents an important mechanism by which lymphocytes traffic to target organs and we therefore wished to explore this pathway in the pathogenesis of chronic GVHD. *Aims.* The study aimed to identify key chemokines associated with the pathogenesis of tissue-specific cGVHD post allogeneic SCT and to determine whether they represent potential therapeutic targets. *Methods.* Patients were recruited onto the study following informed consent prior to undergoing allogeneic SCT. Peripheral blood samples were taken both pre and post transplantation. Additional blood and fresh skin biopsy samples were also received at the time of skin cGVHD. A panel of cytokines and chemokines were analysed by luminex technology in the serum of patients at the time of chronic disease (n=40), and compared to those who did not develop clinical evidence of chronic GVHD (n=18). Equal proportions of patients who underwent sibling and MUD allografts, and who underwent myeloablative and nonmyeloablative allografts were included. Patients included in the cohort had evidence of cGVHD of tissues including the skin, gut, liver, oral and ocular mucosa and the lungs. In addition chemokine receptor expression was then assessed in both the peripheral blood and tissues using 9 colour flow cytometry and immunohistochemistry. *Results.* The CXCR3 specific chemokines CXCL9, 10 and 11 were found to be significantly associated with chronic GVHD of the oral mucosa, skin and eye, and with pulmonary cGVHD respectively, being elevated in the serum of patients at the time of cGVHD. In particular CXCL10 was elevated from approximately 170pg/ml to 350pg/ml in both skin and ocular disease ($p<0.05$). CD4+ CXCR3+ T cells were reduced in the peripheral blood of patients with skin disease from approximately 23% of CD4+ T cells in control patients to just 5% in patients with skin cGVHD (p=0.02, n=5). Levels of both CD4+ and CXCR3+ T cells were also elevated in the skin of patients with cGVHD with CD4+ cells being increased from approximately 16% (n=7) of the T cell population to 38% (n=8) and CXCR3+ cells from 50% to 63% of the T cell population. The nature of conditioning and transplant did not appear to affect the levels of CXCL10 in the serum of patients post 100 days post SCT, and regulatory T cell populations in the peripheral blood expressing



A role for CXCL10/CXCR3 in chronic GVHD pathogenesis; Reduced peripheral blood frequencies of CD4+ CXCR3+ T cells as identified by flow cytometry in patients with chronic skin GVHD compared to patients post SCT without cGVHD.

Figure 1.

CXCR3 were not significantly reduced. *Summary/Conclusions.* The data suggests that CXCL9-11 are associated with cGVHD, and that elevated levels of CXCL10 may result in the migration of effector T cell populations from the blood to the skin where they can cause tissue damage. CXCL10 thus represents a specific chemokine which could be targeted to both prevent or treat the symptoms of cGVHD of the skin.

0457

GENETIC VARIABILITY AT LOCI CONTROLLING GLUTATHION HOMEOSTASIS AFFECTS TRANSPLANT RELATED MORTALITY AND SURVIVAL IN PATIENTS RECEIVING AN ALLOGENEIC HSCT AFTER A BUSULFAN-BASED CONDITIONING REGIMEN

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Background. Busulfan is the most widely used drug for allogeneic conditioning regimens. Busulfan metabolism depends on liver glutathion (GSH) availability. A number of loci controls liver GSH synthesis and consumption, occurring during drug conjugation and oxidative stress response. *Aims.*The impact of polymorphisms at loci involved in GSH balancing on overall survival (OS) and transplant related mortality (TRM) was tested. *Methods.* Here we report on a study population of 331 consecutive patients who received an allogeneic HSCT for haematological malignancies at Institute of Hematology "Seràgnoli"from 2004 onwards. 185 patients received busulfan in the conditioning regimen, mostly at myeloablative intensity, while 146 received a TBI based regimen or a reduced intensity conditioning regimen non busulfan-based; clinical variables were similarly distributed in the two groups. A total of 35 polymorphisms (32 SNPs and 3 insertion/deletions) at 15 candidate genes were analysed by high throughput mass array Sequenom TM platform or by DHPLC. *Results.* We found that a C to G rs2180314 SNP at Glutahione Transferase A2 (GSTA2) locus (Codon 112 which leads to a Ser to Thr aminoacidic transition) impacts OS and TRM in the whole population (CC vs G-carriers: HR=1.604, 95%CI=1.081-2.381, p=0.019 for OS and HR=1.992, 95%CI=1.100-3.609, p=0.023 for TRM). Such an effect was particularly evident in patients who received busulfan (CC vs G-carriers: HR=2.438, 95%CI=1.446-4.108, p=0.0008 for OS and HR=4.580, 95%CI=2.005-10.461, p=0.0003 for TRM). No effect was present in the group not receiving busulfan. The polymorphism at microsomial GST-1 promoter (rs7970208) also affects OS and TRM, although to a lesser extent (AA vs G-carriers: HR=1.405, 95%CI=1.076-1.835, p=0.012 for TRM and HR=1.255, 95%CI =1.050-1.499, p=0.012 for OS). *Summary/Conclusions.* These data point out that genetic variability at loci controlling GSH balancing may affect allogeneic HSCT outcome in busulfan treated patients. These data could be validated on patients populations belonging to controlled clinical trials with the aim to predict toxicity after allogeneic HSCT.

0458

GPI-ANCHOR NEGATIVE MEMORY T CELLS IN PATIENTS AFTER ALEMTUZUMAB-MEDIATED T-CELL DEPLETED HEMATOPOIETIC STEM CELL TRANSPLANTATION

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The anti-CD52 antibody is frequently used in allogeneic hematopoietic stem cell transplantation (HSCT) for *in vivo* T cell depletion (TCD). We have recently shown that CD52 negative donor T-cells reconstitute in patients after HSCT following alemtuzumab-mediated TCD. These T cells persist for years in the peripheral blood. We have also demonstrated that the application of donor lymphocyte infusion (DLI) has the capacity to replenish the CD52-positive T cell compartment. By staining with a fluorescent aerolysin, we have demonstrated that the lack of CD52 expression originates from a loss of glycosyl-phosphatidyl-inositol (GPI) anchors in the T-cell membrane. GPI-anchor negative T cells revealed an altered antigen specific function compared to GPI-anchor positive T cells: They secreted less IFN gamma in response to CMV peptides and allogeneic stimuli. They also showed a decreased lytic response and proliferative capacity. We further stained peripheral blood T-cells from 12 patients of different age with the surface markers CD45RA, CD45RO, CD62L and CCR7 to differentiate memory and naïve CD4 and CD8 T cells. Early after transplantation, the T cells were only of memory phenotype reflecting the proliferation of graft-derived memory T cells. The expression of CD45RO, CD62L or CCR7 did not differ between GPI-anchor positive and negative T cell subpopulations. In many patients, we detected CD45RA-positive naïve T cells later after transplantation even though the time of reconstituting naïve T cells strongly differed between individual patients. The newly reconstituting naïve T cells were always GPI-anchor positive. At the same time, GPI-anchor negative cells were still present among memory T cells. Our data promote the hypothesis, that graft derived GPI-anchor negative memory T cells persist and proliferate in patients following alemtuzumab-mediated T-cell depletion in the context of allogeneic HSCT. The GPI-anchor negative T cells present with an altered antigen specific T cell function. We hypothesize that naturally occurring GPI-anchor negative T cell sub-populations expand under the selective pressure of the anti-CD52 antibody alemtuzumab. These T cells persist for years by homeostatic proliferation. In those patients, were naïve T cells reconstitute from the stem cell compartment, the new naïve T cells are GPI-anchor positive. Since GPI-anchor negative T cells are functionally altered, prophylactic DLI application might help to bridge the state of impaired T cell function through provision of GPI-anchor positive memory T cells.

0459

GENETIC VARIABILITY IN INNATE IMMUNE GENES (NLRP2, NLRP3, TGFB1) IMPACT THE ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) CLINICAL OUTCOME AND INFLUENCE THE INFLAMMATORY CYTOKINE PROFILE PRODUCTION

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Background. Graft versus leukemia effect (GvL) is the main advantage in the allogeneic stem cell transplantation (allo-SCT) setting. However in spite of this, the inflammatory response due to the conditioning regimen and the graft versus host disease (GvHD) are responsible for the transplant related mortality (TRM) incidence. Previous results by our group have shown variants of innate immune genes associated with GVHD, with the incidence of leukemic relapse, and with TRM after allo-SCT. We speculated that the effect of these polymorphisms of innate immune genes was due to an influence on the inflammatory cytokine profile production. *Aims.* To determine the inflammatory cytokine profile in healthy individuals harbouring single nucleotide polymorphisms (SNPs) in genes involved in innate immunity (*NLRP3*, *NLRP2*, *TGFB1*, *IRF3*) which were previously associated with the incidence of relapse, TRM and chronic GvHD in a series of HLA-identical sibling allo-SCT (n=198). *Methods.* Study population for the functional studies consisted of 120 healthy donors. Peripheral blood (PB) was stimulated with phytohaemagglutinin (PHA), incubated overnight at 37°C, and serum was collected by centrifugation 15 min at 2,500 RCF. ELISA was performed to determine the IFN-γ production, whereas IFN-α, IL12, IL13, IL15 and IL17 production were determined using the Human Cytokine II 5-Plex (Invitrogen). *Results.* CC recessive gene variant in rs10925027 in donor in *NLRP3*, associated with lower relapse incidence, was responsible for higher IFN-γ (103 *vs* 171 pg/mL, p=0.029) and lower IL12 (52 *vs* 89 pg/mL, p=0.05) production in healthy individuals. GG dominant variant in rs1043684 in donor in *NLRP2*, associated with higher TRM, was responsible for higher IL12, IL-13 and IL17 production in healthy donors (85 *vs* 88 *vs* 47 pg/mL, p=0.04; 128 *vs* 72 *vs* 43 pg/mL, p=0.038; and 84 *vs* 60 *vs* 47 pg/mL, p=0.04, respectively). Finally, GG recessive gene variant in rs2282790 in donor in *TGFB1*, associated with higher incidence of cGvHD, was responsible for higher IFN-γ production (116 *vs* 116 *vs* 131 pg/mL, p=0.049). *Conclusions.* SNPs in rs1043684 in *NLRP2*, causing high levels of inflammatory cytokines correlate with higher TRM, whereas SNPs in rs10925027 in *NLRP3* and in rs2282790 in *TGFB1* causing high levels of IFN-γ correlate with lower relapse and higher cGVHD incidence. These gene variants could be used as predictors of development of cGVHD and inflammatory complications after allo-SCT.

0460

THE ROLE OF HISTONE DEACETYLASE INHIBITORS IN HEMATOPOIETIC STEM CELL EXPANSION

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Histone deacetylase inhibitors (HDIs) are a new class of potential anticancer agents that are also active against leukemic cell lines and primary leukemia cells. The list of this class of drugs is growing and several are now in phase I/II clinical trials. Although the mechanism of action of HDIs on cancer cells may include enhancement of apoptosis, induction of cell cycle arrest and promotion of cellular differentiation, the exact mechanism is not known. Interestingly, researchers have found that in *ex vivo* expansion cultures addition of HDIs to the cultures delayed differentiation and enhanced stem cell numbers. Therefore, we investigated the effect of HDIs and other small molecules on normal hematopoietic stem cells in expansion cultures. To study this, we used a differentiation model where murine Lin-Sca-1+c-kit+ (LSK) cells are forced to differentiate in response to SCF and GM-CSF. It was found that Valproic Acid (VPA), together with LiCl, delayed hematopoietic stem cell (HSC) differentiation. Morphology and several *in vitro* and *in vivo* assays suggested that VPA and LiCl synergistically delayed or prevented differentiation and this was found also on the level of gene expression. Li did not significantly change gene expression during 7 days of culture, VPA affected expression of approximately 100 genes and the combination lead to altered expression of more than 300 genes. The transcriptional program associated with multilineage differentiation has been affected. Moreover, the combination of VPA and Li enhanced the expression of multipotency- associated genes but reduced the expression of differentiation- associated genes. Our preliminary data also suggest that VPA delayed myeloid and lymphoid differentiation, whereas it enhanced erythroid differentiation. We further investigated whether other HDIs were able to modulate GM-CSF-induced differentiation. Therefore, we have chosen 6 different HDIs, VPA, sodium butyrate, trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA), MS-275 and apicidin and compared their effects. All HDIs influenced cell growth, stem cell phenotype and delayed stem cell differentiation in a dose-dependent manner. Strikingly, expression of the mouse stem cell antigen (Sca)-1 could be upregulated by HDIs and this correlated with an increase in progenitor cell function. Progenitor cells were found to be an important target cell for HDI action because they increased their proliferative capacity in colony and single cell assays. This research implies that HDIs may be used to improve HSC expansion protocols by delaying differentiation and enhancing the potency of hematopoietic progenitors.

0461

REGULATORY T CELLS USE AN IL-10 INDEPENDENT, CAMP DEPENDENT PATHWAY TO SUPPRESS GRAFT-VERSUS-HOST DISEASE

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Allogenic hematopoetic stem cell transplantation (HSCT) is a common curative treatment option for many hematological malignancies. Graft-versus-host disease (GvHD) is a significant contributor to morbidity and mortality after HSCT. A possibility to control GvHD is the use of regulatory FoxP3+ CD4+ T cells (Treg) which are able to suppress conventional T cell activation. We used an acute MHC mismatch mouse model to analyze Treg dependent mechanisms of GvHD amelioration transplanting BALB/c recipients with bone marrow and CD90.2+ T cells from C57BL/6 donors. The resulting severe GvHD was strongly attenuated by cotransfer of donor Treg cells. By *in vivo* studies with IL-10-/- donor and recipient mice we show an important role for IL-10 in moderating GvHD. Following *in vitro* studies adressing the suppressive mechanisms used by Treg reveal that this suppression occurs independent of Treg derived IL-10. In this case Treg cells utilize gap junction intercellular communication (GJIC) to suppress allogenic dendritic cell activation. Inhibition of the cAMP degradating enzyme phosphodiesterase 4 (PDE4) by the drug Rolipram leads to enhanced suppressive capacities of Treg cells suggesting an important role for cyclic adenosine monophosphate (cAMP). In conclusion, we demonstrate important yet distinct roles for IL-10 and Treg cells in suppressing GvHD: Treg mediated suppression of GvHD is IL-10 independent, but contact dependent and can be modulated by drugs modulating cAMP metabolism. Our results may provide the basis for an advanced understanding of the role Treg cells play and pave the way for combined cellular and drug therapies to overcome current limitations of allogenic HSCT. Michael.Weber@unimedizin-mainz.de

0462

THE PHENOTYPICALLY NAIVE T CELL SUBSET OF HEALTHY DONORS CAN BE USED TO EXPAND ACUTE MYELOID LEUKEMIA-REACTIVE CD4+ T CELLS IN VITRO

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Background. In allogeneic hematopoietic stem cell transplantation (allo-HSCT), donor-derived T lymphocytes recognize leukemia-associated and minor histocompatibility antigens (mHag) on recipient leukemia cells, thereby mediating the graft-versus-leukemia (GvL) effect. Here, previous research has mainly focused on CD8+ cytotoxic T cells as GvL effectors. Other studies have demonstrated that CD4+ T cells are not only required to provide help for CD8 responses, but can exhibit direct cytolytic reactivity against leukemia cells. Furthermore, leukemia-reactive CD4+ T cells have been isolated from allo-HSCT patients upon GvL responses, and were successfully used to identify HLA class II-restricted mHag. *Aim.* We aimed at extending our recently developed protocol for CD8+ T cells (Distler *et al*., Exp Hematol 2008; Albrecht *et al*., Cancer Immunol Immmunother 2011) to the *in vitro* generation and expansion of acute myeloid leukemia (AML)-reactive CD4+ T cells from the naive donor T cell repertoire. *Methods.* Naive CD4+ T cells were isolated from PBMC of healthy donors either immunomagnetically (Naive CD4 T Cell Isolation Kit, Miltenyi Biotec) or by FACS sorting according to expression of CD45RA. T cells were stimulated in 96-well mini-mixed lymphocyte-leukemia cultures ("mini"-MLLC) with HLA-DR and -DQ matched irradiated AML blasts isolated from patients at initial diagnosis. AML blasts were pre-cultured overnight in medium only, or for 4 days in medium supplemented with IL-4, GM-CSF, SCF and TNF-α to improve the antigen-presenting cell phenotype. After 2 weekly re-stimulations with AML blasts, mini-MLLCs were screened in split-well IFN-γ ELISpot assays for reactivity against stimulator cells. Leukemia-reactive populations were expanded by further re-stimulations with AML blasts and were characterized for cytokine production, HLA restriction, cytolytic activity, and T cell receptor (TCR) Vβ chain usage. *Results.* In 2 out of 3 AML patient/donor pairs with full HLA-DR and -DQ match several CD4+ T cell populations with strong and persistent AML reactivity could be expanded to cell numbers exceeding >108. Reactivity of these T cells was restricted by HLA-DR, -DQ or -DP alleles as determined by using specific HLA blocking antibodies in IFN-γ ELISpot assays. Since HLA-DP was not yet typed, we cannot exclude allo-HLA-DP mismatch reactivity for HLA-DP-restricted T cell populations. T cells recognized either solely AML blasts, or also EBV-transformed B cells of patient origin in IFN- ELISpot assays. Donor-derived EBV-B cells or K562 cells were not recognized. Chromium-release assays showed that CD4+ T cells lysed primary AML blasts only at moderate levels. Leukemia-reactive CD4+ T cell populations expressed either TCRs with Vβ chains of a single family, indicating clonality, or were oligoclonal with up to 4 different TCR Vβ chains. *Summary/Conclusions.* We show herein that leukemia-reactive CD4+ T cells can be readily isolated and expanded from naive precursors of HLA class II-matched healthy individuals by *in vitro* stimulations with primary AML blasts. Our current effort is to optimize the protocol in further patient/donor combinations. We also plan to investigate the *in vivo* behavior of AML-reactive CD4+ T cells in immunodeficient NOD/SCID-IL2R (null) mice. Moreover, well-expanded leukemia-reactive CD4+ T cell clones can be used to identify potential target antigens for AML immunotherapy.

0463

COMBINATION CELL THERAPY OF EX-VIVO EXPANDED REGULATORY T CELLS AND HUMAN ADIPOSE TISSUE-DERIVED MSCS EFFECTIVELY INHIBITS ACUTE GRAFT-VERSUS-HOST DISEASE IN MURINE MODEL

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Background. Graft-versus-host disease (GVHD) remains the major barrier to the success of allogeneic hematopoietic stem cell transplantation (HSCT). The immunomodulatory effects of MSCs have been proposed as a promising treatment for acute GVHD as well as experimental encephalomyelitis and diabetes. However, the specific mechanisms involved in the immunoregulatory activity of MSCs remain unknown. Although the therapeutic effects of MSCs have varied in preclinical models those were closely related to the involvement of CD4+CD25+Foxp3+ regulatory T cells (Tregs) regardless of antigen specificity. Our previous study showed a negative effect of single infusion of MSCs on a murine model of collagen-induced arthritis (CIA) because MSCs alone did not prevent proinflammatory cytokines. Therefore, the combination cell therapy of *ex-vivo* expanded Tregs and MSCs may be expected synergistically to inhibit acute GVHD in allogeneic HSCT. *Aims.* The present study is aimed to evaluate the therapeutic efficacy of combination cell therapy of *ex-vivo* expanded regulatory T cells and human adipose tissue-derived mesenchymal stem cells (hAd-MSCs) to prevent acute GVHD in murine model. *Methods.* To obtain Treg cells, isolated CD4+ T cells from recipients (BALB/c) were cultured with anti-CD3 (1 ug/ml), anti-CD28 (1 ug/ml), human recombinant transforming growth factor (5 ng/ml) and all-trans retinal (1 uM) for 3 days. In murine models of GVHD, lethally irradiated BALB/c mice are transplanted C57BL/6 BM cells on day 0. Following transplantation, recipients after given once a week 4 times combination of hAd-MSCs (RNLBIO, Seoul, Korea) and Tregs or a single hAd-MSCs injection. All animals were monitored for survival and clinical signs of GVHD. *Results.* The results showed that combination cell therapy of hAd-MSCs and Tregs can act remarkably protected recipients from lethal GVHD and prevented severe tissue damage after allogeneic BM transplantation. A single infusion of hAd-MSCs was less effective than those in preventing GVHD. These therapeutic effects were associated with an increase of Th2 and Tregs for suppressive effect against activated T cells, and decrease of Th1 and Th17 cells. *Conclusions.* These data indicate that combination cell therapy of *ex-vivo* expanded regulatory T cells and hAd-MSCs effectively inhibits acute graft-versus-host disease in murine model. In addition, the results of the ongoing clinical trials will properly assess the therapeutic potential of hAd-MSCs and Tregs.

SIMULTANEOUS SESSION I

Multiple Myeloma - Biology

0464

PHENOTYPIC AND FUNCTIONAL DIVERSITY AND *IN VIVO* CHEMORESISTANCE IN MULTIPLE MYELOMA

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Background. Multiple myeloma (MM) is characterised by accumulation of malignant plasma cells (PC) in the bone marrow (BM). Although it is well established that acquired genetic events in co-operation with the tumour microenvironment activate proliferation and survival pathways thus driving tumour growth, the identity and functional properties of the myeloma propagating cells have been a matter of controversy. *Aims.* To establish the phenotypic diversity in MM and study its functional and clinical significance *ex vivo* and *in vivo*. *Methods.* Multicolour flow-cytometric analysis and sorting were performed on BM and peripheral blood samples using LSRFortessa and FACSAriaII sorter respectively. Clonotypic cells, bearing the patient-specific IgH CDR3 area were quantified within sorted cellular compartments using a highly sensitive genomic DNA Taqman qPCR. Flow-cytometry cell cycle analysis was performed after DAPI staining, while transcription factor mRNA levels were measured by qPCR. For *in vivo* assays, 8-10-week old NOD/SCID/IL-2Rγ-/- (NSG) mice were transplanted with highly purified primary myeloma cell populations. *Results.* Using flow-sorting combined with IgH CDR3 qPCR in a cohort of 30 patients, we established that the immunophenotypic diversity of the clonotypic fractions in MM comprises a hierarchy of at least 5 different populations that display incremental frequency and follow normal late B cell development: CD19+CD27+/-IgD-CD38-CD138- resting memory B cells, CD19+CD27+/-IgD-CD319+CD38++CD138- plasmablasts (PB), a novel CD19-CD38+CD56+CD319+CD138- fraction we termed pre-plasma cells (PPC), and CD19-CD56+CD38+CD319+CD138low and CD138hi PC. Although these clonotypic fractions are morphologically diverse with PPC resembling small lymphocytes, they all share the same oncogenic chromosomal aberrancies that define malignant PC. To test their tumour-propagating potential, highly purified CD138hi PC and PPC were transferred to NSG mice. Both fractions engrafted in the BM and, remarkably, both recapitulated the original, patient BM CD19- hierarchy of CD138low and CD138hi PC as well as PPC suggesting that PC and PPC represent two dynamic and interchangeable phenotypic states of the same cell. Strikingly, engraftment in the spleen and liver of NSG mice preferentially comprised PPC suggesting the presence of a specialised PPC niche in 2o lymphoid organs. These differences were also underpinned by absent or very low expression of Pax-5 mRNA in CD138low/hi PC but its higher expression in PPC and the extramedullary clonotypic fractions, both in patients and NSG mice. To explore the clinical significance of the functional relationship between PC and PPC, we compared their frequency at diagnosis and after treatment in the same 8 patients. We found that while PC frequency was reduced by ~30-fold after treatment, PPC frequency was reduced by only ~4-fold suggesting that PPC are relatively chemoresistant *in vivo*. Consistent with this, PPC were considerably more quiescent than PC. *Conclusions.* The cellular diversity of MM is more extensive than previously recognised and is organised in a dynamic phenotypic and functional hierarchy (memory B cell»PB»PPC"PC138lo"PC138hi). Both PC and the novel PPC fraction display myeloma propagating activity but PPC are more quiescent and chemoresistant *in vivo* than PC and are preferentially present in extramedullary niches. These findings have profound implications for treatment, monitoring disease status and development of new therapeutic strategies in MM.

0465

THE INVESTIGATION OF GENOMIC PROFILES IN PLASMA CELL LEUKEMIAS BY MEANS OF AN INTEGRATIVE MICROARRAY APPROACH

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Background. Multiple myeloma (MM) is a clonal proliferation of malignant plasma cells (PCs) characterized by a marked genomic instability. Plasma cell leukemia (PCL) is an aggressive malignancy that can occur directly (primary, pPCL) or progress from intramedullary MM (secondary, sPCL). Genome-wide studies in PCL are still limited. *Aims.* To provide insights into the genetic lesions and altered molecular pathways characterizing Plasma Cell Leukemia. *Methods.* Highly purified PCs from 54 newly diagnosed MM, 16 untreated pPCL and 6 sPCL patients were characterized for the main chromosomal aberrations by FISH. pPCL cases were recruited in a multicenter GIMEMA clinical trial testing the lenalidomide/low dose dexamethasone combination. Gene expression profiles were generated on Gene 1.0 ST array (Affymetrix) in the complete dataset. Thirty-five MMs and 13 pPCLs were profiled for global miRNA expression on miRNA Microarray V2 (Agilent). Genome-wide DNA profiles of 13 pPCLs were obtained using the 250K Nsp SNP array (Affymetrix); copy number values were inferred through circularly binary segmentation and FISH-based normalization procedures. *Results.* Unsupervised analyses of gene and miRNA expression profiles grouped most of PCLs and MMs into two distinct branches partly according to the major IgH chromosomal translocations. Supervised analysis evidenced 237 differentially expressed genes in PCLs versus MMs, of which 155 positively modulated genes were enriched in cytoskeleton organization, cell adhesion, migration categories and partly (24%) associated to invasion and metastasis processes. As compared to pPCL, in sPCLs we evidenced the overexpression of transcripts mainly concerned in mitosis, spindle organization and chromosome segregation. Thirty upregulated and 21 downregulated miRNAs were identified in pPCLs vs MMs. Some of the overexpressed miRNAs in PCL samples may have a particular importance in the context of B cell dyscrasias as demonstrated by their involvement in B cell development, lymphoproliferative disorders or in sustaining growth of MM cell lines. The genotyping analysis and FISH concordantly detected 13q deletion in 77% and 17p deletion in 58% of cases. The most recurrent copy number alteration specifically identified by SNP-array was 1q gain (61.5%); in addition, losses involving chromosomes 1p (39%), 8p (31%), 14q (39%), 16q (39%) and gains affecting 7q (31%) and 19p (31%) and one amplification at 17q21 (46%) were detected. One case displayed a near tetraploid karyotype and, interestingly, another showed a hyperdiploid pattern. A correlation between the expression levels and the occurrence of allelic imbalances was identified for 199 genes mainly localized in the previously described altered regions. The same integrative approach applied on miRNA expression evidenced 23 miRNAs mostly mapping to chromosomes 1p (22%), 13q (26%) and 19 (22%). These results highlighted a wide gene-dosage effect suggesting that genomic structural abnormalities in pPCL closely reflect expression imbalances. *Conclusions.* Integrative genomics approach in PCL patients allowed the identification of specific gene and miRNA signatures, altered molecular pathways and novel genetic lesions potentially involved in more aggressive forms of PC dyscrasia.

0466

ROLE OF TORC1 AND TORC2 IN MULTIPLE MYELOMA

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Background. Mammalian target of rapamycin (mTOR) is a downstream serine/threonine kinase of the PI3K/Akt pathway that integrates signals from the tumor microenvironment such as cytokines and growth factors, nutrients and stresses to regulate multiple cellular processes, including translation, autophagy, metabolism, growth, motility and survival. Mechanistically, mTOR operates in two distinct multi-protein complexes, TORC1 (Raptor) and TORC2 (Rictor). Activation of TORC1 leads to the phosphorylation of p70S6 kinase and 4E-BP1, while activation of TORC2 regulates phosphorylation of Akt and other AGC kinases. In multiple myeloma (MM), PI3K/Akt plays an essential role enhancing cell growth and survival and is activated by the loss of the tumor suppressor gene PTEN and by the bone marrow microenvironment. Rapamycin analogues have been tested in clinical trials in MM and their efficacy as single agents is modest. Inhibition of Akt and 4E-BP1 signaling requires inactivation of both complexes TORC1 and TORC2. Consequently, there is a need for novel inhibitors that can target mTOR in both signaling complexes. *Aims.* Evaluate the role of TORC1 and TORC2 in MM and the activity and mechanism of action of INK128, a novel and selective TORC1/2 kinase inhibitor. *Methods.* MM cell lines and BM samples from MM patients. The mechanism of action was investigated by MTT, Annexin V, cell cycle analysis, Western-blotting and siRNA assays. For the *in vivo* analyses, OPM2 cells were injected into the tail vein of 30 SCID mice, the percentage of CD138+ cells and the effect in homing were detected by *in vitro* and *in vivo* flow cytometry, respectively. Nanofluidic proteomic immunoassays were performed in selected tumors. *Results.* We examined the protein expression levels of both mTOR complexes and their downstream effectors in MM cells from patients and cell lines. mTOR, Akt, pS6R and 4E-BP1 are constitutively activated in all samples, and the activity was independent of the expression levels of Deptor, Rictor and Raptor. The IC50 of INK128 was in the range of 7.5-30 nM in the eight cell lines tested. Similar results were observed in freshly isolated plasma cells from MM patients. In the bone marrow microenvironment context, INK128 inhibited the proliferation of MM cells and decreased the p4E-BP1 induction. INK128 also showed a significantly greater effect inhibiting cell adhesion to BMSCs and HUVECs compared to rapamycin. These results are in concordance with our *in vivo* homing studies using *in vivo* flow cytometry showing that inhibition of both TORC1 and TORC2 had a significant effect in delaying homing of MM cells to the BM compared with the inhibition of only TORC1. Moreover, oral daily treatment with INK128 highly decreased the percentage of CD138+ tumor plasma cells in mice implanted with OPM2, reduced the levels of p-Akt and p-4EBP1, and induced apoptosis upon treatment. In conclusion, our results show that TORC1/TORC2 and its downstream targets are major regulators of cell cycle, apoptosis and adhesion of MM cells. These results suggest that dual targeting of TORC1 and TORC2 by active-site mTOR inhibitors offers a novel therapeutic approach disrupting the interaction of MM cells with the BM microenvironment.

0467

BKT140 IS A NOVEL CXCR4 ANTAGONIST WITH A POTENT STEM CELL MOBILIZATION CAPACITY AND THE ABILITY TO INDUCE MULTIPLE MYELOMA APOPTOTIC CELL DEATH

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Background. CXCR4-CXCL12 interaction is involved in the retention of normal hematopoietic stem cells (HSCs) in the bone marrow (BM) niches, as well as in localization and adhesion of multiple myeloma (MM) tumor cells to the BM microenvironment. Therefore, blocking CXCR4 may result in mobilization of HSCs and may influence the biology of MM and the disease course. BKT140 is a high affinity CXCR4

inhibitor. The possible usage of BKT140 for mobilization of normal HSCs and as anti-MM compound was evaluated in patients with MM. *Results.* In pre-clinical study, we demonstrated that the CXCR4 antagonist BKT140 but not AMD3100 exhibited a CXCR4-dependent cytotoxicity toward MM cells. BKT140 induced apoptotic cell death of MM *in vitro*, demonstrating increased phosphatidylserine externalization, decreased mitochondrial membrane potential, caspase-3 activation, sub-G1 arrest, and DNA double-stranded breaks. *In vivo*, subcutaneous injections of BKT140 significantly reduced, in a dose-dependent manner, the growth of MM xenografts. Tumors from animals treated with BKT140 were smaller in size and weights, had larger necrotic areas and high apoptotic scores ($p<0.01$). Further, we conducted a phase I/IIa clinical study administrating BKT140 to 16 MM patients (pts), assessing toxicity, mobilization of CD34+ cells, pharmacokinetic (PK), pharmacodynamic and its effect on CD138+ MM cells. BKT140 was administrated at escalated doses (30,100,300,900 μg/kg) following high-dose cyclophosphamide (Cy) (2 g/m2) and G-CSF (5 μg/kg). G-CSF was started on day 5 post Cy and BKT140 was injected subcutaneously once on day 10. BKT140 demonstrated low toxicity and short PK profile. Preliminary results show that BKT140 administration resulted in a significant dose-dependent increase in PB CD34+ cells as well as neutrophils, monocytes and lymphocytes, compared to the Cy/G-CSF individual pt baseline. The mean absolute PB CD34+ cells mobilized following BKT140 administration was 6.6, 7.5, 11.2 and 20.6 x106/kg for the 4 BKT140 administered doses, respectively. Moreover, the number of aphaereses was reduced from 2.5 to 1 procedure at the lowest (30 and 100 μg/kg) and highest (300,900 μg/kg) BKT140 doses, respectively. BKT140 at the highest doses reduced the number of PB CD138+ cells in PB in 3/7 pts with baseline CD138+ cells in their blood, while BKT140 at the lower doses increased the number of circulating CD138+ cells. The BKT140 mobilized grafts were used for AutoSCT in 15 MM pts following 200 mg/m2 melphalan conditioning. Pts received an average of 5.3x106 CD34+ cells/kg. All pts demonstrated rapid engraftment. The median day for neutrophil (>500/mm3) and platelet (>20,000/mm3, >50,000/mm3,) recovery was day 11 (range, 0-13), day 11 (range, 0-14), and day 14 (range, 0-23), respectively. *Conclusions.* BKT140 demonstrated potent anti-MM effect *in vitro* and *in vivo* in mouse xenograft model. Furthermore, first human phase I/IIa clinical trial in MM pts showed that BKT140 can be safely administrated with minimal toxicity and side effects. BKT140 significantly increased HSC mobilization and at higher doses reduced days of aphaeresis. In addition, at higher doses BKT140 released MM cells from the BM to the circulation. Additional studies are warranted to further evaluate the effect of BKT140 as an anti-MM agent.

0468

PROGNOSTIC MARKERS FOR THE PREDICTION OF EARLY RELAPSE IN MYELOMA PATIENTS ACHIEVING COMPLETE RESPONSE AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background. The incorporation of high-dose therapy/autologous-stem-cell-transplantation (HDT/ASCT) and novel agents for the treatment of young multiple myeloma (MM) patients have markedly improved response rates, particularly the achievement of complete response (CR), and survival. The association between higher CR rates and extended survival is unquestionable, but the clinical course of MM patients achieving CR is still heterogeneous; some patients die from disease progression within few months, whereas others live for over 10 years. *Aims.* To identify prognostic parameters for the prediction of early disease progression in patients achieving CR after HDT/ASCT. *Methods.* A total of 241 patients achieving CR at day+100 after HDT/ASCT are the focus of this study. Patients were included in two consecutive GEM/PETHEMA trials: GEM2000 (VBMCP/VBAD, n=140) and GEM2005<65y (Thalidomide/Dexamethasone, n=20; Bortezomib/Thalidomide/Dexamethasone, n=46; VBMCP/VBAD with Bortezomib in the two final cycles, n=35). All cases were referred for minimal residual disease (MRD) assessment by multiparameter flow cytometry (MFC) at day+100 after HDT/ASCT; baseline FISH analysis were available in 110 of the 241 patients. *Results.* Time-to progression (TTP; median, 71 months) and overall survival (OS; 73% at 5-years) of these 241 patients were, as expected, superior than those of the whole series (TTP: median, 52 months; OS: 66% at 5-years). Multivariate analysis including those variables with significant influence in the univariate analysis showed that the best combination of independent predictive parameters for TTP were: MRD status by MFC (P=.007, HR=9.004), FISH cytogenetics (high- vs. standard-risk; P=.009, HR=9.081), and percentage of plasma cells in S-phase (>2%; P=.013; HR=7.094); in turn, for OS MRD status by MFC (P=.001; HR=7.730), FISH cytogenetics (P=.011; HR=5.062) and age (<60 vs. ≥60 years; P=.027, HR=3.420) were selected. We further investigated which parameters could help to identify those cases showing early progressive disease. Of the 241 patients, 30 (12%) progressed within one year after HDT/ASCT. This subgroup of patients showed significantly increased frequency of baseline anemia (48% vs. 26%, P=.013), ISS stage 2 or 3 (86% vs. 55%, P=.003), high-risk cytogenetics (40% vs. 10%, P=.005) and persistent MRD detected by MFC (63% vs. 32%, P=.001). By multivariate analysis, only MRD status by MFC (P=.005, HR=4.686) and FISH cytogenetics (P=.005, HR=4.528) were selected as independent prognostic factors for predicting progressive disease during 1-year after HDT/ASCT. Based on the variables with independent predictive value for early disease progression (MRD status by MFC and FISH cytogenetics), we established a predictive index by assigning 1 point for each adverse factor. Accordingly, 3 risk groups of patients in CR were defined, with significantly different (P<.001) rates of disease progression within one year after HDT/ASCT for patients with no risk factors (4 progressions of 58 cases, 7%), cases with 1 risk factor (9 progressions of 45 cases, 20%), and patients with both risk factors (7 progressions of 7 cases, 100%). *Conclusions.* Patients with high-risk cytogenetics and persistent MRD after HDT/ASCT do not sustain the CR and are candidates for experimental consolidation treatments.

Acute myeloid leukemia - Clinical 1

0469

A VALIDATED DIAGNOSTIC MICROARRAY FOR NEWLY DIAGNOSED ADULT AML PATIENTS

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Background. In newly diagnosed acute myeloid leukemia (AML) gene expression (RNA) profiling (GEP)[1] on Affymetrix GeneChips identifies homogeneous clusters that correlate with all favorable cytogenetic subtypes t(15;17), t(8;21) and inv(16)/t(16;16) and favourable CEBPA gene double mutants.[2] GEP can also detect NPM1 type A/B/D by RNA genotyping with specifically designed probes on the microarray and it can assess the expression levels of individual transcripts with demonstrated prognostic significance (e.g. EVI1 and BAALC RNA expression). *Aims.* We set out to develop and validate an *in vitro* diagnostic microarray for use in diagnostics of newly diagnosed adult AML. *Methods.* A custom Affymetrix microarray, the AMLprofiler, was produced that contains a combination of generic and specially designed probes. The array was tested following hybridisation of 261 AML training cases. Next, the AMLprofiler was evaluated in an independent cohort of 267 unselected newly diagnosed cases of AML (Erasmus University Medical Center & University Ulm). *Results.* During validation in 267 independent cases the AMLprofiler identified 18/17 inv(16), 7/7 t(15;17) and 16/16 t(8;21) AML's and 70/71 NPM1A/B/D cases. There was one false-positive inv(16) namely a t(11;16) translocation concurrent with MYH11 overexpression, suggesting involvement of the 16p13.1 breakpoint like in bona fide inv(16) or t(16;16) which has been infrequently reported in secondary AML. There was 1 false-negative case of NPM1 type-D, which prompted a retraining of the algorithm and subsequently required independent re-validation. This re-validation detected 68/66 NPM1 type AB/D mutants in 143 Normal Karyotype AML cases. One of the two latter false-positive cases carried a non-AD type mutation which is clinically indistinguishable from D mutations. The EVI1 and BAALC cut points were validated according $p < 0.05$ in the logrank test for OS between high versus low expressing intermediate cytogenetic risk cases. *Summary/Conclusions.* We report the development of an AML gene expression RNA microarray for diagnostic use that can be applied by physicians in their own laboratories, to detect core binding AML, PML, NPM1 A/B/D mutant, CEBPA double mutant, high EVI1 and low BAALC AML cases for diagnostic use.

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0470

CLOFARABINE + ARA-C IMPROVES RESPONSE RATES AND EVENT-FREE SURVIVAL, NOT OVERALL SURVIVAL, IN OLDER PATIENTS WITH RELAPSED/REFRACTORY AML COMPARED TO ARA-C ALONE: UPDATED CLASSIC I STUDY RESULTS

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Background. Prognosis among older patients (pts) with relapsed or refractory (R/R) acute myelogenous leukemia (AML) is dismal with median survival of 4.7 months (mos) (Rowe, Blood 2005 106: Abstract 546). For these patients, attainment of a complete remission (CR) is often considered the first treatment objective. Since remission status at the time of hematopoietic stem cell transplantation (HSCT), the only curative approach in R/R AML, is an important predictor of long term outcomes, new agents to increase complete remission rates prior to transplant are needed. *Aim.* Phase III study designed to evaluate the efficacy and safety of clofarabine (CLO) in combination with cytarabine (ara-C) compared to ara-C alone in older adult pts with R/R AML. *Methods.* This prospective, randomized, double-blind, placebo-controlled trial included pts ≥55 yrs with relapsed or refractory AML. Randomization to CLO+ara-C or placebo+ara-C was stratified by duration of remission following the first pre-study induction regimen [REF: refractory or CR1 <6 mos; REL: CR1 ≥6 mos]. Patients received CLO (40

Table 1.

Results

	CLO+ara-C (n=162)	Ara-C alone (n=157)	P-value
Primary/Secondary endpoints			
OS, HR (95%CI)	1.00 (0.78, 1.28)		0.9951
REF	1.13 (0.81,1.57)		0.4674
REL[1]	0.85 (0.58, 1.24)		0.3963
ORR, %	47	23	<0.0001
REF	46	23	0.0022
REL	49	23	0.0019
CR, %	35	18	0.0005
REF	33	18	0.0353
REL	38	18	0.0096
EFS, HR (95%CI)	0.63 (0.49, 0.80)		0.0001
REF	0.67 (0.49, 0.93)		0.0131
REL	0.57 (0.40, 0.83)		0.0022
30-day mortality, %**	16	5	0.0013
REF	16	4	0.0093
REL	15	6	0.0997
Exploratory endpoints			
Pts proceeding to HSCT, %	21	19	--
REF	17	16	--
REL	26	23	--
Pts proceeding to HSCT in Remission,* %	16	9	--
REF	11	10	--
REL	22	8	--

CLO: clofarabine; Ara-C: cytarabine; HR: hazard ratio; Pts: patients; HSCT: hematopoietic stem cell transplant
[1]REF: CLO+ara-C (n=68); Ara-C alone (n=63); REL: CLO+ara-C (n=74); Ara-C alone (n=74); Unknown strata (n=1)
*Remission from study treatment
**n=315 treated patients with known 30-day survival status

mg/m^2 IV) or placebo followed by ara-C 1 g/m^2 IV daily x5 days. The primary endpoint was overall survival (OS). Select secondary endpoints included overall remission rate (ORR=CR+CRi), event-free survival (EFS) and safety; exploratory endpoints included HSCT rates. *Results.* Of the 320 pts with confirmed AML, 162 were randomized to CLO+ara-C and 158 to ara-C alone. The median age was 67 yrs (range: 55-86). Overall, 45% were primary refractory to their initial induction therapy and 49% had adverse cytogenetics. Although there was no difference in OS, CLO+ara-C demonstrated statistical significance across secondary efficacy endpoints, including doubling of remission rates and 37% improvement in EFS [HR: 0.63] (Table 1). Overall the number of patients who underwent HSCT was similar between the 2 arms; 21% in the CLO+ara-C arm vs 19% ara-C alone arms. However, a higher proportion of patients in the CLO+ara-C arm underwent HSCT while in remission from their study treatment (16% vs 9%). Overall 30-day mortality was 16% and 5% in the CLO+ara-C and ara-C alone arms, respectively. Serious adverse events (SAE) occurred in 60% of the CLO+ara-C and 49% of the ara-C alone pts. Serious infections occurred in 38% vs 22% of pts, respectively. The most frequent (≥5%) non-infectious SAE included febrile neutropenia (16% vs 12%) and pyrexia (4% vs 6%). Grade 3 or higher infections occurred in 65% vs 48% of pts, respectively. Grade 3 or higher non-infectious AEs occurring in ≥10% of pts in either arm included febrile neutropenia (47% vs 34%), hypokalemia (18% vs 10%), thrombocytopenia (16% vs 17%), anemia (13% vs 8%), neutropenia (11% vs 9%), increased AST (11% vs 2%) and increased ALT (10% vs 3%). *Summary/Conclusions.* While OS did not differ between arms, CLO+ara-C significantly improved response rates and EFS and allowed more patients to proceed to transplant in remission from study treatment compared to ara-C alone. Study follow-up continues and the role of clofarabine in the treatment of adult patients with AML continues to be investigated in randomized studies by cooperative groups.

0471

HIGH EXPRESSION OF THE BRCA1 COMPLEX MEMBER BRE PREDICTS FAVORABLE PROGNOSIS IN ACUTE MYELOID LEUKEMIA, ESPECIALLY AMONG MLL-AF9 POSITIVE LEUKEMIA

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Background and Aims. Acute myeloid leukemia is a heterogeneous disease. Several recurrent genetic mutations that contribute to disease pathogenesis have an impact on disease outcome. Therefore, treatment strategies are influenced by the presence of mutations that have prognostic impact. Because deregulated expression levels of genes, like EVI1, also correlate significantly with disease outcome, the type of treatment is also influenced based on expression levels of individual genes. However, it is still not possible to classify all patients based on currently known aberrations. Changes in protein ubiquitination have recently been identified to contribute to the pathogenesis of acute myeloid leukemia (AML). To identify new prognostic factors in AML, we studied whether changes in expression of over 1600 ubiquitination-related genes correlated with clinical outcome in 525 adult AML patients. *Methods and Results.* Gene expression from 525 cases with AML, for whom written informed consent was obtained, was analyzed using Affymetrix HG-U133 plus 2.0 arrays. To study the correlation between expression changes of ubiquitination-related genes and overall survival (OS) the Cox proportional hazard model was used. In addition, we performed analyses to identify genes that showed altered expression in a small subset of patients (so called outlier expression) that correlated with survival. A differential expression of 9 genes was found to correlate with OS. Subsequent multivariate analyses identified the level of expression of five of these nine genes (BRE, DNMT3B, EVI1, RNF168 and ZAP) as independent prognostic factors for overall survival. Outlier high expression of one of these genes, BRE, was observed in 3% of the patients and predicted a favorable overall and event free survival (5-year overall survival of 57%). Importantly, high BRE expression was mutually exclusive with FLT3 ITD, CEBPA mutations, EVI1 over-expression, and favorable karyotypes. In contrast, high BRE expression co-occurred strongly with FAB M5 morphology and MLL-AF9 fusions. Strikingly, within the group of MLL-AF9 positive patients, high BRE expression predicted superior survival, while lack of high BRE expression predicted extremely poor survival (5-year overall survival of 80% vs 0%, respectively, p=0.0002). Finally, unsupervised gene expression profiling showed that 86% of the patients with high BRE expression were confined to a previously unrecognized cluster. *Conclusion.* We conclude that high BRE expression defines a novel good risk group among adult AML. This work contributes to further risk stratification and sub-classification of AML and may contribute to individualized treatment strategies.

0472

DNMT3A MUTATIONS IN ACUTE MYELOID LEUKEMIA: FREQUENCY AND PROGNOSTIC IMPACT

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Background. Acute myeloid leukemia (AML) is a heterogeneous neoplasm of the hematopoietic stem cell caused by mutations, deregulated gene expression and epigenetic modifications of genes leading to decreased differentiation of hematopoietic progenitor cells and increased proliferation. Recently, mutations in DNMT3A have been identified to occur in AML. *Aims.* The aim of this study was to analyze the frequency and prognostic significance of DNMT3A mutations in a large cohort of uniformly treated, well characterized AML patients. *Methods.* A total of 489 AML patients younger than 60 years were examined for DNMT3A mutations by direct sequencing. The prognostic impact of DNMT3A mutations was evaluated in the context of other clinical prognostic markers and genetic risk factors (cytogenetic risk group; mutations in NPM1, FLT3, CEBPA, IDH1, IDH2, MLL1, NRAS, WT1, and WT1 SNPrs16754; expression levels of BAALC, ERG, EVI1, MLL5, MN1 and WT1). *Results.* DNMT3A mutations were found in 87 out of 489 patients (17.8 %). The highest mutation frequency was found in cytogenetically normal (CN-) AML (71 of 261 patients, 27.2 %). Patients with DNMT3A mutations were found be older, had higher WBC and platelet counts and more often had a normal karyotype. Additionally, patients with mutated DNMT3A were also more likely to have mutations in NPM1, FLT3, and IDH1 genes, and had higher MLL5 expression levels when compared to patients with wildtype DNMT3A. Multivariate analysis demonstrated that DNMT3A mutations independently predicted a shorter overall survival (OS) (HR 1.59; 95% CI 1.15-2.21; P=.005), but were not associated with relapse-free survival (RFS) or complete remission (CR) rate when the entire patient cohort was considered. In CN-AML patients, DNMT3A mutations independently predicted shorter OS (HR 2.46; 95% CI 1.58-3.83; P<.001) and lower CR rate (OR 0.42; 95% CI 0.21-0.84; P=.015), but not RFS (P=.32). Additionally, within CN-AML patients, DNMT3A mutations had an unfavorable effect on OS, RFS, and CR rate in NPM1/FLT3ITD high risk but not in low risk patients. *Conclusion.* DNMT3A mutations are among the most frequent mutations in younger AML patients, and are associated with an unfavorable prognosis.

0473

NPM1 MONITORING ENABLES EARLY DETECTION OF IMPENDING RELAPSE IN ACUTE MYELOID LEUKEMIA FOLLOWING CONVENTIONAL CHEMOTHERAPY AND POST ALLOGENEIC STEM CELL TRANSPLANTATION

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Background. Relapse of disease remains the major reason for treatment failure in patients with acute myeloid leukemia (AML), even after allogeneic stem transplantation (SCT). Early detection of relapse using molecular techniques could enable early preemptive therapy, but so far, the number of suitable markers is limited. More recently, mutations of the nucleophosmin gene (NPM1) have been described. These mutations are amongst the most common changes in adult AML and have shown potential for minimal residual disease (MRD) detection. However, only few studies on NPM1-based MRD detection have been published. *Aim.* In this study we investigated the suitability of NPM1 as MRD-marker in a large cohort of AML-patients. *Methods.* 184 NPM1-

mutant AML patients (pts) (median age 53.5 yrs. (range, 21-81 yrs.)), treated in protocols of the Study Alliance Leukemia (SAL) were prospectively monitored. We developed an optimized assay for the sensitive cDNA based detection of the three most common NPM1-mutations using a Real-Time-Q-PCR with locked-nucleic acid (LNA) containing primer-probe designs. The threshold for molecular relapse (mol-Rel) was defined as a 10-fold increase of NPM1 transcript level compared to the lowest level achieved or levels with greater than 1%. Molecular non or partial responder were defined as cases without a significant decrease of NPM1 transcript levels or levels > 1% after completion of the first line therapy. *Results.* We studied 184 patients having one of the three most common NPM1-mutations, A (N=156), B (N=17) and D (N=11). A total of 1661 samples (978 BM; 683 PBL) were analyzed, the median number of samples per patient was 7 (range, 3-55), the median molecular follow-up was 453 days (99-1703 days). 65 patients (35.3%) had undergone SCT (12 auto SCT, 53 allo SCT), an FLT3-ITD mutation was present in 68 pts. (37%). According to our criteria, 18 pts were defined as molecular non-responders and none of them achieved durable CR. In 28 pts with hematological relapse (hem-rel) and sufficient molecular follow-up, the rise of MRD preceded the hem-rel by a median of 66 days (range, 0-313 days). Out of 121 pts without mol-rel only one patient relapsed ($p < 0.001$). In a subgroup of 117 patients with available NPM-data in remission generalized linear models were fitted to model the risk of relapse in a defined time span. In a second step ROC-analyses were performed to identify MRD-ranges with different relapse risks. *Conclusions.* In conclusion, our data indicate that NPM1 mutations can serve as markers for MRD monitoring allowing early detection of recurrent disease in a considerable proportion of AML patients. Increasing NPM1 transcript level could trigger preemptive intervention using DLI after allogeneic SCT or targeted therapy within prospective clinical trial.

Non-Hodgkin Lymphoma - From biology to therapy

0474

MICRORNAS PLAY A PIVOTAL ROLE IN REGULATING WALDENASTROM'S MACROGLOBULINEMIA BIOLOGY

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Background. Waldenstrom's Macroglobulinemia (WM) is a low-grade lymphoproliferative disorder characterized by the presence of a lymphoplasmacytic infiltrate in the bone marrow and serum monoclonal immunoglobulin M. Cytogenetic and molecular studies on gene expression analysis at the mRNA level have demonstrated minimal changes in WM cells. Therefore, multi-level characterization of this disease at genetic and epigenetic levels is required to improve our understanding of the underlying molecular changes that lead to the initiation and progression of this disease. We have therefore evaluated how microRNA (miRNA) aberrations may possible modulate WM biology both *in vitro* and *in vivo*. *Aims.* 1) To determine miRNA profiling in primary WM cells. 2) To evaluate the functional role of miRNA-155 and -9* in regulating WM biology both *in vitro* an *in vivo*. *Methods.* miRNA- and gene-expression-profiling have been performed on bone-marrow-derived-CD19+ WM cells, compared to their normal cellular counterparts. Data were validated by stem-loop-qRT-PCR. *In vitro* and *in vivo* functional studies were performed on precursor-anti-miRNA-155 and precursot-miRNA-9*-transfected-WM cells. Effect on signaling cascades have been evaluated by western-blot and immunofluorescence. DNA-proliferation, cytotoxicity, cell cycle, apoptosis were assessed by thymidine incorporation, MTT, PI, Apo2.7 staining, respectively. GFP+ WM cells were transfected using either control-probe or miRNA-155 knockdown probe, and then injected in mice 24 hours after transfection: *in vivo* confocal imaging has been performed. *Results.* WM cells present with a miRNA signature characterized by increased expression of miRNA-155 and decreased expression of miRNA-9* (ANOVA;$P< 0.01$). Potential microRNA-155 target genes were identified using gene-expression-profiling and included genes involved in cell cycle progression, adhesion, and migration. Predicted miRNA-9* included histone-deacetylases (HDAC4; HDAC5) and -acetyltransferases (Myst3). We found that miRNA-155 regulates proliferation and growth of WM cells *in vitro* and *in vivo* by inhibiting signaling cascades including MAPK/ERK, PI3/AKT and NF-kB pathways. In addition, we demonstrated that primary WM cells are characterized by unbalanced expression of HDACs and HATs at gene level, responsible for decreased acetylated-histone-H3 and -H4, at protein level and increased HDAC activity. miRNA-9* played a functional role in regulating histone-acetylation and HDAC activity in WM cells, based on their ability to target HDACs and HATs; leading to induction of toxicity in precursor-miRNA-9*-transfected cells, as shown by reduced proliferation rate, cell cycle arrest, induction of apoptosis, supported by PARP-, caspase-8-, caspase-9-cleavage. In addition, miRNA-9* induced autophagy in WM cells by modulating Rab7 and LC3B. *Conclusion.* These *in vitro* and *in vivo* findings confirm that miRNA-155 and -9* are crucial regulators of WM pathogenesis; and provide the basis for miRNA-based-therapeutical strategies in this disease.

0475

DEPLETION OF TUMOUR ASSOCIATED MACROPHAGES SIGNIFICANTLY RETARDS THE PROGRESSION OF AN AGGRESSIVE AND CHEMORESISTANT MODEL OF B-CELL NON HODGKIN LYMPHOMA

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Background. A large number of infiltrating tumour associated macrophages (TAM) is associated with a poor prognosis in many cancers. *In vitro* studies have established the diversity and plasticity of macrophages, such that they may exhibit classically activated (M1) or alternatively activated (M2) phenotypes. In models of non-lymphoid malignancies, TAM exhibit pro-tumoural features approximating an M2 phenotype. In Non Hodgkin Lymphomas (NHL) there are conflicting reports of the clinical significance of TAM

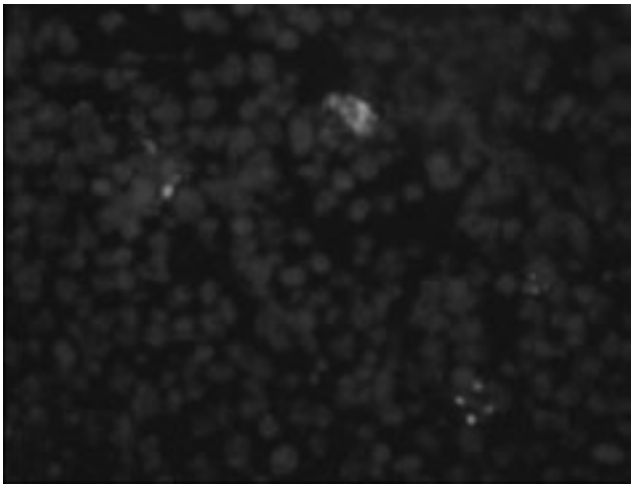

Figure 1. Fluoresecent labeled adoptively transferred BMDM.

numbers. Gene expression analysis of whole lymph node biopsy specimens in Follicular Lymphoma and Diffuse Large B-Cell Lymphoma suggest that macrophage activity might be associated with a poor prognosis. We are testing the hypothesis that TAM play an important role in the progression of NHL, and might therefore constitute a rational and effective therapeutic target. *Aims.* To investigate tumour-macrophage interactions by manipulating TAM numbers and phenotype in a transplantable mouse lymphoma with a view to providing a rationale for further investigation of the therapeutic potential of manipulating TAM in human B-NHL. *Methods.* As a model system to study B-NHL we used a mature B-cell lymphoma arising in Eμ-myc/bcl-2 transgenic mice, which, when intravenously injected into healthy C57BL/6 mice produced a disseminated lymphoma. Macrophage depletion was achieved by intravenous injection of liposomes containing dichloromethylene-diphosphonate (Liposomal Clodronate). A variety of schedules of delivering Liposomal Clodronate were employed to establish the nature and amplitude of effects on progression of lymphoma. Subsequent studies employed macrophage depletion with Liposomal Clodronate combined with adoptive transfer of syngeneic BM derived macrophages (BMDM), *in vitro* polarized to M1 and M2 phenotypes. More specific targeting of TAM, and relative sparing of physiological monocytes and resident tissue macrophages, was attempted by pharmacological inhibition of monocyte recruitment. This strategy was used against growing lymphomas, and in lymphomas relapsing after chemotherapy. Lymphoma growth was assessed by measuring lymph node weight, and cross-sectional area in tissue sections. Gene expression changes in whole lymph nodes with and without lymphoma, and following interventions to manipulate macrophage populations, were determined by real-time PCR. Changes to the cellular composition of the immune microenvironment were assessed by FACS analysis of single-cell suspensions of lymph nodes, and by immunohistochemistry. Circulating monocyte populations and serum cytokine levels were measured by FACS analysis and ELISA, respectively. *Results.* Intravenous delivery of Liposomal Clodronate in mice injected with Eμ-myc/bcl-2 lymphoma successfully depleted macrophages in the bone marrow, lymph nodes, and spleen, and significantly reduced lymphoma mass compared to vehicle controls (29.7%↓). We observed a dose-response reduction in lymphoma growth in Liposomal Clodronate treated animals. Adoptive transfer of M1-polarized BMDM also attenuated lymphoma growth (37.3%↓). Moreover, it resulted in further attenuation of lymphoma growth in mice previously treated with Liposomal Clodronate (47.1%↓). Pharmacological inhibition of macrophage recruitment resulted in reduced lymphoma growth in otherwise untreated lymphomas (19.7%↓), as well as in lymphomas relapsing following cytotoxic chemotherapy, whilst not depleting the circulating monocyte pool. *Summary/Conclusions.* Our *in vivo* studies support a crucial relationship between macrophage numbers/ phenotype, and lymphoma progression. Therefore, targeting TAM provides a very attractive therapeutic opportunity in human B-lymphomas.

0476

TARGETING THE CD20 AND CXCR4 PATHWAYS IN NON HODGKIN LYMPHOMA (NHL) WITH ANTI CD20 AND CXCR4 ANTAGONIST

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Background. Non Hodgkin's lymphoma (NHL) derives from the neoplastic transformation of lymphocytes at different stages of differentiation and may show systemic, nodal and extranodal localization in different sites, including bone marrow. Rituximab is anti-CD20 monoclonal antibody which is widely used in treatment of B-cell malignancies. The overall survival rates of patients with B cell lymphoma improved with rituximab treatment, but the relapsed/refractory rates are still high. Chemokine receptor CXCR4 and its ligand CXCL12 are critically involved in the survival and trafficking of normal and malignant B lymphocytes. The interaction of malignant B cells with stromal cells via CXCR4/CXCL12 signaling may induce chemo-resistance. Therefore, blockade of CXCR4/CXCL12 signaling may antagonize the survival and spreading of lymphoma cells and restore their chemo-sensitivity. *Results.* We evaluated the effect of CXCR4-specific antagonist BKT140 on lymphoma cell growth and rituximab-induced cytotoxicity *in vitro* and *in vivo*. We found that *in vitro* treatment with BKT140 antagonist directly inhibited the cell growth and induced cell death of CD20-expressing lymphoma cell lines and primary lymphoma cells from patients with bone marrow involvement. Combination of BKT140 with rituximab significantly increased the cytotoxic effect (apoptosis) against the lymphoma cells in a dose-dependent manner. We found that rituximab induced CXCR4 expression in lymphoma cell lines and primary lymphoma cells, both on mRNA and cell-surface levels, suggesting the possible interaction between CD20 and CXCR4 pathways in NHL and providing the rationale for CXCR4 targeting in combination with rituximab treatment. To further explore the effect of BKT140 on NHL, we established a xenograft model of B-cell lymphoma with bone marrow involvement in mice. Human CXCR4-expressing B NHL cell line, BL-2, was subcutaneously implanted into NOD/SCID mice and developed aggressive local tumors which specifically spread to the bone marrow. Following the tumor establishment, mice were injected subcutaneously with BKT140, rituximab or with combination of both reagents. BKT140 reduced the local tumor progression of BL-2 generated tumors. Moreover, BKT140 treatment significantly reduced the number of BL-2 tumor cells in the BM by 76% ($p<0.01$) compared to control, and promoted their apoptosis within bone marrow microenvironment. *In vivo* treatment of established bone marrow tumors with BKT140 together with rituximab increased the anti lymphoma effect of rituximab. *Conclusions.* Taking together, these results demonstrate potent anti-lymphoma effect of CXCR4-specific antagonist BKT140 *in vitro* and *in vivo*, suggest the possible interaction between CD20 and CXCR4 pathways in NHL, and provide the rational basis for the development of novel combined CXCR4-targeted therapies for refractory NHL.

0477

CROSSLINKING CD74 AND CD20 WITH NOVEL BISPECIFIC ANTI-CD74/CD20 ANTIBODIES INDUCES POTENT CYTOTOXICITY IN MANTLE CELL LYMPHOMA LINES

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Background. Mantle cell lymphoma (MCL) is an aggressive form of B-cell lymphoma with poor prognosis. The combination of chemotherapy with rituximab increases the overall survival, but the disease relapses in virtually all patients. At present, there is no cure for MCL; thus novel treatments are desirable. The anti-CD20 rituximab or veltuzumab is moderately effective against MCL lines *in vitro* only in the presence of a crosslinking antibody and the observed cytotoxicity can be enhanced with the addition of the anti-CD74 milatuzumab. *Aims.* To determine whether bispecific anti-CD20/CD74 antibodies are more potent against MCL than their parental antibodies alone or in combination. *Methods.* Hexavalent antibodies (HexAbs) comprising four Fabs of one specificity linked to a full IgG of the same or a different specificity can be successfully prepared by the Dock-and-Lock (DNL)

method to retain the binding activity of each constitutive Fab. Cognate CH3-AD2-IgG and CH1-DDD2-Fab modules were generated and combined under mild redox conditions to produce 74-(20)-(20), comprising four Fabs of veltuzumab linked to milatuzumab, and 20-(74)-(74), comprising four Fabs of milatuzumab linked to veltuzumab. The *in vitro* activities of 74-(20)-(20) and 20-(74)-(74) were assessed in three MCL lines (JeKo-1, Mino, and Granta-519) for growth inhibition by cell proliferation assay and for apoptosis by annexin binding assay and the results compared with the monospecific counterparts of 74-(74)-(74) and 20-(20)-(20). In addition, the effects on human B cells and growth of JeKo-1 cells in whole blood were analyzed *ex vivo*. *Results.* Each HexAb was shown to be homogeneous, with >95% purity by size-exclusion HPLC and SDS-PAGE. Both 20-(74)-(74) and 74-(20)-(20) potently inhibited the growth of JeKo-1, Mino and Granta-519 cells at 10 nM. In contrast, neither parental antibody, alone or in combination, nor the two monospecific counterparts, 74-(74)-(74) and 20-(20)-(20), inhibited the growth of JeKo-1 under the same conditions, suggesting the requirement of heteromerization of CD74 and CD20 for the observed cytotoxicity. The two anti-CD20/CD74 HexAbs also induced 25-30% apoptosis in Jeko-1, compared to 10-12% apoptosis with parental IgG, alone or in combination, and similar results were observed in clinical samples obtained from MCL patients. Additional studies revealed that the bispecific HexAbs, but not the parental mAbs, induced strong homotypic adhesion, pronounced phosphorylation of ERKs and JNKs, and reduction of the anti-apoptotic protein Bcl-xl in target cells. Although both bispecific HexAbs were capable of depleting human B cells *ex vivo*, only 20-(74)-(74) inhibited the growth of JeKo-1 cells in blood. We also found that 20-(74)-(74) had a higher antibody-dependent cellular cytotoxicity than 74-(20)-(20), but neither showed complement-dependent toxicity. The *in vivo* efficacy of 20-(74)-(74), given 370 μg twice a week for two weeks, was demonstrated in nude mice bearing JeKo-1 xenografts, resulting in 56% increase in median survival as compared to saline control mice (P<0.0001). *Conclusions.* The promising results obtained for 20-(74)-(74) against MCL lines and patient samples warrant its further preclinical evaluation as potential therapeutic against MCL and B-cell lymphomas that are refractory or poorly responsive to anti-CD20 or anti-CD22 antibodies.

0478

GERMINAL CENTER B-CELL SIGNATURE IS ASSOCIATED WITH HIGHER [18F]-FDG UPTAKE AND IMPROVES THE PROGNOSIS VALUE OF TEP SCAN IN DLBCL TREATED BY RITUXIMAB AND ANTHRACYCLINES-BASED CHEMOTHERAPY

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Background. In addition to the molecular classification, [18F]-Fluorodeoxyglucose positron emission tomography (FDG-PET) imaging is essential to optimise initial staging or to predict prognosis of DLBCL. *Aims.* The aim of the study was to assess the relationship between cell of origin (COO) classification and PET scan features in DLBCL. *Methods.* Fifty seven cases treated by CHOP/CHOP-like+R were retrospectively analysed (median age = 65y, aaIPI 0-1 = 30%, 2-3 =70%). PET scan results at diagnosis (SUVmax), following 3/4 cycles of chemotherapy (interim PET) and at the end of treatment (final PET) were correlated to molecular features. Expression profile of 18 genes related to GCB/ABC signatures and 5 genes coding for glucose transporters (GLUT) was determined from frozen tissues using an Illumina platform and DASL technology (cDNA-mediated Annealing, Selection, Ligation and extension). Phenotypes were also assessed by immunohistochemistry (IHC) according to Hans algorithm. *Results.* Gene expression profiling classified 30 DLBCL in the GCB subtype (2-year PFS=76%) and 27 in the ABC subtype (2-year PFS=51%, p=0.03), giving a concordance rate of 77% with IHC. Expression of GLUT2 was significantly higher in DLBCL with SUVmax ≥ third quartile, regardless the GCB/ABC subtype. At base-line, SUVmax was higher in the GCB subtype as compared to the ABC subtype (p = 0.029) but was not predictive of the outcome. Interim and final FDG-PET (negative / positive) were highly predictive of the prognosis. Using semi-quantitative assessment of SUV decrease at interim PET (SUV) fast (n=36) and slow (n=9) responders (SUV ≥ or < 70%) were defined. In multivariate analysis, GCB/ABC(OR=5.1), aaIPI(OR=7.1) and slow/fast responses (OR=0.1) were independently correlated with PFS and OS. Using the GCB/ABC classification and interim PET, we identified patients with a very favourable outcome (2-year OS/PFS = 100%) characterized by a GCB phenotype and a fast metabolic response. Conversely, in the GCB group (defined by DASL or IHC), slow responders display a very poor prognosis (2-year OS=33%). Similarly, DLBCL with fast metabolic responses but belonging to the ABC subtype displayed an unfavourable outcome (2-year OS = 57%). *Conclusion.* Molecular classification according to COO and interim TEP scan are two strong and independent prognostic factors in DLBCL that should be incorporated in future clinical trials to tailor therapeutic strategies.

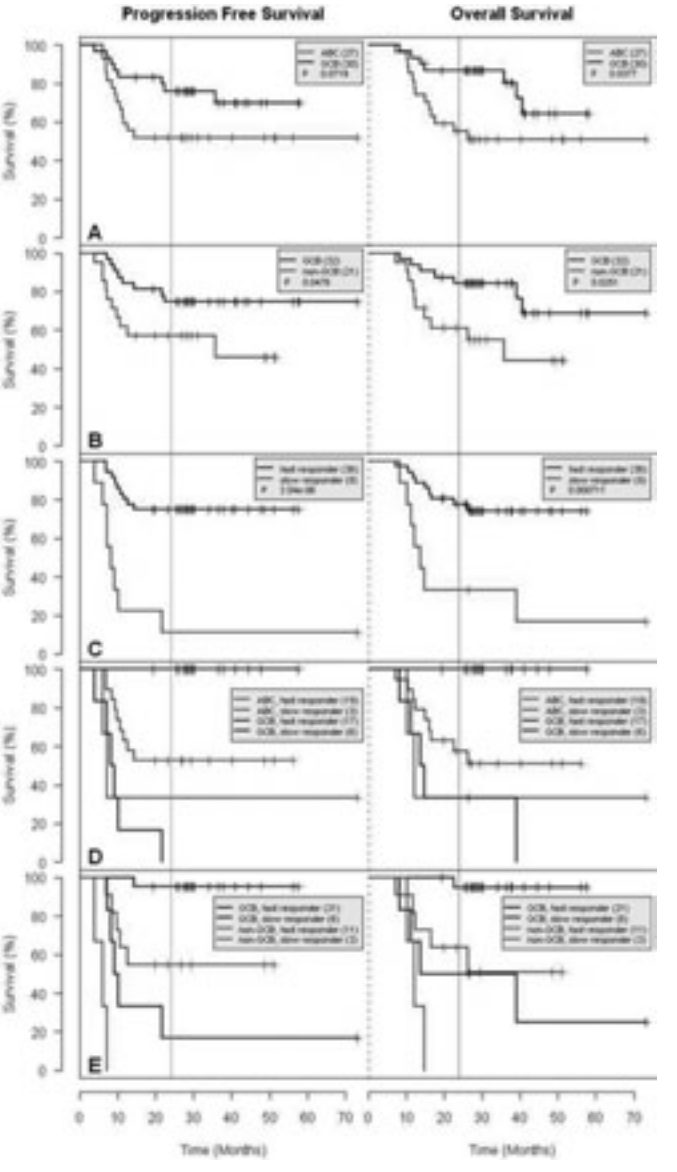

Figure 1.

Myelodysplastic syndromes - Clinical

0479

PROGNOSTIC IMPACT OF PARTIAL OR TOTAL MONOSOMY 7 AS A SINGLE ANOMALY IN PRIMARY MDS

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Introduction. Partial (del(7q)) or total monosomy 7 (-7) is one of the most frequent cytogenetic abnormalities in MDS, occurring as an isolated anomaly in about 5% of abnormal cases in patients (pts) with primary MDS. The IPSS defines any abnormality of chromosome 7 as unfavourable and classifies them, combined with complex abnormalities, into the poor risk cytogenetic subgroup. However, in previous publications from other groups, the prognosis of isolated -7/del(7q) was described as intermediate. The aim of the present study was to re-analyze the prognostic impact of -7/del(7q) as a single anomaly based on a large, international MDS database. *Materials and Methods.* 2901 Patients derived from the international MDS database were screened for monosomy 7. This large international data collection contains patients with MDS, originating from the German-Austrian (GA)-, the International MDS Risk Analysis Workshop (IMRAW), the Spanish Cytogenetic Working group (GCECGH) and the International Cytogenetics Working Group of the MDS Foundation (ICWG). Only patients with primary MDS, age >=16, and bone marrow blasts <=30%, treated with supportive care exclusively, were considered for the analysis. Uni- and multivariate analyses were performed for overall survival (OS) and risk of AML-transformation (AML-t). In multivariate analysis, site, age, gender, bone marrow blast count, date of first diagnosis and number of peripheral cytopenias were defined as co-variables. *Results.* In total, 59 patients (2.1% of all pts/4.6% of abnormal cases) with monosomy 7 were identified (del(7q): n=13; -7: n=46). The median age of these patients was 66.1 years, which is significantly lower compared to patients without monosomy 7 (70.0 years; p<0.01; t-test, 2-sided). Regarding peripheral blood count, the mean hemoglobin in -7/del(7q) pts (9.2 g/dl) as well as ANC (1.7*10^3/ul) did not differ significantly as compared to pts without -7/del(7q) whereas the platelet count in pts with -7/del(7q) was significantly lower (82*103/ul vs. 125*10^3/ul; p<0.01). The median overall survival in -7/del(7q) pts was 16.0 (95% CI 14.0-21.4) months and the Hazard ratio (HR; as compared to a normal karyotype with a median survival of 47.4 (44.0-53.4) months as the reference category) was 1.6 (1.1-2.3; <0.01). Regarding the risk of AML-transformation, the median time to AML was 42.2 (14.4-not reached) months and the HR 1.7 (0.9-3.2; p<0.01). In comparison, this differed significantly from the median survival- (p<0.0001) and time to AML-transformation (p=0.027) for complex abnormalities, which are included with -7/del(7q) in the poor risk IPSS cytogenetic subgroup and were 5.7 (4.7-6.8) and 8.2 (6.4-14.0) months, respectively. The HR for complex abnormalities was 4.3 (3.4-5.4; p<0.01) for OS and 5.2 (3.8-7.5; p<0.01) for AML-transformation. *Conclusions.* The re-analysis of -7/del(7q), based on the largest MDS patient cohort yet published, confirms that the prognostic impact of an isolated total or partial monosomy 7 for overall survival as well as the risk of AML-transformation is not as poor as defined in the IPSS and significantly different from complex abnormalities. This finding is anticipated to be considered in the upcoming revision of the IPSS.

Acknowledgements: The authors like to thank the MDS-Foundation for its support.

0480

A RANDOMIZED TRIAL OF HORSE VERSUS RABBIT ANTITHYMOCYTE GLOBULIN IN SEVERE ACQUIRED APLASTIC ANEMIA

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In patients with severe aplastic anemia (SAA) who are not candidates for stem cell transplantation, immunosuppressive therapy with horse anti-thymocyte globulin (ATG; ATGAM®) plus cyclosporine (CsA) is standard. In comparison to horse ATG, rabbit ATG (Thymoglobulin®) is more potent and has distinct biological effects on the immune system. Thymoglobulin is preferred to ATGAM in some clinical settings, as in the prevention and treatment of kidney allograft rejection. In patients with refractory and relapsed SAA, rabbit ATG used as salvage therapy improves hematopoiesis. We hypothesized that rabbit ATG would be a superior regimen compared to horse ATG as first therapy in SAA. To test this hypothesis, we conducted a prospective randomized study comparing horse ATG/CsA and rabbit ATG/CsA in treatment-naïve SAA (registered at www.clinicaltrials.gov as NCT00260689). Informed consent was obtained according to a protocol approved by the Institutional Review Board of the National Heart, Lung, and Blood Institute. Consecutive patients with SAA who required initial immunosuppressive therapy were eligible; all were enrolled from December 2005 to July 2010 and treated at the National Institutes of Health in Bethesda. The primary endpoint was hematologic response at 6 months, which has been shown to strongly correlate with long-term survival (JAMA 289: 1130, 2003). The current study was powered to detect a 25% difference between arms at a 5% significance level and with 80% power. A total of 120 patients (ages 2-78 years) were randomized between horse and rabbit ATG (60 in each arm). Median follow-up for surviving patients was 891 days (range, 185-1852). All patients are evaluable for the primary endpoint. Demographics and clinical features were well matched between the two groups. The hematologic response rate at 6 months for horse ATG was 68% (95% CI, 56%-80%) and for rabbit ATG 37% (95% CI, 24%-49%; p<0.001). (Almost all patients in both arms had shown response by 3 months: horse ATG 37/60 [62%] and rabbit ATG 20/60 [33%].) Hematologic response rates for horse ATG are similar to our reported experience (Blood 108: 2509, 2006). Despite relatively short follow-up, relapse and clonal evolution to date have occurred with similar frequency; 9 relapses (among 41 responders) and 9 clonal evolutions have been observed in the horse ATG arm, and 2 relapses (among 22 responders) and 7 evolutions in the rabbit ATG group. There have been 4 deaths in the horse ATG arm and 14 in the rabbit ATG arm, resulting in different survival proportions for the two regimens mainly due to high-risk therapies required in patients who failed initial immunosuppression (see Figure). We infer from our results that rabbit ATG/CsA is markedly inferior to horse ATG/CsA as first treatment in SAA, as measured by the critical parameter of early hematologic response. Horse ATG is presently not available in Europe, Japan and South America, and therefore these data have immediate implications for the treatment of SAA worldwide, as well as to the mechanism of action of polyclonal antisera in general and in particular in this disease.

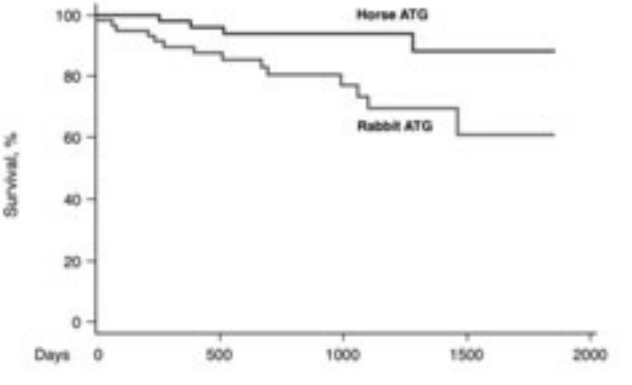

Figure 1. Overall survival for horse and rabbit ATG.

0481

PREDICTIVE FACTORS FOR OVERALL SURVIVAL (OS) AND AML PROGRESSION IN A LARGE COHORT OF PATIENTS WITH LOW-/INT-1-RISK MDS WITH DEL(5Q) TREATED WITH LENALIDOMIDE (LEN)

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Background. Most studies evaluating prognosis in MDS have focused on untreated patients from all subgroups. Higher transfusion burden and bone marrow blast percentage, and complex cytogenetics at baseline have been associated with reduced OS and increased AML progression in MDS patients (Greenberg P, *et al*. Blood. 1997;89:2079-88; Malcovati L, *et al*. J Clin Oncol. 2007;25:3503-10). Evaluation of predictive factors specific to disease-modifying drugs such as LEN, and to MDS subtypes such as Low-/Int-1-risk groups with del(5q) is needed. *Aim.* To identify predictive factors for OS and AML progression in RBC-transfusion dependent patients with IPSS-defined Low-/Int-1-risk MDS with del(5q), treated with LEN in 2 multicenter trials"[1]MDS-003 (phase 2 single-arm) and MDS-004 (phase 3 randomized, double-blind). *Methods.* Patients who provided informed consent received LEN 5 mg on days 1-28, or 10 mg either on days 1-21 or 1-28 of 28-day cycles. Cox proportional hazards models assessed the impact of baseline characteristics and RBC-transfusion independence (TI) for ≥26 weeks, on OS and time to AML progression (calculated from study entry/randomization); RBC-TI for ≥26 weeks was included as a time-dependent covariate. Once potentially significant risk factors were identified, a multivariate model simultaneously determined the most important prognostic variables using a backward elimination variable-selection approach. The Table shows the univariate model with individual variables and the final model based on backward model selection. *Results.* From the 2 studies, 286 LEN-treated patients were included in the intent-to-treat population. Median age was 69 years (range 36-95); 70% of patients were female; 70% had isolated del(5q) and 26% had del(5q) plus ≥1 additional abnormality; 31%, 42%, and 6% had IPSS-defined Low-, Int-1-, and Int-2-/High-risk MDS, respectively. FAB subtypes were: 63% RA/RARS; 19% RAEB/CMML; and 19% other/missing. At baseline, median transfusion burden was 6 units/8 weeks (range 1-25) and median platelet count was 235×10^9/L (range, 14-1401). Median follow-up duration for OS was 38.4 months (range, 0.3-81.9) in MDS-003 and 36.1 months (range, 0.4-59.4) in MDS-004. Results of the Cox proportional hazards model are in the Table. Achieving RBC-TI for ≥26 weeks and higher baseline platelet counts were associated with significantly reduced relative risks of death (64% and 13% reductions per platelet count increase of 100×10^9/L, respectively). Additionally, significant increases in the relative risk of death were reported with RAEB/CMML (63% increase), higher transfusion burden (6% increase per 1 unit/8 weeks), and older age (5% increase per year) at baseline. Higher transfusion burden and del(5q) plus ≥1 additional abnormality were associated with significantly increased relative risks of AML progression. *Summary.* In LEN-treated patients with Low-/Int-1-risk MDS with del(5q), higher baseline transfusion burden was associated with an increased relative risk of AML progression and death. Older age and RAEB/CMML at baseline were associated with reduced OS, whereas del(5q) plus ≥1 additional abnormality was associated with an increased risk of AML progression. Achievement of RBC-TI for ≥26 weeks and higher baseline platelet counts were associated with significantly increased OS in this large patient cohort, confirming the findings in previous, smaller-scale studies.

Table 1.

Variables*	HR Univariate Model (Individual Variable)		HR Final Model	
	OS	AML Progression	OS	AML Progression
Age, years	1.0358 P < 0.001	0.9915 P = 0.451	1.0468 P < 0.001	
Sex (male vs female)	1.8288 P < 0.001	1.4419 P = 0.169		
IPSS risk (Int-1-/Int-2-/High-risk vs Low-risk)	1.2276 P = 0.252	1.3561 P = 0.320		
FAB classification (RAEB/CMML vs RA/RARS)	1.6218 P = 0.011	1.2744 P = 0.466	1.6260 P = 0.012	
Time since diagnosis, years	1.0159 P = 0.427	1.0057 P = 0.871		
Transfusion burden, units/8 weeks	1.1189 P < 0.001	1.1184 P = 0.001	1.0643 P = 0.013	1.1255 P < 0.001
Bone marrow blasts (≥5% vs <5%)	1.4885 P = 0.049	1.1579 P = 0.679		
No. of cytopenias (2 or 3 vs 1)	1.2113 P = 0.221	0.9590 P = 0.872		
Platelet count, per 100×10^3/L	0.8009 P < 0.001	1.0122 P = 0.847	0.8713 P = 0.026	
Absolute neutrophil count, per 1×10^3/L	0.9880 P = 0.710	1.0352 P = 0.399		
Hemoglobin level, g/dL	0.9047 P = 0.162	0.9146 P = 0.450		
del(5q) (plus ≥1 additional abnormality vs isolated)	1.5319 P = 0.013	1.9532 P = 0.014		2.1205 P = 0.006
WPSS risk (High-/Very High-risk vs Low-/Int-risk)	1.4574 P = 0.032	1.5593 P = 0.124		
RBC-TI ≥26 weeks (yes vs no)	0.3330 P < 0.001	0.5868 P = 0.040	0.3584 P < 0.001	

Variables are continuous except when specified.
*Variables are baseline except for RBC-TI ≥26 weeks.
CMML, chronic myelomonocytic leukemia; FAB, French-American-British; HR, hazard ratio; RA, refractory anemia; RAEB, RA with excess blasts; RARS, RA with ring sideroblasts.

0482

A GENE EXPRESSION BASED RISK SCORE IN MDS PATIENTS PREDICTS AML TRANSFORMATION

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Background. High expression levels of MN1, ERG, BAALC and EVI1 have been found to be associated with an adverse outcome in patients with acute myeloid leukemia (AML). Whether expression levels of these genes also have a prognostic influence in myelodysplastic syndromes (MDS) remains unknown. *Aims.* The aim of this study was to develop a scoring system that is predictive for progression of MDS to AML based on gene expression levels of MN1, ERG, BAALC and EVI1 in patients with MDS. *Patients and Methods.* MN1, ERG, BAALC and EVI1 transcript levels were analyzed by quantitative RT-PCR in 140 MDS patients and assessed for their prognostic significance in the context of other clinical and molecular markers. Expression data of the four genes were combined in an additive score, which was validated in an independent patient cohort of 110 MDS patients. *Results.* Patients with high compared to low expression of the individual genes MN1, ERG, BAALC or EVI1 expression showed a significantly worse overall survival (OS, P=.001; P<.001, P<.001, and P=.044, respectively) and shorter time to AML progression (MN1, ERG, and BAALC, P<.001, EVI1, P=.001). Multivariate analysis revealed that a high 4-gene expression score, defined as expression above the median of at least two of the four genes predicted a significantly shorter OS (HR 2.29, 95%CI 1.29-4.08, P=.005) and time to AML progression (HR 4.83, 95%CI 2.01-11.57, P<.001) compared to a low 4-gene expression score, independent of karyotype, transfusion dependence, percentage of bone marrow blasts, ASXL1, and IDH mutation status. In a validation cohort of 110 MDS patients, a high 4-gene expression score predicted shorter OS (HR 1.77; 95%CI 1.04-3.0, P=.034) and time to AML progression (HR 3.0, 95%CI 1.17-7.65, P=.022). *Conclusion.* A high 4-gene expression score is an unfavorable prognostic marker in MDS and is associated with a high risk for progression to AML. This prognostic marker may become useful for risk and treatment stratification.

0483

BONE MARROW RESPONSE AND OVERALL SURVIVAL IN ON 01910.NA TREATED PATIENTS WITH REFRACTORY ANEMIA AND EXCESS BLASTS WHO FAILED PRIOR HYPOMETHYLATING THERAPY

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Background. ON 01910.Na is a new potent and selective mitotic inhibitor which inhibits the cell cycle progression at the G2/M interface, as well as the alpha and beta subunits of PI3-Kinase resulting in reduced levels of Cyclin D, C-myc and other regulatory proteins. It is also selective in cancer cells vs. normal cells. *Methods.* We analyzed bone marrow (BM) response and overall survival (OS) in 31 patients (pts) with refractory anemia with excess blasts (RAEB) -1,- 2 or -t, previously treated with azacitidine or decitabine who signed informed consent and were enrolled in 4 independent clinical trials. Pts received ON 01910.Na administered as a continuous intravenous infusion (CIV) from 2 to 6 days weekly or every other week (wk) with BM response initially assessed per protocol by wk 4 or 8 and every 8 wks thereafter. *Results.* Median OS was 36 wks and reached 49 wks in patients treated with ON 01910.Na 1800 mg dosing per 24h over 3-day infusions every other wk. A BM complete response (CR) (>50% decrease from baseline BM blast and decrease below 5% for at least 4 wks, per MDS IWG 2006 criteria) or a > 50% decrease of BM blasts was documented in 13/24 (54%) treated pts with at least one follow-up BM evaluation (green line) and was associated with a 44-wk median overall survival (OS) by the method of Kaplan-Meier (Fig. 1). Patients with stable disease (N=9; orange line) had a 40-wk median OS. Two patients progressed (median OS=16 wks; brown line) and 7 were not assessed (median OS=7 wks; blue line). Five pts had complete BM response and 5 patients had a hematological improvement (IWG 2006). Best results were found with 3-day infusions. Overall, ON 01910.Na infusions were well tolerated and no myelotoxicity was found when analyzing bone marrow cellularity. *Conclusion.* The median survival of MDS pts who failed to respond to prior treatment with azacitidine or decitabine has been reported to be approximately 4 to 6 months. These results and the apparent predictive value of BM response to ON 01910.Na for estimating overall survival of these patients have led to the initiation of a randomized survival trial of ON 01910.Na 3-day infusions vs. best supportive care in RAEB 1,2,t pts who failed or progressed after receiving hypomethylating agents.

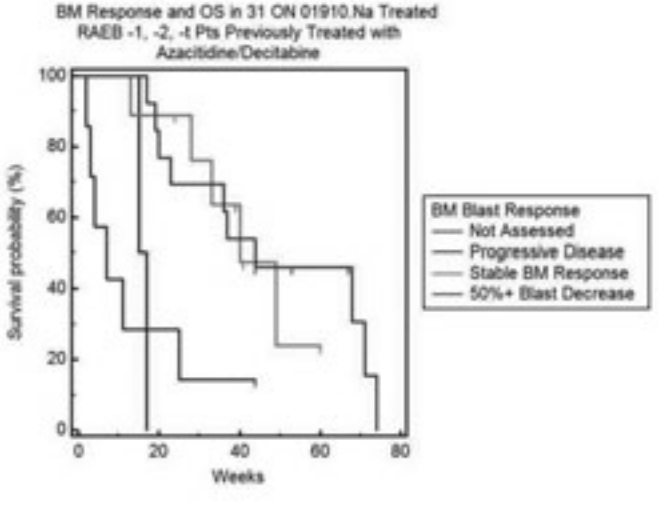

Figure 1.

Chronic myeloid leukemia - Clinical 1

0484

SUPERIOR EFFICACY OF NILOTINIB COMPARED WITH IMATINIB IN NEWLY-DIAGNOSED PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC (CML-CP): ENESTND MINIMUM 24-MONTH FOLLOW-UP

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Background. In ENESTnd, nilotinib has demonstrated superior efficacy to imatinib in patients with newly-diagnosed Philadelphia chromosome-positive (Ph+) CML-CP at 12 and 18 months. *Aims.* Here, we report results with a minimum patient follow-up of 24 months. *Methods.* 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 (n = 283). Major molecular response (MMR; ≤ 0.1% BCR-ABL^{IS}) and complete molecular response (CMR^{4}, ≤ 0.01%^{IS}; CMR$^{4.5}$, ≤ 0.0032%^{IS}) by 24 months, time to progression to accelerated phase/blast crisis (AP/BC) on treatment, progression-free survival (PFS) on treatment, and overall survival (OS) were measured. *Results.* Significantly higher rates of MMR by 24 months were reported for both doses of nilotinib (71% and 67% on nilotinib 300 mg and 400 mg BID, respectively) compared with imatinib (44%, $P < .0001$ for each comparison) (Table). Rates of MMR were superior

Table 1.

	Nilotinib 300 mg BID (n = 282)	Nilotinib 400 mg BID (n = 281)	Imatinib 400 mg QD (n = 283)
CCyR, %			
By 24 mo	87 *P* = .0018*	85 *P* = .0160*	77
MMR, %			
By 24 mo	71* *P* < .0001*	67* *P* < .0001*	44
Best Molecular Response at any time, %			
≤ 0.01% IS (CMR^{4})	44 *P* < .0001*	36 *P* < .0001*	20
≤ 0.0032% IS (CMR$^{4.5}$)	26 *P* < .0001*	21 *P* = .0004*	10
Freedom from Progression to AP/BC, (%)			
Estimated rate at 24 mo Excluding clonal evolution	99.3 *P* = .0059**	98.1 *P* = .0196**	95.2
Including clonal evolution	99.3 *P* = .0003**	97.3 *P* = .0089**	93.2
PFS, %			
Estimated rate at 24 mo	96.0 *P* = .0736**	97.7 *P* = .0437**	95.2
OS, %			
Estimated OS rate at 24 mo	97.4 *P* = .6485**	97.8 *P* = .2125**	96.3
Estimated OS rate at 24 mo considering only CML-related deaths	98.9 *P* = .1930**	98.9 *P* = .0485**	96.7

* Cochran-Mantel-Haenszel test stratified by Sokal vs imatinib.
** Log-rank test stratified by Sokal vs imatinib.

for nilotinib at both doses (vs imatinib) regardless of Sokal risk score. Rates of CCyR by 24 months were also significantly higher on both nilotinib arms (87%, 85%) vs imatinib (77%; *P* = .0018 and .0160 for comparison with nilotinib 300 mg BID and 400 mg BID, respectively). Rates of CMR[4] and CMR[4.5] at any time were significantly higher for both doses of nilotinib vs imatinib (CMR[4]: 44%, 36%, and 20% for nilotinib 300 mg and 400 mg BID and imatinib, respectively, *P* < .0001 for both; CMR[4.5]: 26%, 21%, and 10%, *P* < .0001 and .0004 for nilotinib 300 mg BID and 400 mg BID vs imatinib, respectively). Progressions to AP/BC (without clonal evolution) continued to be less frequent on nilotinib, occurring in 2, 3, and 12 patients on the nilotinib 300 mg BID, 400 mg BID, and imatinib arms, respectively. At 24 months, OS remained similar in all groups, but there were fewer CML-related deaths on both nilotinib 300 (n = 5) and 400 mg BID (n = 3) vs imatinib (n = 10). Twice as many patients had emergent BCR-ABL mutations on imatinib (n = 20) vs nilotinib (n = 10 and 8 for 300 mg BID and 400 mg BID, respectively) and mutations were more frequent in patients with high or intermediate Sokal risk. Overall, 72 (26%), 61 (22%), and 92 (33%) of patients in the nilotinib 300 mg and 400 mg BID and imatinib arms, respectively, discontinued treatment. Both drugs were well tolerated, with the fewest discontinuations due to adverse events/laboratory abnormalities in the nilotinib 300 mg BID arm (9% vs 13% and 10% for the nilotinib 400 mg BID and imatinib arms, respectively). There has been no change in the safety profile of nilotinib in the second year of treatment. *Conclusions.* With a minimum follow up of 24 months, nilotinib continues to demonstrate superior molecular responses and improved disease control compared with imatinib. These data continue to support nilotinib as a potential new standard of care in newly-diagnosed patients with Ph+ CML-CP.

0485

THE BELA TRIAL: BOSUTINIB VERSUS IMATINIB IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA; 18-MONTH FOLLOW-UP

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Background. Bosutinib is an orally active, dual Src/Abl tyrosine kinase inhibitor with minimal inhibitory activity against PDGFR or c-kit. *Aim.* The phase 3 BELA study compared bosutinib with imatinib in patients with newly diagnosed Philadelphia chromosome positive (Ph+) chronic phase chronic myeloid leukemia (CP CML). *Methods.* Patients (N = 502) with Ph+ CP CML stratified by Sokal score and region were randomized to oral bosutinib 500 mg/day (n = 250) or imatinib 400 mg/day (n = 252). Study design and endpoints have been described (Gambacorti, ASH 2010). Safety analyses included all treated patients; efficacy analyses included all randomized patients (intent-to-treat [ITT] population). *Results.* Median treatment duration was 16.6 months for bosutinib and 16.8 months for imatinib; 69% and 78% of patients, respectively, were still receiving therapy. Common treatment-emergent adverse events (TEAEs; ≥20% of patients) observed with bosutinib and imatinib, respectively, were diarrhea (68%, 22%), vomiting (31%, 14%), nausea (31%, 35%), rash (21%, 16%), and muscle cramps (4%, 20%). Pleural effusions were seen in 3% of bosutinib patients (no imatinib patients). Grade ≥3 TEAEs (≥2% of patients) seen with bosutinib were diarrhea (10%), vomiting (3%), pneumonia (3%), and dyspnea (2%). Median cumulative duration of diarrhea was 33 days for bosutinib and 17 days for imatinib. Grade ≥3 laboratory abnormalities (≥10% of patients) with bosutinib and imatinib, respectively, were elevated alanine aminotransferase (23%, 3%), thrombocytopenia (14%, 14%), elevated aspartate aminotransferase (11%, 3%), neutropenia (9%, 21%), and hypophosphatemia (4%, 17%). Twenty-two percent of bosutinib patients and 6% of imatinib patients discontinued due to AEs. Deaths occurred in 4 (1.6%) bosutinib patients and 12 (4.8%) imatinib patients; overall, 81% of these died from disease progression, with CML-unrelated deaths reported for only 1 bosutinib patient and 2 imatinib patients. In efficacy analyses, complete cytogenetic response (CCyR) rates for bosutinib and imatinib, respectively, at 1 year were 70% and 68% for the ITT population, and 78% and 68% for the evaluable population (P = 0.026). Cumulative CCyR rates by 1 year were 79% (bosutinib) and 75% (imatinib). Major molecular response (MMR) rates at 1 year were higher for bosutinib versus imatinib (39% vs 26%; P = 0.002), as were cumulative MMR rates by 1 year (47% vs 32%; P <0.001). Time to CCyR and MMR were significantly shorter with bosutinib (P <0.001 for both). Transformation to accelerated/blast phase occurred in 4 (2%) patients on bosutinib and 10 (4%) patients on imatinib (P = 0.053). Treatment failures were reduced in the bosutinib group compared with the imatinib group (3% vs 10%; P <0.001). Event-free survival rates were similar between groups (92% for bosutinib and 90% for imatinib). *Summary/Conclusions.* Safety and efficacy were consistent with previously reported results. Bosutinib had a distinct and acceptable toxicity profile. Bosutinib showed a significantly higher MMR rate at 1 year, significantly faster times to CCyR and MMR, and a borderline significantly lower transformation rate versus imatinib. In conclusion, bosutinib may provide a new therapeutic option in patients with newly diagnosed Ph+ CP CML. Data for the 18-month follow-up will be presented.

0486

BCR-ABL KINASE DOMAIN MUTATIONS IN IMATINIB AND IN SECOND- GENERATION TYROSINE KINASE INHIBITOR ERAS: A REVIEW OF SEVEN YEARS OF MUTATION ANALYSIS BY THE GIMEMA CML WORKING PARTY

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Background. Over the years, Bcr-Abl kinase domain (KD) mutation analysis has more and more extensively been applied in Ph+ patients receiving tyrosine kinase inhibitors (TKIs). *Aims.* We reviewed the database recording the mutation analyses performed in our laboratory from January 2004 to January 2011 in order to: i) assess how the clinical relevance of mutations (overall and individually) has changed from the imatinib to the 2nd-generation TKI era; ii) understand how the role of mutation analysis in routine clinical management of patients has evolved over time; iii) elucidate how frequently physicians may expect to find mutations in specific settings. *Methods.* 3285 bcr-Abl KD mutation analyses were performed by D-HPLC and/or direct sequencing; 1439 patients were analyzed (CML, n=1275; Ph+ ALL, n=164). *Results.* Since 2006, mutation analysis of CP-CML patients on imatinib has usually been triggered by failure or suboptimal response according to ELN definitions. Only 41/142 (29%) failures and 19/222 (10%) suboptimal responses we analyzed harbored mutations; the likelihood of mutation detection varied across different subcategories. In particular, no mutations were detected in any of the 42 CCyR patients who failed to achieve MMR at 18 months. Over time, more and more physicians asked for mutation analysis of their CP-CML patients because of a Bcr-Abl transcript increase at a single RQ-PCR test; mutations were detected in 0/26 patients who experienced <1-log increase without MMR loss, 0/41 patients who experienced ≥1-log increase without MMR loss, 1/36 (3%) patients who experienced <1-log increase with loss of MMR and 2/41 (5%) patients who experienced ≥1-log increase with loss of MMR but not CCyR. The 25 additional patients analyzed after a transcript increase confirmed by two subsequent RQ-PCR assess-

ments were negative as well. Among imatinib-resistant CML patients receiving a 2nd-generation TKI (dasatinib, nilotinib) 38 analyses were triggered by provisional ELN criteria for failure or suboptimal response; again, failures were more frequently associated with mutations (57%) than suboptimal responses (21%). In addition, 71% of patients who lost a previously achieved response (HR or CyR) were positive for mutations. The ten most frequent mutations conferring resistance to dasatinib/nilotinib in CML and Ph+ ALL included T315I (30.3%), F317L (16.2%), Y253H (16.2%), F359V (7.1%), V299L (7.1%), E255K (6.1%), E255V (5.1%), F359I (4%), T315A (3%), F359C (2%) - detected either alone (56% of patients), or combined (29%), or together with other mutations (15%). Some physicians ask for mutations analysis of their newly diagnosed, TKI-naïve patients - mutations were detected in 1/58 CP CML, 3/12 BC CML and 3/60 Ph+ ALL patients. *Summary.* Bcr-Abl KD mutations contribute differently to different types of 'resistance', and this happens both in the setting of imatinib first-line and in the setting of 2nd-generation TKIs second-line - although the mutation frequency is, overall, higher in the latter. In CP CML patients on imatinib who show Bcr-Abl transcript increase, only loss of MMR is a reasonable trigger for mutation analysis - although <3% of molecular suboptimal responders can be expected to harbour mutations. Prevalence of individual mutations is changing. Additional analyses will be presented.

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0487

BCR-ABL FUSION TRANSCRIPT AND OUTCOME OF CHRONIC MYELOID LEUKEMIA PATIENTS IN EARLY CHRONIC PHASE TREATED WITH IMATINIB: A GIMEMA CML WP ANALYSIS

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Background. Chronic myeloid leukemia (CML) is characterized by the BCR-ABL fusion gene. Different types of BCR-ABL transcripts can be found, due to different genomic breakpoints and alternative splicing. The most frequent transcripts are the e13a2 (b2a2) and the e14a2 (b3a2). Occasionally, both transcripts may be present. In the imainib (IM) era, few data about the prognostic significance of the transcript type are available, particularly in the setting of early chronic phase (ECP): one study suggested that patients with the b2a2 transcript may be more sensitive to IM (de Lemos *et al*. Genet Mol Res 2005), while two larger studies suggested that patients with b3a2 transcript may have better responses to IM (Vega-Ruiz *et al*. ASH 2007; Lucas *et al*. Haematologica 2009). No systematic evaluations in large prospective clinical trials have been performed. *Aims.* To investigate the influence of the BCR-ABL transcript type on the responses and the outcome of ECP CML treated with IM. *Methods.* Analysis of 3 concurrent clinical trials of the GIMEMA CML WP (Clin Trials Gov. NCT00514488, NCT00510926 and observational trial CML/023). Response monitoring: conventional cytogenetic examination (bone marrow) and Q-PCR (peripheral blood). Definitions: Major Molecular Response (MMR): BCR-ABL/ABL ratio <0.1% (International Scale); failures: revised European LeukemiaNet criteria (Baccarani *et al*. J Clin Oncol 2009); events: failure or treatment discontinuation for any reason. All the calculations have been made according to the intention-to-treat principle. *Results.* 559 consecutive ECP CML patients were enrolled. Patients expressing rare transcript types (e1a2 and e19a2) and patients with both b2a2 and b3a2 transcripts were excluded: 493 out of 559 patients were evaluable, 203 (41%) with b2a2 transcript and 290 with b3a2 transcript (59%). The 2 groups were comparable (no significant differences in sex, age, Sokal/Hasford score distribution, clonal chromosomal abnormalities in Ph+ cells), except for the proportion of patients treated with IM 800 mg/daily: 20% and 28% (p=0.034) in patients with b2a2 and b3a2, respectively. The median observation time was 60 months. In patients with b2a2 and b3a2 transcript, the observed 12-months CCgR rates were 75% and 79%, respectively, with a cumulative CCgR incidence of 89% and 88%, respectively (no significant differences). The time to MMR was significantly shorter for patients with b3a2 transcript and the overall estimated probability of MMR was significantly lower for patients with b2a2 transcript (85% vs 90%, p<0.001, fig.1). The probability of Overall Survival (OS), Progression-Free Survival (PFS), Failure-Free Survival (FFS) and Event-Free Survival (EFS) was 86% and 91% (p=0.064), 82% and 90% (p=0.027), 70% and 76% (p=0.095), 58% and 70% (p=0.027) in patients with b2a2 and b3a2 transcript, respectively (fig. 1). *Summary/Conclusions.* In patients with b2a2 and b3a2 transcript the CCgR rates were comparable, but the overall estimated probability of MMR was significantly lower for patients with b2a2 transcript. OS, PFS, FFS, and EFS were uniformly lower in patients with b2a2 transcript (PFS and EFS: p< 0.05).The b2a2 transcript is a candidate adverse prognostic factor in ECP CML patients treated with IM frontline.

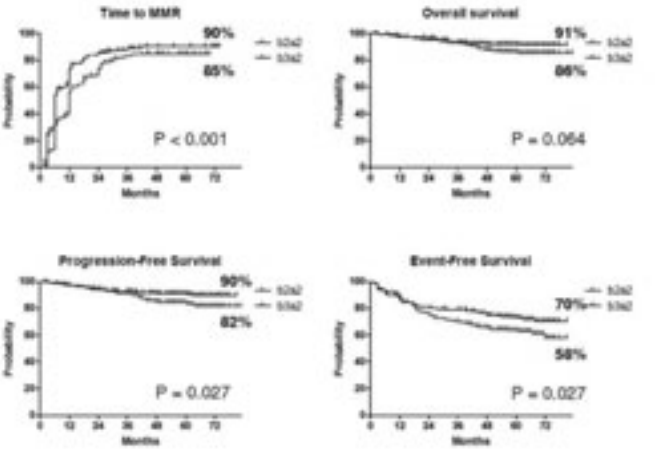

Figure 1. Response and outcome.

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0488

CANCEROUS INHIBITOR OF PP2A (CIP2A) AT DIAGNOSIS OF CHRONIC MYELOID LEUKAEMIA IS A CRITICAL DETERMINANT OF DISEASE PROGRESSION

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Background. Prospective identification of patients whose chronic myeloid leukaemia (CML) will progress to blast crisis is currently not possible. PP2A is a phosphatase and tumour suppressor which regulates cell proliferation, differentiation and survival. Cancerous inhibitor of PP2A (CIP2A) is a recently described novel inhibitor of PP2A with an unknown biological role in CML. *Aims.* The aim of this study was to investigate the role played by CIP2A in CML and whether PP2A, or its inhibitors CIP2A and SET, could predict clinical outcome. *Methods.* In 31 newly diagnosed chronic phase patients PP2A, phosphorylated PP2A (as a measure of PP2A activity) and CIP2A proteins were assessed by flow cytometry at diagnosis and following 12 months of treatment, or at transformation. TaqMan gene expression assays were used to measure PP2A and CIP2A gene expression, and CIP2A siRNA was transfected into K562 cells. Results were analysed in patients stratified according to their eventual clinical outcome - cytogenetic responders (CCR), Non-responders (No-CCR) and blast crisis (BC). *Results.* PP2A is functionally inactive in MNC and CD34+ cells taken at diagnosis in patients destined to progress to BC compared to those patients who

achieve a CCR. With SET, protein levels were significantly higher at diagnosis in the CCR and No-CCR groups compared to normal values (p=0.001 and p=0.01 respectively), but in patients who subsequently progressed to BC there was a trend for lower SET levels - suggesting that SET was not solely responsible for inhibiting PP2A in destined to progress into BC. At diagnosis of CML, patients who later progress to BC have significantly higher levels of CIP2A protein (p=<0.0001) than patients who do not progress. There was no correlation between CIP2A levels and patients' Sokal score. CIP2A protein was not suppressed by imatinib treatment *in vivo*. During disease progression, CIP2A protein levels increased further, suggesting that PP2A function is increasingly impaired in these patients. Chronic phase patients who have high CIP2A protein at diagnosis have a 100% probability of progressing to BC - with the mean time to progression being 13 months. Knockdown of CIP2A resulted in increased PP2A activity and decreased BCR-ABL1 tyrosine kinase activity. mRNA expression of CIP2A, SET and PP2A had no prognostic value. These data show that two mechanisms control PP2A activity in CML. In patients who clinically respond well SET appears to be the controlling inhibitor, while in patients who progress to BC PP2A activity appears to be impaired predominantly by CIP2A. Importantly these two signalling mechanisms can be detected at diagnosis. *Summary/Conclusion.* CIP2A is a prospective biomarker of BC in CML and may be a useful therapeutic target.

Acute lymphoblastic leukemia - Biology

0489

DEREGULATED EXPRESSION OF MICRORNA125B BY IGH TRANSCRIPTION ELEMENTS CAUSES ACUTE LYMPHOBLASTIC LEUKEMIA IN VIVO

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Background and Aims. Chromosomal translocations involving the immunoglobulin heavy chain gene (IGH) locus play a pivotal role in the pathogenesis of human B-cell malignancies. IGH translocation brings target genes positioned on different chromosome loci into close apposition with transcription elements within the IGH locus, resulting in deregulated expression of the target genes. We previously reported an insertion of microRNA125b1 (hearafter,miR125b1) into the IGH locus in a patient with B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Other groups identified fusion sequences of IGH and miR125b1 from t(11;14)(q24;q32) indicating that chromosome translocation involving IGH and miR125b1 loci is a recurrent event in human BCP-ALL However, *in vivo* oncogensis of miR125b in B-cells have not been fully understood. To confirm that deregulated expression of miR125b by IGH regulatory elements induces B-cell tumor, we generated transgenic mouse (Emu/miR125b1 TG mouse) mimicking the t(11;14)(q24;q32). *Methods.* The transgene consisted of human intronic enhancer of IGH

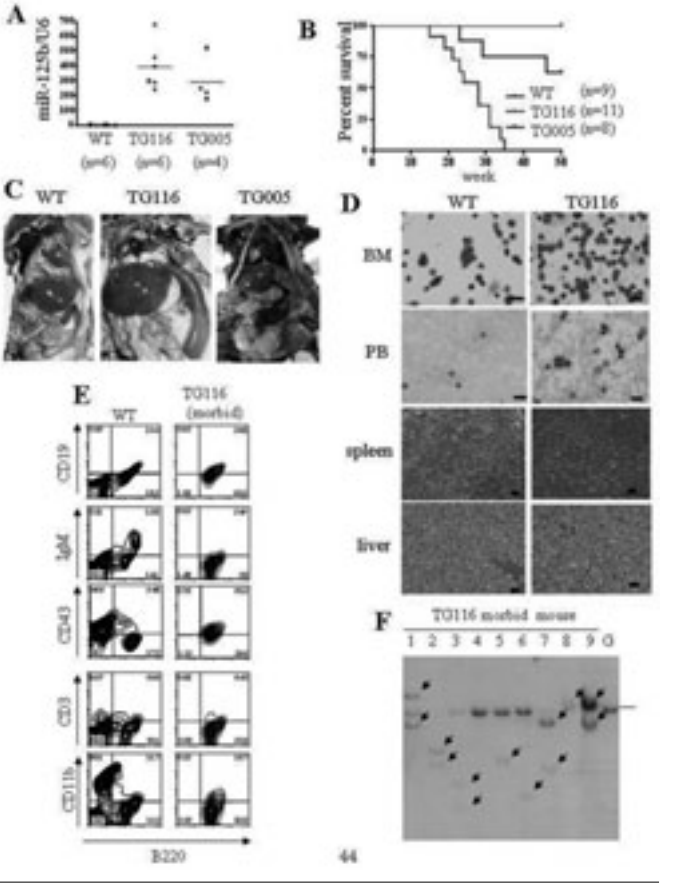

Figure 1.

(Emu), mouse VH promoter, and rat beta-globin including mouse genomic sequence containing pre-miR125b. Two stable Emu/miR125b1 mice (TG116 and TG005) were established and subsequently examined in morphologic, phenotypic and genotypic characteristics. We performed a bioinformatics search using Targetscan for candidate targets of the miR125b. MiR125b expression was analyzed in various hematological tumor samples using qRT-PCR. *Results.* Both TG mice developed lethal B-cell malignancies. Most tumor cells of the morbid TG116 mice were B220+/CD19+/IgM-/CD43+ B-cells; whereas those of the morbid TG005 mice were B220+/CD19+/IgM+/CD43- B-cells with clonal B-cell proliferation as shown by Southern blot analysis. We found several molecules for the target of miR125b, such as BAK1, which has been previously reported as a candidate. Among them, we focused on trp53inp1, a pro-apoptotic gene. MiR125b binds to tpr53inp1 3'UTR with seed sequence and B-cells obtained from the TG116 mice tended to reduce trp53inp1 at the mRNA and protein levels compared to WT mice. 32Dcl3 cells overexpressing miR125b or B-cells of TG116 became resistant to apoptosis induced by cytokine depletion or serum starvation, respectively. Of clinical relevance, overexpression of miR125b was found in various human hematological tumors including B-ALL, AML and MDS, especially in BCR/ABL positive B cell leukemia (four out of eight BCR/ABL positive ALL and one B-lymphoid crisis from CML). *Conclusion.* Deregulated expression of miR125b controlled by IGH regulatory elements causes BCP-ALL in TG mice. The phenotype of the tumor cells seen in the TG mice was similar to those of human disease. MiR125b binds to trp53inp1 and its overexpression is suggested to confer anti-apoptotic characteristics on cells. Overexpression of miR125b might be associated to BCP-ALL derived from BCR/ABL hematopoietic clone.

0490

CLONAL SELECTION IN XENOGRAFTED HUMAN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA RECAPITULATES GAIN OF MALIGNANCY AT RELAPSE

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Background. Despite major therapeutic improvements, a fraction of patients with acute lymphoblastic leukemia (ALL) still relapse and experience eventual refractory leukemia, pointing to the need for relevant preclinical models to design new therapeutic strategies. Genomic studies in human ALL have revealed intra-clonal heterogeneity at diagnosis and clonal evolution at relapse, suggesting that genetic subclones with distinct functional properties may preferentially contribute to tumor progression. *Aims.* Primary human ALL cells can be transplanted and expanded into immunodeficient mice, leading to xenograft human leukemia. We hypothesized that re-initiation of leukemia in mice could favor clonal evolution similarly to relapse in patients. We aimed to use xenograft to investigate the genomic and functional mechanisms of tumor progression in T-ALL patients. *Methods.* A series of 26 diagnosis T-ALL samples was transplanted into immunodeficient mice (NOD/Scid or NSG), and paired xenograft and diagnosis T-ALL samples were compared by high-density DNA arrays and oncogene resequencing. For 8 patients, relapse samples could also be analyzed. Large scale gene-expression profiling, lentiviral-mediated shRNA knockdown in patient's primary leukemic cells followed by competitive *in vivo* experiments, and *in vitro* assays for drugs response were performed to analyze functional features of clonal selection in xenograft leukemias. *Results.* We show that leukemia in recipient mice frequently re-initiated from minor subclones pre-existing at diagnosis and bearing additional genomic lesions of human cancer genes like PTEN, MYC, MYB, WT1, CDKN2A, and NOTCH1, reminiscent of clonal selection towards relapse in patients. Gene-expression profiling identified a robust signature of cell cycle and mitosis in the xenograft leukemia samples compared with the corresponding diagnosis samples. Importantly, Gene Set Enrichment Analysis (GSEA) found that this signature was also highly enriched in the cells at relapse in two independent series of human ALLs. Mimicking the effect of an additional genomic lesion in patient's primary leukemia cells by shRNA-mediated knock-down conferred a selective advantage in competitive engraftment experiments, demonstrating that these lesions can be drivers of increased leukemia-initiating activity. Finally, xenograft leukemia cells had an overall diminished sensitivity to glucocorticoids and gamma-secretase inhibitor. *Summary/Conclusions.* The establishment of human T-ALL in immunodeficient mouse is associated with the selection and expansion of a more aggressive leukemia with enhanced proliferation, recapitulating the process of leukemia progression in patients. This approach using leukemia xenotransplantation shed light on the mechanisms underlying tumor progression and should contribute to the design of novel strategies to prevent or treat T-ALL relapse in patients. Finally, these results suggest that genetic heterogeneity of ALL and subsequent clonal selection in immunodeficient mouse have to be considered when characterizing human ALL leukemic stem cells.

0491

MUTATION OF THE HEDGEHOG PATHWAY IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. The Hedgehog (HH) signaling pathway plays an important role in cell growth and differentiation and is involved in the development of many tissues, including T cell development. Based on this important function, it is not surprising that several human diseases are caused by mutation in components of the HH pathway. Many studies have revealed mutation of this pathway in basal cell carcinoma, medulloblastoma, and other cancers. Also in chronic myeloid leukemia stem cells, activation of the HH pathway was suggested, but mutation in HH pathway components have not been identified in hematological malignancies. *Aims.* Upon mutation analysis of 97 candidate oncogenes in T-cell Acute Lymphoblastic Leukemia (T-ALL), we identified PTCH1 (patched) mutations in several T-ALL cell lines (see abstract by V. Gianfelici *et al.*). The aim of this project was to confirm the involvement of the HH pathway in the pathogenesis of T-ALL and to determine the sensitivity of T-ALL cell lines to HH pathway inhibitors. *Methods.* We performed a mutation analysis of the different HH components in 17 T-ALL cell lines and 57 T-ALL patients. In addition, we treated a set of T-ALL cell lines with 4 different HH antagonists (Cyclopamine, GDC-0449, GANT61 and Itraconazole) and tested the effect on proliferation, cell cycle and apoptosis. *Results.* Sequence analysis of PTCH1, PTCH2, SMO, SUFU, GLI1 and GLI3 revealed a number of mutations in at least one of these genes in 9/17 (53 %) cell lines and 9/57 (15%) primary T-ALL samples. In agreement with this, T-ALL cell lines showed sensitivity to HH pathway antagonists. Itraconazole, a natural antifungal triazole, that was recently discovered as a new inhibitor of the HH pathway was identified as the most potent HH inhibitor in T-ALL cell lines with IC50 values below 500 nM. Apart from a reduction of the proliferation, it also induced apoptosis and blocked the cell cycle. *Conclusion.* Our data demonstrate an important role of the HH pathway in the pathogenesis of T-ALL, and show potent inhibitory activity of HH pathway inhibitors on T-ALL cell proliferation and survival.

0492

THE ERYTHROPOIETIN RECEPTOR (EPOR) IS DEREGULATED BY ETV6-RUNX1 (TEL-AML1) IN EARLY B LINEAGE PROGENITOR CELLS

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Background. ETV6-RUNX1 (TEL-AML1) fusion is a pre-natal and initiating event in childhood acute lymphoblastic leukaemia (ALL). The erythropoietin receptor gene (EPOR) is consistently highly expressed ectopically in TEL-AML1+ ALL but any role in leukemogenesis driven by TEL-AML1 remains to be confirmed. *Aims.* To assess the impact of TEL-AML1 expression on candidate 'pre-leukaemic' stem cells and to demonstrate the presence of functional, ligand binding EPOR on TEL-AML1+ ALL cells that may provide these cells with a survival signal. *Methods.* Biotinylated erythropoietin (EPO) and flow analysis were used to assess cell surface EPOR protein levels. Quantitative PCR, ChIP, luciferase reporter assays and EMSA showed that TEL-AML1 directly regulates EPOR. Standard tissue culture techniques were used to show cell survival in the presence of EPO alone and western blot to assess the mechanism of cell signalling. *Results.* Biotinylated EPO and flow analysis showed that the pre-B ALL TEL-AML1+ cell line REH has higher levels of EPOR than similar non-TEL-AML1 cell lines. A "blind screen"of CD19+ cells isolated from 10 patients with pre-B ALL, identified five patients with high expression of ligand-binding EPOR, four of which were subsequently identified as TEL-AML1+. The inducible

expression of TEL-AML1 in lymphoid BaF3 cells, or its constitutive expression in a murine transgenic model was sufficient to increase expression of EPOR. EMSA and ChIP experiments within the EPOR promoter confirmed occupancy of AML1 consensus binding sites by TEL-AML1 and luciferase reporter assays in the presence of the fusion protein showed up-regulation of EPOR promoter activity. Given the proposed pro-survival properties of EPO on non-erythroid cells, we asked if the observed increase in expression of the EPOR could correlate with increased cell survival in the presence of EPO. Cell survival experiments including growth curves, propidium iodode staining and analysis of anti-apoptotic gene markers revealed that IL3-dependent cells expressing TEL-AML1 showed a prolonged survival in the presence of EPO alone. Turning off TEL-AML1 in these cells resulted in cell death even in the presence of EPO, suggesting that this effect is a consequence of TEL-AML1 expression alone. Signalling through EPOR in the presence of EPO was confirmed by phosophorylation analysis of JAK and AKT pathways, analysis of STAT5B activity and the concomitant up-regulation of BCL-XL. EPOR functionality in the presence of EPO was also demonstrated in TEL-AML1+ patient cells. In our model of human pre-leukemia, normal human CD34+ cord blood cells were transduced *in vitro* with a lentivirus capable of expressing both TEL-AML1 and GFP and cells were 'primed' for pre-B lineage commitment. These TEL-AML1+ cells also showed increased levels of functional cell surface EPOR and activated survival gene targets - again suggesting a role for enhanced cell survival through the EPO-EPOR axis. *Summary.* These data support the contention that TEL-AML1 directly activates ectopic expression of a functional EPOR, which provides cell survival signals that contribute critically to persistence of the pre-malignant clone in patients.

0493

MICRORNA SIGNATURES IN NORMAL AND MALIGNANT T-CELL DEVELOPMENT

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Background. T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy of thymocytes. In order to comprehensively assess the action of microRNAs (miRNAs) in T-ALL, we compared miRNA expression patterns in 50 human T-ALL samples and 5 distinct subsets of normal developing T-cell progenitors with an unbiased miRNA library screen followed by computational target identification and functional assessment of the most relevant candidate miRNAs in a murine T-ALL model. *Methods.* Using high-throughput quantitative stem-loop RT-PCR, 430 miRNAs were profiled in a T-ALL patient cohort including 12 HOXA, 15 TAL/LMO, 10 TLX3 and 5 TLX1 rearranged patient samples as well as in 5 different subsets of sorted T-cell populations from human thymus. An unbiased miRNA library screen was performed in myc-transduced MEFs, based upon rescue for myc-induced apoptosis, followed by validation for individual miRNAs in FL5-12 lymphocytes and an *in vivo* NOTCH1-sensitized murine T-ALL model. *Results.* A total of ten miRNAs were highly expressed in the entire cohort of T-ALLs, i.e. miR-223, miR-19b, miR-20a, miR-92, miR-142-3p, miR-150, miR-93, miR-26a, miR-16 and miR-342. High expression of this subset of 10 miRNAs was confirmed in a series of 18 T-ALL cell lines. Cross-comparison with the miRNA library screen allowed the identification of five T-ALL promoting miRNAs (miR-19b, miR-20a, miR-26a, miR-92 and miR-223). Remarkably, these miRNAs produce overlapping and cooperative effects on validated target genes with known tumor suppressor function in T-ALL, including IKAROS (IKZF1), PTEN, BIM, PHF6, NF1 and FBXW7. In addition, specific sets of differentially expressed miRNAs were delineated for each of the genetic T-ALL subgroups. Finally, the current mRNA/miRNA profiles also provide insight into key regulatory events controlling normal T-cell development. *Conclusion.* A comprehensive and unbiased analysis of miRNA action in T-ALL and normal developing thymocytes reveals a cooperative role for a small set of miRNAs in suppression of key T-ALL suppressor genes, identifies miRNA signatures in genetic T-ALL subgroups and provides insights into miRNA controlled regulation of thymocyte maturation.

Thrombosis

0494

MELISSE, A LARGE MULTICENTRIC OBSERVATIONAL STUDY TO DETERMINE CRITERIA AND RISK FACTORS OF THROMBOEMBOLISM FOR PATIENTS WITH MULTIPLE MYELOMA TREATED WITH IMMUNOMODULATOR DRUGS

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Background. Immunomodulator drugs (IMiDs) are new and very promising oral agents in Multiple Myeloma (MM); however, IMiDs are also associated with an increased risk of thromboembolism events (TE) which necessitates routine prophylaxis. Controversies remain regarding the best choice of TE prophylaxis in MM patients treated with IMiDs-based therapy and the criteria for the TE risk definition. *Aims.* We designed a large multicentre observational study aimed at prospectively evaluate the incidence and risk factors of venous thromboembolisms associated with IMiDs [either lenalidomide (Len) or thalidomide (Thal)] therapy in MM. *Methods.* A total of 519 patients with MM treated with IMiDs-based therapy at first to third line of therapy were included in this study. VTE prophylaxis was recommended to start prior to start IMiDs, the choice was left at the discretion of the investigator. Patients gave written informed consent according to the declaration of Helsinki. Various patient characteristics were recorded, such as age, sex, criteria of vascular complications, including adjuvant treatment and previous history of vascular complications. The physicians were to record the risk of VTE occurrence, based on guidelines and their own appreciation of the risk. Occurrence of any thrombosis event (either venous or arterial) was to be recorded along with the descriptive characteristics of the event, how the event was managed and the outcome of the patient. The data were collected at entry in the study, and then at 4 and 12 months. *Results.* Out of the 519 patients, 35.66% had Thal-based and 64.34% had Len-based therapy. Overall, median age was 71, with 65% >65 years old and sex ratio was 249 male/268 female, similar in the 2 groups (data missing for n=2). One hundred and eighty patients were in first line therapy, 169 in second line therapy and 153 in third line therapy (data missing for n=17). Patients were treated with VTE prophylaxis as follow (data missing for n=8); 293 (57.34%) aspirin, 91 (17.81%) LWMH and 46 (9.00%) vitamin K antagonists. Surprisingly, 16% had no VTE prophylaxis. Aspirin was primarily administered in 70% of low risk patients and 58% of moderate risk patients, and LWMH in 46% of high risk patients along with 20% aspirin. Investigators recorded 17 (3. 5%) VTE at 4 months. Of the VTE, 50% of the patients had aspirin and 20% had LWMH. Interestingly, patients with VTE in the aspirin group were considered low risk, and patients in the LWMH group were considered high risk. The occurrence of VTE was unrelated to the IMiD-based therapy and the line of therapy. None of the patients with PE had LWMH. *Conclusion.* VTE is low in IMiDs-based treated MM patients upon VTE prophylaxis. However, despite VTE prophylaxis, we observed occurrence of VTE, not related to the VTE risk stratification recommended in guidelines. It is therefore needed to determine risk factors of VTE upon VTE prophylaxis. Final results will be proposed with updated results at EHA 2011.

0495

THE OPTIMAL DURATION OF ANTICOAGULANT THERAPY IN PATIENTS WITH CANCER-RELATED DEEP VEIN THROMBOSIS: THE ADVANTAGE OF USING RESIDUAL VEIN THROMBOSIS (THE CANCER-DACUS STUDY)

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Type and duration of anticoagulation is still matter of debate in cancer patients with acute Deep Vein Thrombosis (DVT) of the lower limbs. Residual Vein Thrombosis (RVT) has been proven to be effective for assessing the optimal duration of oral anticoagulants in non cancer patients (Siragusa S *et al* Blood 2008:112:511-5). In the present study we evaluate the role of a RVT-based management of anticoagulation with Low-Molecular Weight Heparin in cancer patients with acute DVT. *Materials and Methods.* Patients with active cancer and a first episode of DVT were treated with LMWH for 6 months (the first month at full dosage followed by dose reduction of 25% in the next 5 months). At the end of treatment, they were managed according to RVT findings: those with RVT were randomized to continue anticoagulants for 6 additional months (Group A1) or to stop it (Group A2), while patients without RVT stopped LMWH (Group B). Outcomes were recurrent venous thromboembolism and/or major bleeding; patients were followed up for one year after LMWH discontinuation. *Results.* Over a period of 36 months, 409 patients were evaluated; 62 were excluded (refusal, need for continuing anticoagulation, etc). In total, 347 were included in the study (Table 1). RVT was detected in 242 (69.7%) patients; recurrent events occurred in 21.9% of those randomized to discontinue and 14.2% of those who continued LMWH. In patients without RVT (105, 30.3%), recurrent events occurred in 3 cases (2.8%) (Table 2 and Figure 1). The adjusted Hazard Ratio (HR) for age and sex between RVT groups (Group A2 vs A1) was 1.58 (95% confidence interval [CI], 0.85-2.93; P=.145). The adjusted HR between group A1 versus RVT-negative group (B) was 4.54 (CI 2.3-6.66; P =.028). Five major bleeding events occurred in Group A1 and two events both in Group A2 and B (Table 2). Overall, 89 (25.6%) patients died due to cancer progression after a median follow-up of 10.2 months after heparin withdrawn. *Conclusions.* The Cancer DACUS is the first ever study evaluating an individual marker for assessing duration of anticoagulation in active cancer population. Final results of the study show that absence of RVT identifies a group of patients at low risk for recurrent thrombosis who can safely stop LMWH after 6 months.

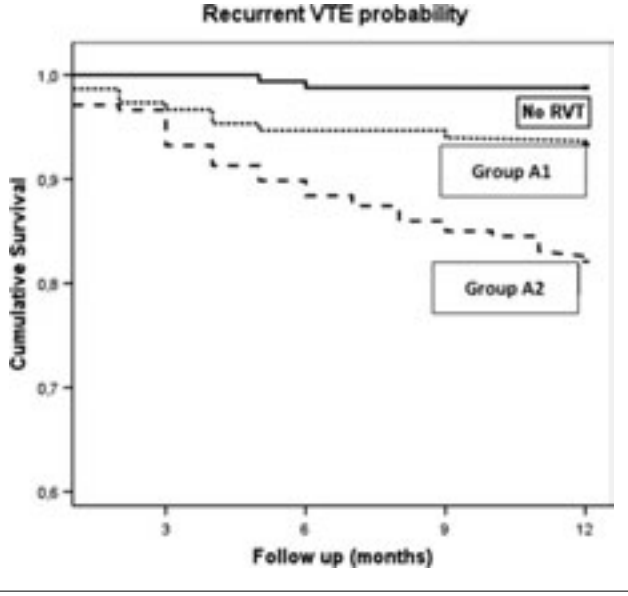

Figure 1.

0496

THROMBIN GENERATION AND PROANGIOGENIC PROPERTIES OF BREAST CANCER CELLS ARE MODULATED BY HEPARINS

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Background. Breast cancer is the most frequent solid tumor among women and is associated to an increased thrombotic risk during antitumor treatments. Tumor cell-associated procoagulant activity contributes to the thrombotic risk and to the disease progression in cancer patients. Reducing tumor procoagulant activity may improve the outcome of the disease. In this setting, low molecular weight heparins (LMWH) have shown a beneficial effect on both thrombosis rate and survival from cancer. *Aim.* In this study we investigated whether two LMWH (i.e. dalteparin, mean MW 6.0 KDa and bemiparin, mean MW 2.6 KDa) and unfractioned heparin (UFH, mean MW 15 KDa) might affect the thrombin generation potential (TG) and the proangiogenic activity of the breast cancer cells. *Methods.* MDA.MB.231 breast cancer cells were lysed (150,000 cells/ml) by 3 cycles of freezing and thawing and TG was evaluated by the Calibrated Automated Thrombogram (CAT) and compared to the activity of a standard TF preparation (i.e. 1 and 5 pM, Thrombinoscope). Parameters evaluated were: time to generate thrombin (Lagtime, min), endogenous thrombin potential (ETP, nM*min), peak height (Peak, nM) and time to reach the peak (ttPeak, min). The inhibition capacity of heparins was evaluated after addition of increasing concentrations of these drugs (from 0.01 to 1 IU/ml) to the experimental system. The anti-angiogenic activity of heparins was evaluated as the capacity of these agents to inhibit endothelial cells capillary-like tube formation in Matrigel. Specifically, endothelial cells (HMEC-1) were incubated for 24 hours with MDA.MB.231 conditioned medium in the presence or absence of heparins (from 0.01 to 1IU/ml), and the total length of the network formed was measured. *Results.* MDA.MB.231 showed a high TG, with values comparable to those obtained with 5pM standard TF. Heparins significantly and dose-dependently reduced the breast cancer cell-induced TG. Among the different TG parameters, ETP and Peak were the most affected by heparins. Particularly, at 0.4 IU/ml concentration, TG was completely inhibited by UFH, while ETP and Peak were reduced by 60% and 80% by dalteparin, and 30% and 50% by bemiparin, respectively. All the heparins studied also reduced in a dose dependent way the proangiogenic activity of tumor cells in the Matrigel assay. Starting from a concentration of 0.1 IU/ml, a significant ($p<0.05$) reduction of capillary network formation was observed with all the three heparins. The pattern of inhibition was the opposite of that of TG, i.e. 100% for bemiparin, 75% for dalteparin and 35% with UFH, showing an inverse relation with the heparin molecular weights. *Conclusion.* The effect of LMWH on the control of two crucial steps of breast cancer invasiveness, i.e.TG and the proangiogenic capacity, supports the role of these agents as adjuvant therapy for cancer treatment.

0497

THROMBOPROPHYLAXIS FOR NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH LENALIDOMIDE-BASED REGIMENS: A RANDOMIZED PHASE III STUDY OF ASPIRIN VS ENOXAPARIN

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Background. Thalidomide-based regimens are associated with an increased incidence of thrombosis in newly diagnosed myeloma (MM) patients. Preliminary studies on MM patients treated with a combination of lenalidomide (R) plus dexamethasone have shown an increased risk (between 11% and 75%) of thromboembolic events as well. *Aims.* In a prospective, multicenter phase III trial (RV-MM-PI-209) newly diagnosed patients were treated with lenalidomide and low-dose dexamethasone (Rd) induction and subsequently randomized to receive consolidation with lenalidomide + melphalan + prednisone (MPR) or high dose melphalan (MEL200). In this substudy, we compared the efficacy and safety of aspirin (ASA) or low-molecular weight heparin (LMWH) as thromboprophylaxis in newly diagnosed MM patients younger than 65 yrs during Rd induction and MPR consolidation. Primary end-points were incidence of venous thromboembolism (VTE), acute cardiovascular events, sudden death, major and minor bleeding. *Methods.* a total of 402 transplantation candidates received four 28-day cycles of lenalidomide (25 mg d 1-21) and low-dose dexamethasone (40 mg d 1,8,15,22) (Rd) as induction and randomized to consolidation with 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) (N=202) or tandem melphalan 200 mg/mq with stem-cell support (N=200). A total of 342 patients without clinical indication or contraindication for a specific antiplatelet or anticoagulant therapy, were enrolled in this substudy and randomly assigned to receive Aspirin 100 mg/d (N=176) or Enoxaparin 40 mg/d (N=166) during induction with Rd and consolidation with MPR. *Results.* patient characteristics and distribution of major risk factors were similar in the two groups. During induction, the overall incidence of any 3-4 thrombotic events was 2,27% in the ASA group and 1,20% in the LMWH group (p=0.685). Deep vein thrombosis were equally distributed in the two groups (1.13% Vs 1.20%, p=0.466), while pulmonary embolism was observed only in the ASA group (1.70%). Compared with LMWH, the absolute risk difference was +0.5% (95%CI=-2.5 to 3.5, p=0,605) in the ASA group. All thromboembolic events occur in early phase of treatment (median 1,3 months). Only 1% of minor bleeding was detected in the LMWH group and no cardiovascular events were observed. During MPR consolidation only one thrombotic event was seen in the LMWH group. *Conclusion.* our data indicate a low overall incidence of thrombotic events in all groups. Both ASA and LMWH show similar safety and efficacy in reducing thromboembolic events in newly diagnosed multiple myeloma patients treated with lenalidomide-based regimens.

0498

TIMING OF TISSUE FACTOR (TF) MRNA AND HYPERCOAGULABILITY DOWNREGULATION BY ALL-TRANS-RETINOIC ACID (ATRA) IN ACUTE PROMYELOCYTIC LEUKEMIA (APL)

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Background. Among the mechanisms responsible for intravascular clotting activation in APL, a key role is played by the procoagulant activities, including TF expressed by the promyelocytic blasts. Differentiating therapy with ATRA or Arsenic Trioxide (ATO) achieves 90% complete remission rate and corrects the hyperactivity of the coagulation and fibrinolytic systems. The beneficial effects on the coagulopathy are at least in part due to the capacity of ATRA and ATO to downregulate TF expression in APL cells. However, it is not known whether the persistence of TF mRNA expression in peripheral APL cells might be a marker of hypercoagulability in these patients. *Aim.* In this study we evaluated the levels of TF mRNA in peripheral mononuclear cells (PBMC) obtained from 9 APL patients: 4 treated with ATRA+Idarubicin (IDA) and 5 treated with ATRA+ATO. *Results.* of TF mRNA were correlated to the plasma levels of thrombin antithrombin complexes (TAT), as an index of thrombin formation and inhibition, and of activated factor VII-Antithrombin complex (FVIIa-AT), as an index of TF exposure and inhibition on blast cells. Five healthy subjects acted as the control group. *Methods*. Blood samples were obtained at the onset of the disease (T0) and after 7 (T1), 14 (T2) and 28 (T3) days of treatment. PBMC were isolated from whole blood and TF mRNA was assessed using real time-polymerase chain reaction. Results were normalized versus T0. Platelet-free plasma was obtained by two serial centrifugations (both 4,000rpm for 15 min) of citrated whole blood. ELISA methods were used for the measurement of plasma levels of TAT (Siemens) and FVIIa-AT complexes (STAGO). *Results.* The levels of TF mRNA of APL PBMC were significantly elevated at the onset of the disease compared to controls ($p<0.05$). In patients treated with ATRA+IDA the levels of TF mRNA decreased by 68% at T1, by 70% at T2 and by 90% at T3; similar reductions were observed in patients treated with ATRA+ATO, i.e. 64% at T1, 83% at T2 and 82% at T3. Plasma concentration of TAT complex of APL patients significantly decreased ($p<0.05$) from T0 (48.9±6.43 mg/L) to T3 (8.13±0.92 mg/L). Similarly, plasma concentration of FVIIa-AT significantly decreased from T0 (287.10±36.51 pM) to T3 (168.31±16.62 pM). The statistical analysis revealed significant correlations between TF mRNA and TAT at T1 and T3 (ATRA+IDA: T1 R2=0.691; T3 R2=0.927; ATRA+ATO: T1 R2=0.685) and between TF mRNA and FVIIa-AT at T1 and T2 (ATRA+IDA: T1 R2=0.767; T2 R2=0.672; ATRA+ATO T1 R2=0.828; T2 R2=0.817). *Summary/Conclusions.* Our results show that TF expression is elevated in PBMC of APL patients at the onset of the disease and is downregulated either by ATRA+IDA or ATRA+ATO treatment following a similar pattern of reduction. The significant correlation between TF mRNA reduction and the decrease of the two markers of hemostatic system activation (TAT and FVIIa-AT) suggests that TF mRNA as a useful surrogate marker of hypercoagulation in multicenter studies.

Experimental stem cell transplantation

0499

REGULATION OF ACUTE GRAFT VERSUS HOST DISEASE BY MICRORNAS

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Background. Acute Graft-Versus-Host disease (aGVHD) is a frequent complication of allogeneic bone marrow transplantation (BMT) in which donor T cells destroy HLA mismatched host tissues. Recent studies indicate that microRNA (miR)-155 is induced upon CD4+ cell activation and promotes Th1 differentiation. *Aims.* Based on the importance of T cells in aGVHD pathogenesis and the regulation of immune responses by miR-155, here we propose to investigate whether miR-155 expression is deregulated in donor T cells during aGVHD and whether miR-155 is involved in the modulation of this process. *Methods*. We used a MHC mismatched BMT aGVHD model in which spleen cells (20X106) and T cell depleted bone marrow (t-BM;5X106) from C57BL/6 (B6) donors were transferred i.v. into lethally irradiated B6D2F1 recipient mice. Control groups included mice that received t-BM only or received no cell infusion. CD4+CD62L- effector cells were isolated from the spleen, RNA extracted and miR-155 expression measured by qRT-PCR. *Results.* CD4+CD62L- cells isolated from mice with aGVHD exhibited increased miR-155 expression with respect to the same cell populations obtained from the t-BM only group (4 fold increase, $p<0.001$). To confirm that a causal relationship exists between miR-155 and aGVHD severity, we repeated the MHC mismatched murine experiment using B6 mice deficient for miR-155 expression as donors. Mice receiving donor spleen cells from BIC/miR-155 KO mice exhibited dramatically lower mean GVHD scores and improved survival compared to those receiving WT spleen cells (87% vs. 13% of mice alive at 70 days, respectively; $p<0.001$). GVHD histological scores in the spleen, liver or gut were remarkably lower in recipients from miR-155 KO (none grade III-IV). Overall survival, GVHD scores and histological GHVD findings were similar between miR-155 KO and WT t-BM only group. Mice receiving miR-155 KO spleen cells also had significantly lower TNF-α levels than WT controls (14 pg/ml vs. 48 pg/ml, $p=0.005$). To further establish the regulatory role of miR-155 in aGVHD, we generated a transgenic mouse that over-expresses miR-155 in T cells under the LCK promoter (B6 LCK-miR-155). Using splenocytes from LCK-miR-155 TG mice we performed the mismatched MHC experiments as described previously. Recipients of miR-155 over-expressing splenocytes developed hyper-acute GVHD (confirmed by pathology) and died shortly after transplant (within 2-3 weeks), while recipients of WT cells developed lethal aGVHD significantly later ($p=0.03$). Relevant to human aGVHD, we measured miR-155 expression in the colon tissues of aGVHD patients (n=5) or controls (n=4) using DIG-tagged anti-miR-155 LNA and found a dramatic up-regulation of miR-155 in the mucosa of aGVHD patients, while it was negative in healthy controls. Finally, we performed the B6 into F1 MHC-mismatched transplants as described and treated 6 mice with antisense miR-155 (LNA anti-155) and 6 mice with a scrambled control (n=6) 5 mg/kg twice a week (I.V) x 2 weeks starting at day+7. The mice that received LNA anti-155 showed higher survival rate compared to mice that received the scramble control ($p<0.03$). *Conclusions.* Collectively, our data indicate that miR-155 modulates aGVHD, and thus point to miR-155 as a potential target for therapeutic intervention for aGVHD.

0500

IKAROS-NOTCH SIGNALING IN HOST ANTIGEN PRESENTING CELLS REGULATES EXPERIMENTAL GRAFT-VERSUS-HOST DISEASE (GVHD) AND GRAFT-VERSUS-LEUKEMIA (GVL) RESPONSES

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Background. Host antigen presenting cells (APCs) are necessary for induction of graft-versus-host (GVH) responses. But the APC autonomous molecular mechanisms that are critical for modulation of GVH are not known. Because Ikaros (Ik) is known to negatively regulate certain dendritic cell (DC) responses (the most potent APCs), we hypothesized that its deficiency in host APCs will reduce GVHD. *Methods and Results.* We generated [B6[ARROWRIGHT]B6] and [Ik-/-B6[ARROWRIGHT]B6] chimeras and utilized them as recipients in C3H.SW B6 model of acute GVHD. The [Ik-/-B6 [ARROWRIGHT] B6] animals showed significantly worse survival, GVHD specific clinical severity and histopathological damage than the allogeneic [B6[ARROWRIGHT] B6] animals ($P < 0.001$). *In vitro*, CFSE and annexin labeling studies demonstrated that Ik-/-DCs caused greater proliferation without altering the rate of apoptosis. To characterize the molecular mechanisms we evaluated the role of putative molecular targets of Ikaros, the Notch signaling pathway. Ik-/- DCs, at steady state, showed an increase in the expression of several Notch target genes such as Hes-1, Hex-1, etc. Blockade of Notch signaling with γ-secretase inhibitor (DAPT) mitigated the enhanced allo-stimulatory capacity of the Ik-/-DCs *in vitro* and decrease donor T cell expansion and improved body weight loss *in vivo*. We next hypothesized that given the enhanced GVHD response that was associated with increased proliferation and preserved cytotoxicity of allo-T cells, the GVL response will also be enhanced in the recipients with Ik-/- APCs. Unexpectedly, the [B6[ARROWRIGHT] B6] and [Ik-/-B6 [ARROWRIGHT] B6] chimeras when transplanted with tumor cells demonstrated equivalent GVL responses despite greater severity of GVHD. *Conclusions.* Together our data demonstrate differential regulation of GVHD and GVL at the level of host APCs and show a role for a novel molecular pathway, the Ik-Notch axis, in the host APCs as an important modulator of GVH responses.

0501

ANALYSIS OF MIIRNA EXPRESSION PROFILE AFTER HAEMATOPOIETIC STEM CELL TRANSPLANTATION: A PROMISING TOOL FOR PREDICTING ACUTE GVHD

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Background. Allogeneic haemopoietic-stem-cell transplantation (HSCT) is the treatment of choice for many malignant and non-malignant disorders. Despite the recent advances in post-transplant immunosuppressive therapy, Graft-versus-Host Disease (GVHD) still represents the major life-threatening complication. Recent studies have indicated that microRNAs (miRNAs) circulate in a stable, cell-free form in the bloodstream and that the abundance of specific miRNAs in plasma or serum can serve as biomarkers of cancer and other diseases. *Aim.* This study was aimed at the prospective analysis of miRNA expression profile in the plasma of allo-transplanted patients in order to detect specific miRNAs with predictive role for acute GVHD (aGVHD). *Methods.* After informed consent, we collected plasma samples from 10 healthy donors and 22 patients (median age: 59 and 41 years) who received unmanipulated HSCT (18 from Matched Unrelated Donors and 4 from HLA-matched siblings). Blood samples were collected weekly after HSCT and patients were monitored to assess aGVHD onset. Three of 22 patients developed intestinal GVHD (grade 2) while 9 of 22 patients developed cutaneous GVHD (grade 2-3). MicroRNAs were isolated from the plasma of patients using a modified mirVana® miRNA Isolation Kit (Ambion Inc). The miRNA expression profile was examined using a quantitative PCR-method (TaqMan ® Human microRNA

Cards, Applied Biosystems) that allows the analysis of 384 human miRNAs by low density array technology. The results obtained were subsequently validated with specific miRNA Single Assays (Applied Biosystems). Hsa-miR-16 was used for data normalization due to its stability in all the samples analyzed. Relative quantification of miRNA expression was calculated with the $2^{-\Delta\Delta Ct}$ method. Cluster analysis was performed using an agglomerative hierarchical algorithm with average linkage as a distance measure. Differential miRNA expression profile was investigated with the Mann-Whitney test. *Results.* Circulating miRNAs are detectable and amplified in all samples analyzed. Unsupervised hierarchical clustering of miRNAs present before the onset of GVHD, showed that specific circulating miRNA expression signatures discriminate between patients who will develop GVHD from those who will not. By comparing the miRNA expression profiles of GVHD patients and non-GVHD patients, we identified a group of 13 miRNAs upregulated in the plasma of GVHD patients ($p<0.05$). We then aimed at assessing whether this 13-miRNA panel provided information regarding the involvement of specific target organs. MiR-194 and miR-367 are overexpressed in patients developing intestinal GVHD ($p<0.05$). Of interest, both miRNAs are implicated in the differentiation of the gastrointestinal epithelium. A significant upregulation of miR-203 was observed prior to the onset of cutaneous aGVHD ($p<0.001$). The involvement of miR-203 in cutaneous aGVHD is supported by recent papers demonstrating that it targets p63, a protein required for keratinocytes differentiation, and SOCS3, a protein involved in the pathogenesis of GVHD (Hill *et al*. Blood, 2010). *Conclusions.* Our results demonstrate that the analysis of miRNA expression profile after HSCT is a promising tool for predicting aGVHD onset. Significantly higher plasma levels of miR-203 characterize patients who will develop skin-only GVHD whereas an upregulation of plasma levels of miR-194 and miR-367 may be used to predict the risk of developing intestinal aGVHD.

0502

IN VITRO-ESTABLISHED ALLOANTIGEN-SPECIFIC CD8+ CTLS MEDIATE GRAFT-VERSUS-TUMOR ACTIVITY IN THE ABSENCE OF GRAFT-VERSUS-HOST DISEASE

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Background. Allogeneic bone marrow transplantation (BMT) is a curative treatment modality for hematopoietic malignancies such as acute and chronic leukemias and lymphomas. Mature donor T cells in the allograft support engraftment, promote early T cell immunity of the recipient and mediate the graft-versus-leukemia (GVL) effect. However, these donor T cells are also responsible for the induction of graft-versus-host disease (GVHD) by attacking recipient tissue such as liver, skin, and bowel leading to significant morbidity and mortality. While depletion of mature T cells from the allogeneic donor graft significantly reduces the risk of GVHD it also abrogates the beneficial effect of GVL and delays immune reconstitution of the host, thereby supporting opportunistic infections. *Aims.* A major challenge in allogeneic BM transplantation is the identification of T cell subpopulations mediating the GVT-effect in the absence of GVHD induction. *Methods.* By repetitive alloantigen stimulation we established alloantigen-specific $CD8^+$ cytotoxic T cells (CTLs) with highly efficient cytotoxicity towards alloantigen-expressing target cells. In two MHC-mismatched BM transplantation models where induction of lethal GVHD is either dependent on the presence of $CD4^+$ or $CD8^+$ T cells we tested the effects of these *in vitro*-generated alloantigen-specific CTLs on GVHD induction and GVT-effect. *Results. In vitro*-derived CTLs exhibited an effector/memory phenotype and showed strong cytotoxicity towards alloantigen-expressing target cells *in vitro*. However, these alloantigen-specific CTLs did not induce GVHD in both MHC-mismatched BM transplantation models. They did not induce the expression of GVHD-associated cytokines IFN-gamma, and TNF-alpha in transplanted recipient mice. No clinical or histological signs of GVHD were detected and CTL-transplanted mice exhibited a survival rate above 90%. Inability to induce GVHD was not due to a decreased survival or impaired effector function of CTLs *in vivo* since they lysed antigen-expressing target cells *in vitro* when re-isolated fourteen days after transplantation. Also a five-fold increase in the number of transplanted CTLs could not induce GVHD. In contrast to alloantigen-activated CTLs, transplantation of unstimulated $CD8^+$ T cells, which were not primed for the alloantigen *in vitro*, induced severe, lethal GVHD in both transplantation models. Surprisingly, alloantigen-specific CTLs efficiently eradicated Bcr-Abl transformed B cell leukemias or mastocytomas showing that they maintain their GVT-reactivity. *Summary/Conclusions.* To our knowledge we show for the first time in a $CD4^+$ and $CD8^+$ T cell-dependent MHC-mismatched BMT model that *in vitro*-generated alloantigen-specific CTLs do not induce GVHD but mediate an efficient anti-tumor effect indicating that adoptive transfer of such effector cells might provide a treatment strategy for tumor eradication in the context of allogeneic BMT in the absence of GVHD-induction. Testing CTLs in a chimeric human/mouse xenogeneic GVHD model will further elucidate whether this stimulation protocol might be applicable in the clinic.

0503

LOOKING FOR MOST SUITABLE CELLS FOR T-CELL RECEPTOR RNA TRANSFER - MEMORY T CELLS OFFER CONSIDERABLE ADVANTAGES

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Background. Reactivation of latent Cytomegalovirus (CMV) infection is a common and sometimes life-threatening complication following allogeneic hematopoietic stem cell transplantation (HSCT). Long-term virus control requires the re-establishment of protective antiviral T-cell immunity in the host. The latter is challenging, particularly if the donor is CMV-negative and thus, no CMV-reactive T cells are being transferred to recipient during HSCT. *Aims.* Grafting nonreactive T cells of CMV-negative donors by virus-antigen specific T-cell receptors (TCR) may be an efficient means to transfer CMV specific T-cell function into HSCT recipients. To overcome the limitations of vector-based gene transfer that hamper clinical translation, we used *in vitro* transcribed RNA encoding CMV-specific TCR for electroporation of non-reactive human T cells. Due to the instability of the introduced RNA molecules, repetitive administration of TCR redirected T cells appears mandatory. However, this approach might be hampered by the induction of serious alloreactivity through the repeated transfer of polyclonal donor T cells with unknown endogenous specificity. To address this concern, our study aimed at the analysis of the CMV-specific as well as allo-HLA-reactive effector potential of different naive and memory T-cell populations. *Methods/Results.* We have reprogrammed T cells of CMV-negative donors with human TCR RNA recognizing the immunodominant HLA-A*0201-binding epitope 495-503 derived from the CMV pp65 protein. This procedure resulted in transient expression of the introduced TCR for up to one week. To compare different T-cell populations with regard to their alloreactive potential, we generated TCRpp65 transfected pure naive and memory T-cell subsets. The latter have been reported to induce less alloreactivity due to a more restricted endogenous TCR repertoire. Although both naive and memory T-cell subsets showed comparable expression of TCRpp65, memory $CD8^+$ T cells mediated superior cytotoxicity and IFN-γ production against CMV-infected fibroblasts for up to one week. Alternatively, we generated EBV/HLA-A*0201 peptide-specific T-cell lines and transfected them with TCRpp65 RNA to obtain EBV/CMV-bispecific T cells. As with TCR redirected memory T-cell subsets, EBV/CMV-bispecific $CD8^+$ T cells showed strong reactivity against CMV-infected fibroblasts for up to one week without hampering the endogenous EBV peptide-specific effector function. To analyze the allo-HLA-reactivity of the naive, memory and EBV-specific T-cell populations, we assayed their IFN-γ-secretion upon stimulation with different HLA-mismatched donor EBV-transformed B cells (EBV-LCL) as well as CD40 ligand activated B-cells. Although we tested only a small panel of 6 HLA-mismatched donors, alloreactivity was solely mediated by T-cell populations of naive phenotype. Moreover, no differences were obtained with either TCRpp65 RNA transfected or untransfected T cells, assuming that mixed dimer formation between introduced and naturally expressed TCR chains did not induce additional allo-HLA-reactivity in our studies. *Summary.* Our data demonstrate that memory T-cell populations from CMV-negative donors can be easily redirected with TCRpp65 RNA, thereby gaining CMV-specific T-cell effector function for a considerable time period. Due to their decreased alloreactivity, we believe that TCRpp65 RNA redirected memory T-cell populations have the potential to be further developed as a therapeutic 'off-the-shelf' reagent for CMV-positive patients who undergo allogeneic HSCT from CMV-negative donors.

Presidential Symposium

0504

RITUXIMAB MAINTENANCE SIGNIFICANTLY PROLONGS DURATION OF REMISSION IN ELDERLY PATIENTS WITH MANTLE CELL LYMPHOMA. FIRST RESULTS OF A RANDOMIZED TRIAL OF THE EUROPEAN MCL NETWORK

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Background. R-CHOP induction is considered the standard regimen for elderly patients with mantle cell lymphoma (MCL), but remissions are of short duration. Maintenance with interferon-alfa has been suggested to be effective, but side effects were serious. Rituximab seemed a promising candidate for improvement. *Aims.* In the European MCL Elderly trial we studied different induction regimens as well as the role of maintenance therapy. Here, the results of the maintenance are reported. *Methods.* Eight countries participated in this trial. Patients >60 yrs not eligible for high dose therapy with stage II-IV MCL were included. Initially, patients were randomized between 8 cycles of 3-weekly R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) or 6 cycles of 4-weekly R-FC (rituximab, fludarabin, cyclophosphamide). Subsequently, patients in complete or partial remission (CR, CRunconfirmed or PR) underwent a second randomization between maintenance with rituximab 375 mg/m^2 every 2 months or interferon-alfa 2a or 2b (IFN) (regular IFN weekly 3x3 MIU or pegylated IFN 1x1 μg/kg). Second randomization was stratified for induction regimen, study group, age, international prognostic index (IPI) and response (CR/CRu vs PR). Both maintenance regimens were continued until progression. *Results.* Randomization was closed Oct 2010. Out of 308 responding patients randomized for maintenance, data from 223 patients are currently evaluable. Median age was 70 yrs, 68% male, 79% stage IV, 48% intermediate and 43% high risk MIPI. Sixty-one percent of patients had a CR/CRu upon induction therapy. Fifty-eight percent had received R-CHOP induction. After a median follow-up of 30 months, patients randomized for rituximab maintenance had a significantly longer remission duration compared to IFN (51 vs 24 months; p=0.0117; HR 0.56; 0.36-0.88). Overall survival (OS) was not different between both arms. However, the subcohort R-CHOP-treated patients appeared to show an advantage after rituximab maintenance (3-yr OS 85% vs 70% after IFN; p=0.0375). Hematologic grade 3-4 toxicity was higher in the IFN arm (leukocytopenia 36% vs 17%; thrombocytopenia 16% vs 7%); non-hematologic grade 3-4 toxicity was rare, except for infections (7% IFN; 7% rituximab). R-FC followed by rituximab resulted in the highest infection rate (all grades 48% vs 30%). Sixty-one percent of patients on IFN stopped maintenance - although not progressive vs 30% on rituximab. Patients in CR/CRu or PR after induction who did not receive any maintenance for various reasons, mainly based upon patient's decisions or ongoing cytopenia after induction (n = 106) had a poor outcome (median remission duration 26 months; 3-yrs OS 52%). *Conclusions.* Rituximab maintenance after R-CHOP induction should be considered the new standard for elderly patients with MCL, to which new regimens should be compared.

0505

RESULTS OF COMFORT-I, A RANDOMIZED, DOUBLE-BLIND PHASE III TRIAL OF THE JAK1 AND JAK2 INHIBITOR RUXOLITINIB (INCB018424) VERSUS PLACEBO FOR PATIENTS WITH MYELOFIBROSIS

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Background. Dysregulated JAK-STAT signaling is a key feature in myelofibrosis, which is characterized by splenomegaly, debilitating symptoms, cytopenias and shortened survival. There are currently no effective drug therapies for myelofibrosis. Ruxolitinib is a selective JAK1 and JAK2 inhibitor with demonstrated clinical activity in myelofibrosis. *Aims.* COMFORT-I was designed to determine the safety and efficacy of ruxolitinib relative to placebo in patients with myelofibrosis. *Methods.* All study participants signed informed consent. Patients with intermediate-2 or high-risk myelofibrosis were randomized to start placebo or ruxolitinib at a dose of 15 or 20 mg PO BID depending on baseline platelet count (100-200 × 10^9/L or >200 × 10^9/L, respectively). The dose was optimized for efficacy and safety during treatment. The primary endpoint was the proportion of patients with ≥35% reduction in spleen volume at week 24 of therapy, assessed by blinded central review of spleen MRI or CT. Secondary endpoints were durability of spleen response, changes in symptom burden as assessed daily by the modified Myelofibrosis Symptom Assessment Form (MFSAF) v2.0, and survival. Additional exploratory endpoints included change in quality of life (QoL) measured by the EORTC-QLQ-C30, fatigue measured with the PROMIS Fatigue Scale, molecular and serum biomarkers, and transfusion dependence. *Results.* 309 patients were randomized: 155 to ruxolitinib and 154 to placebo. Median follow-up was 32.2 weeks. The proportion of patients with ≥35% reduction in spleen volume at 24 weeks was 41.9% vs 0.7% (ruxolitinib vs placebo, p<0.0001). Median duration of response has not been reached. At week 24, the proportion of patients with ≥50% improvement in symptom score was 45.9% vs 5.3% (ruxolitinib vs placebo, p< 0.0001) and the mean percent change in total symptom score was an improvement of 46.1% vs a worsening of 41.8% (ruxolitinib vs placebo, p<0.0001). There were 10 vs 14 deaths (ruxolitinib vs placebo, HR 0.67, p=0.33). Improvement in symptoms was rapid, with the majority of patients showing significant benefit within the first 4 weeks. Changes in both QoL and fatigue mirrored changes in the modified MFSAF v2.0 symptom score over time, and all showed improvement relative to placebo regardless of changes in hemoglobin. The most common AEs of any grade seen in >20% of patients on either arm of the study were (ruxolitinib vs placebo) abdominal pain (10.3% vs 41.1%), thrombocytopenia (34.2% vs 9.3%), fatigue (25.2% vs 33.8%), anemia (31% vs 13.9%), diarrhea (23.2% vs 21.2%), and peripheral edema (18.7% vs 22.5%). Anemia and thrombocytopenia were manageable and rarely (0.6% ruxolitinib vs 0.7% placebo, each) led to withdrawal from the study. *Summary/Conclusion.* In this study, ruxolitinib demonstrated

marked clinical benefits in spleen size, debilitating symptoms, and QoL that were rapid in onset and sustained. Anemia and thrombocytopenia were among the most common AEs but they were manageable, as demonstrated by the low withdrawal rate due to these events. The overall safety profile relative to placebo in myelofibrosis was acceptable.

0506

DYNAMIC MUTATION PROFILES OF LEUKAEMIC CELL POPULATIONS IN RESPONSE TO TREATMENT ARE REVEALED BY GENOME-WIDE SEQUENCING OF SEQUENTIAL SAMPLES FROM PATIENTS WITH B-CLL

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B-cell chronic lymphocytic leukaemia (B-CLL) is the most common leukaemia in the Western World. It is characterized by clinical and biological heterogeneity and a chronic relapsing course making it an ideal model to study the molecular events underlying relapse and the development of treatment resistance. Whole genome sequencing (WGS) has the potential to identify molecular pathways perturbed in cancer. However, the clinical significance of this information is not clear. Our aim was therefore to (1) establish the molecular profiles of sequential samples from patients with B-CLL at every relapse using WGS and (2) correlate findings with clinical outcome. Patients undergoing multiple rounds of treatment including second-generation monoclonal antibody treatment were selected for study. WGS was carried out to an average of >30-fold depth on each of five sequential peripheral blood samples per patient plus matched germline buccal swab controls. Sequencing employed SBSv5 chemistry on the HiSeq2000 instrument. Paired 100-base reads were aligned to the human reference GRCh37.1/hg19 and candidate single nucleotide variants (SNVs), insertions, deletions and copy number variants (CNVs) were detected in all genomes. Candidate somatic variants were called in each tumour sample by subtraction of all germ-line variants (i.e. those present in the matched normal sample for the individual). Somatic variants were clustered based on the profiles of allele frequency along the time-course of the entire tumour set within an individual. Targeted deep sequencing (TDS) of amplicons containing selected non-synonymous SNVs was carried out to a depth of ~10-50,000-fold and mutant allele frequencies were calculated by establishing the fraction of reads containing the mutant allele. An average of 4,600 mutations per sample were identified in each patient. On average, just 20 mutations were non-synonymous, nonse<<nse or frameshift mutations. On the basis of their varying mutant frequency profiles over time, the mutations could be clustered into up to 4 classes depending on patient: (I) those that disappear after treatment with purine analogues; (II) those that are present throughout the course of the disease and therefore resistant to treatment; (III) those that are initially detected at low frequency and then expand (Class III); and (IV) those not detected until later stages. So far, the data suggest that Class I and Class II sub-clones are characterized by mutations in pathways regulating innate immune responses and apoptosis (eg ADAD1, SAMHD1, BCL2L13) whereas Class III and IV sub-clones carry mutations in general cancer pathways (eg MEK1, ASXL1, FAT3, NRG3). *Conclusions.* This is the first sequential WGS analysis of B-CLL samples. We reveal that the molecular composition of this leukaemia alters with treatment. We identify mutations that are eradicated by or resistant to treatment including mutations important in regulation of innate immune response and cancer progression. These findings and the results of ongoing studies of other B-CLL cases will direct future clinical trials and therapeutic decisions.

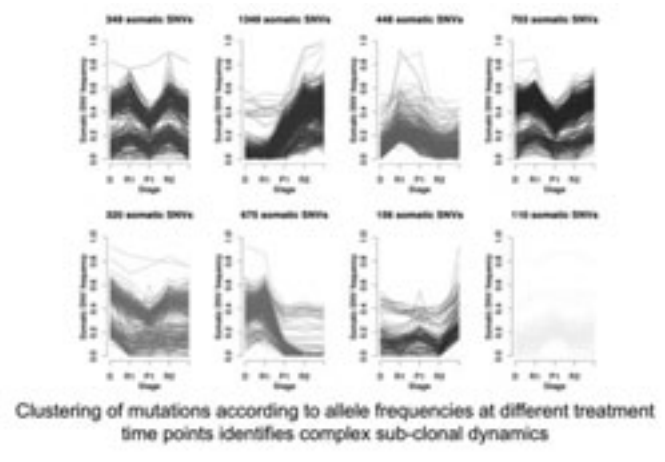

Figure 1.

0507

SILENCING OF RHOA NUCLEOTIDE EXCHANGE FACTOR, ARHGEF3 REVEALS ITS UNEXPECTED ROLE IN IRON UPTAKE

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Background. Genome-wide association meta-analysis studies (GWAS) have identified over 100 independent genetic loci associated with blood cell indices, including volume and count of platelets and erythrocytes. *Aims.* Although several of these loci encode known regulators of haematopoiesis the mechanism by which the majority of sequence variants exert their effect on blood cell formation remains elusive. *Methods.* A recent meta-analysis of the GWAS in 68,000 individuals of European ancestry has identified 68 genomic loci (53 new) associated with platelet count and volume. Here we demonstrate a functional validation for five of these novel loci in *Danio rerio* by the means of gene silencing. *Results.* Our experiments in *Tg(cd41:EGFP)* transgenic fish reveal important functions for genes encoding guanine nucleotide exchange factors *(arhgef3)*, tropomyosins *(tpma)* and previously uncharacterised transcriptional regulators (*rnf145* and *jmjd1c*), in regulating thrombopoiesis. In addition, lineage relatedness of megakaryocytes and erythrocytes prompted us to explore the putative role of these genes in erythropoiesis and silencing of all five genes but tpma (transcription of its human homologue *TPM1* in blood is restricted to megakaryocytes) also exerted an effect on primitive erythropoiesis. The silencing of *arhgef3* on erythropoiesis was the most profound and this prompted further detailed studies. Examination of peripheral blood from *arhgef3* morpholino (MO) injected zebrafish embryos revealed microcytic and hypochromic erythrocytes, a classic feature of iron deficiency. Anemia was rescued by intracellular supplementation of iron to *arhgef3* MO injected embryos, demonstrating that *arhgef3* depleted erythroid cells, once provided with intracellular iron are fully capable of haemoglobinisation. Disruption of the arhgef3 target, RhoA, also produced severe anemia, which was again rescued by iron injection. Moreover, silencing of ARHGEF3 expression in the erythromyeloblastoid cell line K562 revealed that the uptake of transferrin, the main blood plasma protein for iron transport, was severely impaired. *Conclusions.* Taken together, this is the first study to provide evidence for ARHGEF3 being a regulator of transferrin and iron uptake in erythroid cells, through activation of RhoA. Taken together, our findings demonstrate the value of pursuing GWAS signals as a new and exciting forward genetics approach in identifying novel regulatory molecules and signalling pathways in haematopoiesis.

0508

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

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Background. High-dose chemotherapy with haemopoietic stem-cell improves quality of response and survival in multiple myeloma (MM).The introduction of novel agents questions the role of autologous stem-cell transplantation (ASCT). *Aims.* to compare in a prospective randomized study conventional chemotherapy plus novel agents (MPR) with tandem high-dose melphalan (MEL200) and ASCT in newly diagnosed MM patients younger than 65 years. *Methods.* All patients (N=402) received four 28-day cycles of lenalidomide (25 mg, d1-21) and low-dose dexamethasone (40 mg, d1,8,15,22) (Rd) as induction. Patients (N=202) were then randomized to MPR [six 28-day cycles of melphalan (0.18 mg/kg d1-4), prednisone (2 mg/kg d1-4) and lenalidomide (10 mg d1-21); pts (N=200)] or MEL200 [tandem melphalan 200 mg/m2 with stem-cell support]. All patients enrolled were stratified according to International Staging System (ISS) (stages 1 and 2 vs stage 3) and age (<60 vs >60 years). Primary end point was PFS. Data were analyzed in intention-to-treat. *Results.* Response rates were similar in the two groups (MPR vs MEL200): >VGPR (60% vs 58%, p=0.24) and CR (20% vs. 25%, p=0.49). After a median follow-up of 20 months, the 18-months PFS was 68% in MPR and 78% in MEL200 (HR=0.58, p=0.006). CR prolonged the 18-months PFS in the MPR (90% vs 66%) and in the MEL200 group (87% vs 76%). The 18-months OS was similar (91% vs 95%, p=0.073). In the MPR and MEL200 groups, G3-4 neutropenia was 55% vs 89% (p <.001); G3-4 infections were 0% vs 17% (p <.001); G3-4 gastrointestinal toxicity was 0% vs 21% (p<.001); second tumors were 0.005% in both arms. DVT was 2.44% vs 1.13% (p=0.43). *Conclusions.* PFS was significantly prolonged in MEL200 group compared to MPR, although toxicities were significantly higher. This is the first report showing a PFS advantage for ASCT in comparison with combinations including novel agents. At present OS is not significantly different in the two groups.

0509

BRAF MUTATIONS IN HAIRY CELL LEUKEMIA

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Background. Hairy cell leukemia (HCL) is a well defined clinico-pathological entity whose underlying genetic lesion is still obscure. *Methods.* We searched for HCL-associated mutations by massively parallel sequencing of the whole exome of leukemic and matched normal mononuclear cells purified from the peripheral blood of one patient with HCL. *Results.* Whole exome sequencing identified 5 missense somatic clonal mutations that were confirmed at Sanger sequencing, including a heterozygous V600E mutation involving the BRAF gene. Since the BRAF V600E mutation is oncogenic in other tumors, further analyses were focused on this genetic lesion. Sanger sequencing detected mutated BRAF in 46/46 additional HCL patients (47/47 including the index case; 100%). None of the 193 peripheral B-cell lymphomas/leukemias other than HCL that were investigated carried the BRAF V600E mutation, including 36 cases of splenic marginal zone lymphomas and unclassifiable splenic lymphomas/leukemias. Immunohistological and Western blot studies showed that HCL cells express phospho-MEK and phospho-ERK (the downstream targets of the BRAF kinase), indicating a constitutive activation of the RAF-MEK-ERK mitogen-activated protein kinase pathway in HCL. *In vitro* incubation of BRAF-mutated primary leukemic cells from 5 HCL patients with PLX-4720, a specific inhibitor of active BRAF, led to marked decrease of phosphorylated ERK and MEK. *Conclusions.* The BRAF V600E mutation was present in all HCL patients investigated. This finding may have relevant implications for the pathogenesis, diagnosis and targeted therapy of this hematological disorder (Funded by the Associazione Italiana Ricerca Cancro and others).

First line therapy in Multiple Myeloma

0510

BORTEZOMIB-THALIDOMIDE-DEXAMETHASONE COMPARED WITH THALIDOMIDE-DEXAMETHASONE AS CONSOLIDATION THERAPY AFTER DOUBLE AUTOLOGOUS STEM-CELL TRANSPLANTATION IN NEWLY DIAGNOSED MULTIPLE MYELOMA

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We prospectively compared thalidomide-dexamethasone (TD) with bortezomib plus TD (VTD) as induction therapy before, and consolidation therapy after, double autologous stem-cell transplantation (ASCT) in patients with newly diagnosed multiple myeloma (MM). 474 patients randomized to the VTD (n=236) or TD (n=238) arm were analyzed on an intention-to-treat basis. After three 21-d cycles of induction therapy, the rate of complete response (CR) plus near CR (nCR), the primary study endpoint, was threefold higher with VTD compared to TD (31% vs 11%, respectively; p<0.0001). Post-ASCT consolidation therapy comprised two 35-d cycles of either VTD (V, 1.3 mg/m2 once-weekly; T, 100 mg/d through d 1 to 70; D, 320 mg/cycle) or TD (same doses as in VTD), according to the initial randomization. After consolidation therapy, the rate of CR-nCR, a secondary study endpoint, was 62% in the VTD and 45% in the TD arm (p=0.0002). A per-protocol analysis of 323 patients who actually received VTD (n=161) or TD consolidation therapy (n=162) was performed to evaluate the activity and toxicities of the two regimens. McNemar test results showed that VTD consolidation significantly increased the rate of CR (p=0.005) and CR-nCR (p=0.01), an objective failed by TD consolidation (p=0.07 and p=0.2 for upgraded CR and CR-nCR rates, respectively). Overall, the probability to upgrade high-quality response from less than CR before consolidation to CR after consolidation was twofold higher with VTD compared to TD (11% vs 6%, respectively). Non hematologic grade 3-4 adverse events were 11% with VTD and 10% with TD, including peripheral neuropathy (1% vs 0%, respectively) and skin rash (0.6% in each of the two treatment arms). Dose adherence of study drugs was very close to that planned; in particular, patients in the VTD arm received 93% of planned doses of bortezomib and thalidomide, while the corresponding value for thalidomide in the TD arm was 97%. In a substudy, post-consolidation molecular detection of minimal residual disease (MRD) was evaluated by means of patient-specific primers. VTD consolidation significantly increased the rate of molecular negativity, up to the 64% value (p=0.007 using the McNemar test), a benefit not seen with TD consolidation (48% of molecular negativity; p=0.06). Quantitative analysis of MRD after the 2 planned cycles of VTD consolidation therapy showed a 5-log reduction in residual clonal cells. By the opposite, consolidation therapy with TD yielded a 1 log reduction in MRD. Attainment of molecular negativity after consolidation therapy was a strong predictor of favourable clinical outcomes. In particular, the 3-year estimate of progression-free survival was as high as 89% for patients with undetectable MRD after consolidation therapy, while the corresponding value for patients with molecular positivity was 47%% (p=0.04). It is concluded that VTD consolidation was more effective than TD in upgrading the rate of high-quality responses and yielding unprecedented high rate of molecular remission, in excess of 60%. Achievement of molecular negativity favourably affected clinical outcomes and should be the primary goal of ASCT incorporating the novel agents.

0511

BORTEZOMIB-BASED INDUCTION AND MAINTENANCE THERAPY IMPROVES OUTCOME IN MYELOMA PATIENTS WITH DELETION 17P - A SUBGROUP ANALYSIS OF THE HOVON65/GMMG-HD4 TRIAL

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Background. Chromosomal aberrations are important prognostic parameters in multiple myeloma (MM). By using interphase fluorescent *in situ* hybridization (FISH) on CD138-enriched plasma cells, specific changes in interphase cells can be detected, overcoming the lack of dividing cells required for conventional cytogenetics. *Aims.* We evaluated the association of FISH results and outcome of a subgroup of patients (pts) within the HOVON-65/GMMG-HD4 trial, a prospective, randomized phase III trial for pts with newly diagnosed MM stage II or III according to Salmon & Durie up to 65 years. *Methods.* Pts were randomized to receive three cycles of VAD (arm A; vincristine, adriamycin, dexamethasone) or PAD (arm B; bortezomib, adriamycin, dexamethasone). All pts received one or two cycles of high dose melphalan (200 mg/m²) with autologous stem cell transplantation followed by maintenance therapy with thalidomide 50 mg daily (arm A) or bortezomib 1.3 mg/m2 once every 2 weeks (arm B), respectively, for a maximum of 2 years. Sites in Germany, the Netherlands and Belgium participated in this trial (n=833 pts). For the German pts (GMMG, n=399) FISH was performed in a single laboratory prior to start of treatment. Cytospins of CD138 purified plasma cells were subjected to FISH with two-color probe sets for the detection of numerical changes for the following chromosome regions: 1q21/8p21, 6q21/15q22, 9q34/22q11, 11q23/13q14, and 17p13/19q13, as well as for the translocations t(4;14), t(11;14), and t(14;16). *Results.* For this analysis, FISH results from 354 (89%) of all GMMG pts were available (n=182 in arm A; n=172 in arm B). For all pts the median follow-up time from randomization was 38.9 months (mo.). The most pronounced impact on prognosis was seen for t(4;14), del13q14, del17p13, and gain1q21, each significantly associated with poor prognosis with respect to progression free survival (PFS) and overall survival (OS). However, deletion of chromosome 13q as exclusive chromosomal aberration without the presence of del(17p) and t(4;14) indicates no impact on outcome. A multivariable Cox PH Model identified t(4;14), del17p13, gain1q21, and ISS III as independent factors for PFS and OS. When comparing pts in the two arms for PFS and OS, we found that bortezomib-based treatment improves significantly the outcome in myeloma patients with del(17p) (3yr-PFS rates: A: 16%, B: 27%, p=0.030; 3yr-OS rates: A: 17%, B: 69%, p=0.014). Patients with t(4;14) or gain1q21 showed also a favourable outcome in arm B, although this difference was not of statistical significance. A multivariable Cox PH Model showed that t(4;14), del17p13, and gain1q21 are independent factors for PFS and OS in arm A, whereas only t(4;14) were of borderline significance for OS in arm B (p=0.049). *Conclusions.* Our analysis confirms the significant negative prognostic impact of del17p13, t(4;14) and gain 1q21 on PFS and OS for pts with newly diagnosed MM. All patients with high-risk chromosomal aberrations benefit at least in part from bortezomib-based treatment. The negative impact of del17p13 on PFS and OS could be significantly improved by the bortezomib-based treatment, suggesting that long-term administration of bortezomib should be considered as a standard of care for these patients.

0512

PROGNOSTIC RELEVANCE OF 18F-FDG PET/CT NEGATIVITY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background. 18F-FDG PET/CT has been reported to be a careful technique for a widespread screening of myelomatous lesions at the onset of MM. Moreover, FDG-PET has been identified as a valuable methods to carefully monitor response and predict clinical outcomes in various tumors, particularly in lymphoma. The incorporation of novel agents into ASCT allowed the achievement of unprecedented high rates of complete response (CR) in young multiple myeloma (MM) patients. Availability of new techniques to identify minimal residual disease (MRD), such as multiparametric flow cytometry or molecular biology, led to the demonstration of a correlation between the depth of response and prognosis. *Aims.* Aim of the present study was to prospectively analyze the prognostic relevance of PET/CT negativity after induction and ASCT in 192 newly diagnosed MM patients. *Methods.* By study design, all patients were studied with 18F-FDG PET/CT at baseline, after induction treatment, after ASCT, once/year during post-ASCT follow-up and at the time of relapse. Bone marrow involvement was described as negative, diffuse or focal. The number of focal lesions (FL), as well as size and associated standardized uptake values (SUV) were recorded. Extramedullary disease (EMD), if present, was described by location, size, number and SUV. *Results.* Twenty four percent of the patients had a negative PET/CT scan at diagnosis. Among PET/CT-positive patients, 44% showed ≥ 3 focal lesions (FLs), 46% had SUV values > 4.2 and in 6% EMD could be detected. These 3 variables adversely affected 4-year estimates of PFS and OS. Thirty seven percent of the patients were negative after induction, while PET/CT was negative in 65% after 3 months from ASCT. Persistence of severe FDG uptake (SUVmax still > 4.2) after induction predicted for shorter PFS at 4 years (P= 0.004). Complete FDG suppression post-ASCT conferred superior PFS and OS in comparison with persistence of FDG uptake. In particular, 4-year estimates of PFS and OS for negative patients were 66% and 89%, respectively, as compared to 45% and 65% for positive patients (P 0.02 both for PFS and OS). In multivariate analysis, both severe PET/CT involvement at diagnosis (SUV > 4.2 and/or EMD) and persistence of FDG uptake after ASCT were independent predictors of worst PFS (SUV > 4.2= HR: 2.0, 95%CI: 1.13-3.72; EMD= HR: 15.0, 4.0-55.8; FDG uptake after ASCT= HR: 2.12, 1.19-3.77) and OS (EMD= HR: 6.99, 2.28-21.46; FDG uptake after ASCT= HR: 3.57, 1.03-12.39). *Conclusions.* These results provide demonstration that PET/CT at diagnosis and after treatment is a reliable tool for predicting prognosis in autografted MM patients and to identify patients at different risk of progression. In particular, post-ASCT complete FDG suppression is associated with extended PFS and OS. Based on these data, aims to evaluate MRD should include also imaging techniques such as PET/CT.

0513

EFFECTS OF ZOLEDRONIC ACID (ZOL) VERSUS CLODRONATE (CLO) ON MYELOMA-RELATED ORGAN OR TISSUE IMPAIRMENT (ROTI) IN PATIENTS WITH MULTIPLE MYELOMA (MM) IN THE MRC MYELOMA IX STUDY

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Background. A key feature of MM is related organ or tissue impairment (ROTI), manifest as CRAB: hypercalcemia, renal impairment, anemia, bone lesions and infection. In the Medical Research Council (MRC) Myeloma IX study, ZOL significantly improved overall and progression-free survival versus CLO in patients undergoing initial therapy for MM (hazard ration [HR] = 0.84, *P* = .0118 for survival; HR = 0.88, *P* = .0179 for progression-free survival; Morgan *et al*. *Lancet*. 2010), but the effects on CRAB have not been reported. *Aims.* These exploratory analyses investigated whether ZOL provided benefits beyond CLO for CRAB in the Myeloma IX study. Both agents are indicated for the prevention of skeletal complications from MM in the United Kingdom; prevention of CRAB is, therefore, off label. *Methods.* Patients newly diagnosed with MM were assigned either to intensive or non-intensive treatment pathways and randomized to CVAD vs CTD or to MP vs CTDa, respectively. Within each treatment arm, patients were randomized to intravenous ZOL (4 mg q3-4wk, adjusted based on renal function) or oral CLO (1,600 mg/d), each of which was continued at least until disease progression. Safety was assessed by continuous adverse event monitoring and standard laboratory and imaging evaluations. All patients provided informed written consent. *Results.* Among the intent-to-treat population (N = 1,960), at a median follow-up 3.7 years, a total of 104 new osteolytic lesions were reported in 95 (9.7%) CLO-treated patients compared with only 53 new osteolytic lesions in 46 (4.7%) ZOL-treated patients (*P* < .0001). Overall, more patients treated with ZOL versus CLO had complete or very-good partial responses (*P* = .018 for the non-intensive pathway). During the first 3 months on study, 16 (1.6%) CLO-treated but no ZOL-treated patients died of renal failure (*P* < .0001), and 42 (4.3%) CLO-treated and 27 (2.8%) ZOL-treated patients died of infections (*P* = .08). During the course of the study, 47 (4.8%) CLO- and 43 (4.4%) ZOL-treated patients died of renal failure (*P* = .67). In the same time period 123 (12.6%) CLO- and 92 (9.4%) ZOL-treated patients died of infections related to MM or its treatment (*P* = .025). In each group, 28 (2.9%) patients had hypercalcemia, reported as a serious adverse event (SAE) for 6 (0.6%) per group (*P* = .99). A pre-specified univariate Mantel-Haenszel test identified no statistically significant between-group differences in hypercalcemia (*P* = 0.99). There was no difference in time to hypercalcemia with ZOL versus CLO (*P* = .60). Recovery data were only available for 9 of the hypercalcemia SAEs, and showed complete resolution for 4 patients per group and death for 1 patient on CLO. A total of 18 (1.8%) ZOL- and 19 (1.8%) CLO-treated patients developed grade 3 or 4 anemia. Summary/conclusions: Consistent with previous reports of objective treatment benefits with ZOL versus CLO in patients receiving initial treatment for MM, ZOL also reduced the incidence and severity of some ROTI compared with CLO, especially early in the course of treatment in the Myeloma IX study.

0514

INCIDENCE OF SECOND PRIMARY MALIGNANCY (SPM) IN MELPHALAN-PREDNISONE-LENALIDOMIDE COMBINATION FOLLOWED BY LENALIDOMIDE MAINTENANCE (MPR-R) IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS = 65 YEARS

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Background. MM patients have an 8-10 fold higher risk of developing acute myeloid leukemia (AML) than the general population. Among persons ≥ 65 years, incidence rate (IR) of invasive cancer is 2.1 per 100 person-years. MM-015 compared continued MPR-R vs fixed-duration melphalan and prednisone (MP) or melphalan, prednisone, and lenalidomide (MPR) in transplant-ineligible patients ≥ 65 years. *Aim.* MM-015 is a phase 3 trial comparing MPR-R vs MP or MPR in transplant-ineligible patients aged ≥ 65 years. This post hoc analysis evaluated IRs for SPMs and risks of SPM relative to progression risk. *Methods.* Potential SPMs were identified (non-melanoma skin cancers excluded). IRs per 100 person-years were calculated for AML and solid tumor malignancies. Progression-free survival (PFS) and relative hazard of progression in MPR-R vs MP were contrasted against an event-free survival (EFS) model that considered SPM an event. *Results.* As of February 11, 2011, the total number of SPM cases during active treatment and extended follow-up was 7/150 in the MPR-R arm, 8/152 in MPR, and 2/153 in the MP arm. Median onset of SPM was 28.2 months (range 2.4-42.5). These include 5 hematologic SPMs in the MPR-R arm (IR: 1.44; 3 AML, 1 T-cell acute lymphoblastic syndrome [ALL], 1 myelodysplastic syndrome [MDS]), 4 in MPR (IR: 1.17; 2 AML, 2 MDS), and 0 in the MP arm (IR: 0). These also include 2 solid tumor SPMs in the MPR-R arm (IR: 0.58), 4 in the MPR arm (IR: 1.17), and 2 in the MP arm (IR: 0.56). A total of 11 patients had adverse complex karyotype of bone marrow plasma cells at diagnosis of MM; all were randomized to receive lenalidomide (MPR-R or MPR arms). Of these, 3 developed AML and 2 developed MDS. Solid tumors were of heterogeneous tumor types. No B-cell malignancies were reported. In addition, 2 cases of AML and 4 solid tumors been reported after February 11, 2011 in the MPR-R/MPR arms as well as 1 case of MDS and 1 solid tumor in MP arm. Continued therapy with lenalidomide in the MPR-R arm provided sustained disease control. Median PFS of MPR-R vs MP was 31 months vs 13 months (P < .001; median follow-up: 25 months), with a 60% reduction in the relative risk of progression (hazard ratio [HR] = 0.395; P<.0001). The benefit of lenalidomide treatment was preserved when SPMs were added as events to the PFS analysis (HR = 0.408; P < .0001). *Conclusions.* An imbalance in the incidence of AML/MDS was observed in MPR/MPR-R vs MP, although the incidence was low (3.6% vs 0.7%, respectively); with 5 of 11 adverse complex karyotype patients developing AML/MDS. Solid tumor IRs were also low. Lenalidomide maintenance significantly reduced the risk of progression. Importantly, SPMs did not impact PFS when added as an event. Longer follow-up will further characterize SPM risk in newly diagnosed MM patients receiving lenalidomide and melphalan.

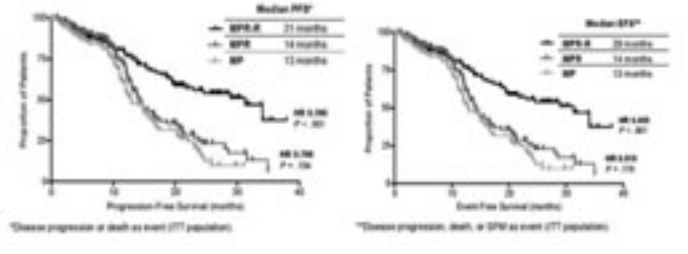

Figure 1.

Hodgkin Lymphoma - Biology & clinical

0515

LESTAURTINIB INHIBITION OF THE JAK/STAT SIGNALING PATHWAY IN CLASSIC HODGKIN LYMPHOMA (CHL) CELLS AND IN PATIENTS LYMPH NODES INHIBITS PROLIFERATION AND INDUCES APOPTOSIS

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Background. Standard cytotoxic chemotherapy for cHL has changed little in 30 years; the treatment for patients with relapsed or refractory disease remains challenging and novel agents are under development. JAK/STAT constitutive activation plays an important role in the pathogenesis of cHL. Lestaurtinib is an orally bioavailable multikinase inhibitor that has recently been shown to inhibit JAK2 in myeloproliferative disorders. Its potential role in cHL therapy is unknown. *Aims.* Firstly, to analyze the *in vitro* effectiveness of Lestaurtinib in five cHL cell lines and its role in the JAK2/STAT5 signaling pathway. Secondly, to analyze for the first time the effect of Lestaurtinib in lymph nodes from cHL patients by flow cytometry. *Methods.* Five cHL cell lines, L-428, L-1236, L-540, HDLM-2 and HD-MY-Z were assayed for proliferation and apoptosis after 48h of treatment with Lestaurtinib or DMSO (control). Cell growth was determined using CellTiter 96 AQueous One Solution Cell Proliferation Assay and apoptosis was measured using CaspaseGlo 3/7 kit (Promega). After 1 hour of incubation with Lestaurtinib, the levels of the JAK2 pathway proteins, JAK2, phospho-JAK2 (Tyr1007/1008), STAT5, phospho-STAT5 (Tyr694), STAT3 and phospho-STAT3 (Tyr705), were analyzed by Western Blot. BCL-xL mRNA levels were quantified using TaqMan Gene Expression assays (Applied Biosystems). cHL patients were diagnosed at the Haematology Department from the University Hospital del Mar, Barcelona. Median age was 29 (range 24-43); 3 were nodular sclerosis and one lymphocyte-rich subtype. All patients were EBV- and 2 were stage IIA and 2 stage IIIA. By flow cytometry, we evaluated 750,000 cells from lymph nodes cultured for 24 hours with 300nM of Lestaurtinib or DMSO. Hodgkin Reed-Sternberg (HRS) cells were gated by the expression of CD40-PE-Cy5, CD95-Pacific Blue and CD30-PE, and the absence of CD3-APC-Cy7 (BD Bioscience). Viability was analyzed using FITC AnnexinV (BD Bioscience). Samples were analyzed on a FACS CANTO II (Becton Dickinson). *Results.* At 48h, a dose-dependent cell growth inhibition (23-66% at 300nM) and apoptotic increment (10-64% at 300nM) were observed in all cell lines. Moreover, Lestaurtinib inhibited JAK2, STAT5 and STAT3 phosphorylation and reduced the mRNA expression of its downstream antiapoptotic target Bcl-xL. Additionally, we have analyzed the effect of Lestaurtinib in lymph nodes from four cHL patients. We have evaluated the effect of treatment with 300nM of Lestaurtinib in the subpopulation of lymph node cells CD30+, CD40+, CD95+ and CD3-, which contain HRS cells. After 24h, cell viability had decreased in three of the four cases by 22%, 35% and 24% versus control cells. In the non-responder patient, we increased the treatment dose to 1μM and then we observed a reduction in cell viability by 12%. In order to shed light on the potential toxicity of Lestaurtinib, we have also analyzed cell viability in lymph node CD3+ cells after treatment with 300nM of Lestaurtinib and observed no decrease of viability (mean versus control=100.5%; range: 90%-119%). *Summary/Conclusions.* Our findings provide, for the first time, a molecular rationale for testing JAK2 inhibitors, specifically Lestaurtinib, in HL patients.

0516

LONG TERM FOLLOW-UP OF PATIENTS TREATED WITH INVOLVED-FIELD COMPARED WITH EXTENDED-FIELD RADIOTHERAPY AFTER CHEMOTHERAPY FOR HODGKIN'S LYMPHOMA: 10 YEAR-ANALYSIS OF THE HD8 TRIAL OF THE GHSG

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Background/Aims. In the HD8 trial of the German Hodgkin Study Group (GHSG), we investigated whether radiotherapy (RT) can be reduced without loss of efficacy from extended field (EF) to involved field (IF) after four cycles of chemotherapy. The current 10 year follow-up analysis was conducted to assess the influence of the two modalities with regard to long-term outcome rates and toxicities including secondary malignancies. *Methods.* Between 1993 and 1998, patients with newly diagnosed early-stage unfavorable HL were randomized to receive four cycles of chemotherapy followed by either RT of 30 Gy EF + 10 Gy to bulky disease (arm A) or 30 Gy IF + 10 Gy to bulky disease (arm B). Of 1,204 patients randomly assigned to treatment, 1064 patients were informative and eligible for the arm comparison (532 patients in each treatment arm; drop-outs before RT excluded). *Results.* Patients demographics and clinical characteristics were well balanced between the two treatment arms. The median observation time was 114 months. Survival rates at 10 years after start of radiotherapy revealed no differences for arms A and B with respect to freedom from treatment failure (FFTF; 79.8% and 79.7%), progression free survival (PFS; 79.8% and 80.0%) and overall survival (OS; 86.4% and 87.3%). Non-inferiority of the experimental arm (IF-RT) was proven with statistical significance for the primary endpoint FFTF (95%CI for HR 0.72-1.25). However, patients 60 years or older had an inferior outcome when treated with EF-RT compared with IF-RT. Acute toxicity from radiotherapy including nausea, leucopenia, thrombocytopenia, gastrointestinal and pharyngeal toxicity were more frequent in the EF arm. A total of 15% of patients in arm A and 12.2% of patients in arm B died; causes of deaths were mainly secondary malignancies (5.3% vs. 3.4%), HL (3.2% vs. 3.4%), and cardiovascular disease (1.7% vs. 2.1%). Interestingly, there were more secondary malignancies (n=58 vs. n= 45), especially AMLs (n=11 vs. n=4) after combined modality treatment including EF-RT than IF-RT. However, longer follow-up is needed for statistic significance and evaluation of other long-term side effects. *Summary/Conclusions.* Radiotherapy volume size reduction from EF to IF after chemotherapy does not result in inferior long-term outcome and produces less acute and long-term toxicities in patients with early-stage unfavorable HL.

0517

TARC BIOMARKER TRENDS OBSERVED IN A PIVOTAL PHASE II STUDY OF ORAL PANOBINOSTAT IN PATIENTS WITH RELAPSED/REFRACTORY HODGKIN LYMPHOMA FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANT

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Background. Panobinostat is an oral pan-deacetylase inhibitor (pan-DACi). TARC (thymus and activation-regulated chemokine) production by Hodgkin lymphoma Reed-Sternberg cells drives intra-tumoral accumulation of reactive cells. TARC levels have been studied in relation to DACi in Hodgkin lymphoma cell lines and are known to reflect disease activity in patients. In a pivotal Hodgkin lymphoma study, panobinostat achieved durable responses in heavily pretreated relapsed/refractory patients after autologous stem cell transplant (ASCT). *Aims.* This prospective analysis was conducted to evaluate TARC as a biomarker for disease activity and to explore the correlation with the response and progression of patients with relapsed/refractory Hodgkin lymphoma following ASCT treated with panobinostat. *Methods.*

Baseline and Cycle 1 Day 15 TARC Data by Best Overall Response

	CR n=5	PR n=31	SD n=70	PD n=14	Unknown n=9
Baseline					
n	n=4	n=25	n=62	n=12	n=6
Median, pg/mL	1122	2089	1479	1318	955
IQR*	568-2188	1288-2951	708-2951	1202-2884	513-1950
Cycle 1 Day 15					
n	n=3	n=25	n=51	n=10	n=4
% of baseline	72%	78%	90%	100%	90%
IQR*	58%-92%	71%-98%	87%-98%	95%-101%	79%-101%

*IQR, interquartile range (interval between 25th and 75th percentile).

Figure 1.

Panobinostat was administered 40 mg three times per week, every week in 21-day cycles. Response was assessed every 2 cycles by CT/MRI scan. Blood was collected at screening, days 5, 8, and 15 of cycle 1, day 1 of cycles 2-4, and end of treatment. Milliplex Map Human Cytokine/Chemokine panel II (Millipore) was used to analyze TARC. *Results.* A total of 129 patients were enrolled and treated, and 36 responses were seen (5 complete [CR], 31 partial [PR]). Stable disease (SD) was observed in 70 patients (for a disease control rate [CR+PR+SD] of 82%), and 14 patients had progressive disease (PD). Samples were collected from 117 patients for analysis. Statistical analysis showed that baseline TARC levels were in similar range across patients with CR, PR, SD, and PD (see table). Reduction of TARC on treatment was observed as early as cycle 1 day 8. Percent change, calculated as the geometric mean ratio of TARC levels at cycle 1 day 15 to baseline, showed a reduction of TARC level to 72% in CR, 78% in PR, 90% in SD, and no change in PD patients. Patients with more than the median TARC reduction (high reduction) were at less risk of progression compared to patients with less than the median of TARC reduction (low reduction); hazard ratio 2.27 [95% CI; 1.30, 3.98]. Median progression-free survival (PFS) in the high-reduction group was 9.9 months vs 4.9 months for the low-reduction group. At cycle 1 day 15, the hazard ratio of PFS for each 1-log reduction in TARC from baseline was 0.44 (95% CI; 0.23, 0.83). Similar trends were observed at other time points. *Summary/Conclusions.* Early TARC reduction was observed post-panobinostat treatment in Hodgkin lymphoma patients with disease control. The level of TARC reduction at cycle 1 day 15 was highest in patients achieving CR and PR, providing further evidence to support the mechanism of action of DACi in Hodgkin lymphoma. Further analysis is ongoing to correlate changes in TARC vs time to response and target lesion reduction, and these updated results may support TARC as a potential biomarker for monitoring Hodgkin lymphoma response to panobinostat.

0518

OBJECTIVE RESPONSES WITH BRENTUXIMAB VEDOTIN (SGN-35) IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN LYMPHOMA (HL) WHO REFUSED OR WERE INELIGIBLE FOR AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT)

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Background. Brentuximab vedotin (SGN-35) is an anti-CD30 antibody conjugated to the highly potent antimicrotubule agent, monomethyl auristatin E (MMAE), by a plasma-stable linker. Brentuximab vedotin binds to CD30 on the cell surface, internalizes, and releases MMAE inside the cell via lysosomal degradation. Binding of MMAE to tubulin disrupts the microtubule network, induces cell cycle arrest, and results in apoptotic death of the CD30-expressing tumor cell. Brentuximab vedotin induced durable objective responses in association with manage-

able adverse events in patients with relapsed or refractory HL after ASCT (Chen *et al*., ASH 2010). *Aims.* To characterize the safety and efficacy of brentuximab vedotin in a population of patients with relapsed or refractory HL who refused or were ineligible for ASCT ("pre-ASCT"patients). *Methods.* Pre-ASCT patients were enrolled per study entry criteria in 2 phase 1 multicenter studies. In Study SG035-0001, patients received brentuximab vedotin IV q3 weeks at: 0.1, 0.2, 0.6, and 1.2 mg/kg (1 patient each); 1.8 mg/kg (2 patients); and 2.7 mg/kg (4 patients). In Study SG035-0002, patients received brentuximab vedotin IV q1 week, 3 out of 4 weeks, at: 0.4 mg/kg (2 patients), 0.8 mg/kg (1 patient), 1.0 mg/kg (3 patients), 1.2 mg/kg (1 patient), and 1.4 mg/kg (3 patients). Informed consent was obtained for all patients. *Results.* Twenty pre-ASCT patients were enrolled across the 2 studies. The median age was 31.5 years (range, 12-87), 65% were male, baseline ECOG performance status was 0 (50%), 1 (30%) or 2 (20%). The median number of prior systemic chemotherapy regimens was 3 (range, 1-7), and 45% of patients had received prior radiotherapy. Relative to the post-ASCT population, a higher proportion of pre-ASCT patients had primary refractory disease and were refractory to their most recent prior therapy. The incidence of bone marrow involvement and baseline B symptoms was also higher in the pre-ASCT population. Adverse events (AEs) of any grade in ≥25% of patients were fatigue, nausea, pyrexia, diarrhea, vomiting, back pain, decreased appetite, anemia, night sweats, and weight decreased. Eleven of 20 patients (55%) experienced AEs with a maximum severity of Grade 3 and treatment-related serious AEs were reported in 3 patients (15%). No deaths occurred within 30 days of the last dose of brentuximab vedotin. Objective responses (Cheson 2007) were observed in 6 of the 20 pre-ASCT patients (30%); 2 CR and 4 PR. Median duration of objective response could not be estimated because only 1 of the 6 patients had disease progression or death by the time of study closure. Censored response duration in the remaining 5 responders ranged from 29.6+ to 60.1+ weeks. *Summary/Conclusions.* Brentuximab vedotin was associated with manageable adverse events in a population of patients with relapsed or refractory HL who refused or were ineligible for ASCT, with a safety profile comparable to that observed in post-ASCT patients. The demonstration of objective responses suggests that anti-tumor activity is not limited to patients who received brentuximab vedotin after ASCT and warrants further studies in earlier lines of therapy.

0519

HIV-RELATED HODGKIN'S LYMPHOMA (HIV-HL): RESULTS OF PROSPECTIVE MULTICENTER TRIAL

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Background. The outcome of patients (pts) with HIV-HL has improved since the introduction of highly active antiretroviral therapy (HAART). However, standard therapy for HIV-HL has not been defined. *Aims.* The current trial was initiated to investigate a risk adapted treatment strategy in pts with HIV-HL as established in HIV-negative pts with HL. *Methods.* Pts. were planned to receive 2x ABVD + 30 Gy involved field (IF) radiation for early stage (ES) favourable HL (stage I/II without risk factors), 4x BEACOPP baseline + 30 Gy IF for ES unfavourable HL (extranodal involvement, large mediastinal mass, ≥ 3 lymph node areas involved), and 6-8 x BEACOPP baseline for advanced stage HL. BEACOPP should be replaced by ABVD in pts with far advanced HIV-infection. HAART was given concomitantly with chemotherapy. The primary endpoint was tolerability and treatment related mortality. Secondary endpoints include event free survival (EFS) and overall survival (OS). *Results.* From 03/2004 to 12/2010 105 pts (8 females) were included in the trial. 23/105 pts (22%) had ES favourable HL, 14 (13%) ES unfavourable HL, and 68 (65%) advanced stage HL. B-symptoms were present in 69 pts (66%) and the mixed cellularity subtype was found in 63 pts (60%). 28 pts (27%) had a prior AIDS defining illness. The median CD4 count at HL diagnosis was 223/µl (range 7-967) and 57 pts (54%) had an HIV-viral load below the detection limit. The median time from HIV diagnosis to HL diagnosis was 5.8 yrs (range 0 - 26). In advanced stage HL grade 3/4 toxicity occurred in 13/13 pts under ABVD and 45/60 pts (75%) under BEACOPP with non haematological toxicity being more frequently observed under BEACOPP than under ABVD (48% vs. 39%). 5 pts died of neutropenic sepsis after the 1st, 7th (n=2) and 8th cycle of BEACOPP, and after 1 cycle of ABVD, respectively. So far response data are available from 101 pts. After a median follow-up of 23.1 months 20/21 pts (95%) with ES favourable HL and 13/13 pts (100%) with ES unfavourable HL achieved a CR/CRu. In pts with advanced HL the CR/CRu rate was 87% (58/67). Of 6 pts with relapsed/refractory HL 2 received an autologous stem cell transplant resulting in a 2nd remission and 4 pts died of progressive disease. 10 of 105 pts (9.5%) have died. Apart from neutropenic sepsis causes of death were progressive HL (n=4) and progressive HIV-infection (PML, n=1). The 2-year OS of the entire study population is 90.6% without significant differences between early, intermediate and advance stage HL. However, pts with both, advanced HL and advanced HIV-infection had a significantly worse OS (p=0.023). *Conclusions.* In pts with HIV-HL risk-adapted CT and concomitant HAART is feasible and effective. However, pts must closely be monitored for neutropenic infections. These data suggest that the prognosis of HIV-HD may approach results achieved in the HIV-negative population with HL.

Chronic myeloid leukemia - Biology

0520

THE TUMOR SUPPRESSOR PP2A AS A THERAPEUTIC TARGET FOR ERADICATION OF TKI-RESISTANT PH+ LEUKEMIC STEM CELLS

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Background/Aims. The success of tyrosine kinase inhibitors (TKIs) depends on the addiction of Philadelphia-positive (Ph+) CML progenitors to BCR-ABL1 kinase activity. However, CML quiescent hematopoietic stem cells (HSC) are TKI-resistant and represent an active disease reservoir. We hypothesize that this innate drug-resistance depends on inhibition of the tumor suppressor protein phosphatase 2A (PP2A). PP2A can be reactivated by FTY720, a drug that targets CML but not normal progenitors. Here we investigated the mechanism controlling survival/self-renewal of quiescent leukemic HSCs and their sensitivity to PP2A-activating drugs. *Methods.* HSCs from CML (n=68) and healthy (n=12) donors were FACS-isolated, and the biologic importance of PP2A inhibition and pharmacologic PP2A activation on their survival/self-renewal was assessed by BM serial transplantation; CFSE and Annexin-V staining; LTC-IC and CFC/replating assays; lentiviral shRNA/cDNA-transduction; LEF/TCF and proximity-ligation assays; Western blot, confocal microscopy and FACS analyses. *Results.* We observed increased BCR-ABL1 expression with impaired kinase activity in quiescent CML HSCs, in which BCR-ABL1 per se is required for induction of JAK2 that subsequently activated beta-catenin and inhibited PP2A. In fact, PP2A was suppressed in CML but not normal CD34+/CD38-/CD90+ HSCs. FTY720 and/or its non-immunosuppressive (S)-FTY720-OMe derivative markedly reduced survival and self-renewal of CML but not normal quiescent HSCs through BCR-ABL1 kinase-independent and PP2A-mediated JAK2 and beta-catenin inhibition. Importantly, FTY720 also strongly diminished BCR-ABL1+ LT-HSC frequency in serial BM transplantation assays. *Conclusions.* The pharmacologic targeting of the newly-identified BCR-ABL1 kinase-independent JAK2/ -catenin interplay in quiescent HSCs with FTY720 and its derivatives, might lead to cessation of lifelong patient dependence on TKIs.

0521

COMBINATION OF THE HEDGEHOG PATHWAY INHIBITOR LDE225 AND NILOTINIB TARGETS THE LEUKEMIC STEM CELL POPULATION IN CHRONIC MYELOID LEUKAEMIA

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Background. Although effective in inducing cytogenetic and molecular remission in chronic myeloid leukaemia (CML), tyrosine kinase inhibitors do not eliminate leukaemia stem cells (LSC), potentially resulting in relapse/progression. Therefore alternative strategies are required for eradication of CML LSC. The Hedgehog (Hh) pathway, a developmental pathway regulating stem cell fate, is active in BCR-ABL+ LSC and contributes to CML LSC maintenance via up-regulation of Smoothened (SMO). Thus inhibition of SMO may specifically target CML LSC. *Aim.* To assess the effect of LDE225 - a clinical grade SMO inhibitor (Novartis) - alone and in combination with nilotinib in chronic phase (CP)-CML. *Methods.* Baseline global gene expression was assessed in primitive CD34+ normal and CP-CML cells using the HuGene 1.0 ST array and Taqman® qRT-PCR. CD34+ primary CP-CML cells were cultured in the presence of LDE225±nilotinib prior to analysis, inoculation into colony forming cell assays (CFC) or long-term culture initiating cell assays (LTC-IC) or transplantation into NOD-SCID γ-chain (NSG) mice. The Scl-tTa-BCR-ABL murine model of CP-CML was used to assess effects of *in vivo* treatment with LDE225 (80mg/kg/day) ±nilotinib (50mg/kg/day) or vehicle over 21 days (Koschmieder *et al*; Blood 2005;105:324-334). *Results.* We detected differential expression of key Hh elements and targets between primitive CD34+ normal and CP-CML cells. Furthermore, Hh targets (Gli1 and Ptch2) in CD34+ CP-CML cells were inhibited following 72 hours exposure to LDE225. Increasing concentrations of LDE225 (1nM-1μM) had no effect on viability, proliferation or cell cycle status on primary $CD34^+$ CP-CML cells compared to untreated controls. LDE225 did not affect CFC readout after 14 days, however, we noted a significant reduction in secondary colony formation following re-plating after exposure to LDE225 (50nM LDE225; 62% $p<0.02$) alone or combined with nilotinib (LDE225 10nM & nilotinib 5μM; 73% $p<0.03$). LDE225 also reduced the LTC-IC recovery of CD34+ CP-CML cells compared to untreated controls (40%; $p<0.03$) and combination treatment (LDE225 10nM & nilotinib 5μM) resulted in significantly reduced LTC-IC frequency versus nilotinib alone (85%; $p=0.007$). $CD34^+$ CP-CML cells, exposed to 10nM LDE225, 5μM nilotinib or combination for 72 hours were transplanted into NSG mice. Combination treatment reduced engraftment of CML $CD45^+$, $CD34^+/45^+$ and CFC compared with control ($p=0.06$ / $p=0.02$ / $p=0.005$ respectively). FISH demonstrated a 1.3-fold and 1.8-fold reduction in engrafted leukaemic cells with nilotinib and LDE225 respectively but a 6-fold reduction following combination treatment. LDE225±nilotinib did not affect engraftment of normal $CD34^+$ cells. In the Scl-tTa-BCR-ABL murine model of CML, LDE225±nilotinib reduced the number of splenic $Lin^-Sca\text{-}1^+Kit^+Flt3^-CD150^+CD48^-$ (LT-HSC) cells ($p<0.01$) compared to control, but did not affect bone marrow (BM) LT-HSC. Nilotinib did not reduce LT-HSC numbers in spleen or BM. Mice treated with combination therapy demonstrated enhanced post-treatment survival compared with other experimental arms. Secondary transplantation experiments indicated that recipients of BM or splenic cells from combination treated mice had a reduced incidence of leukaemogenesis. *Conclusion.* LDE225 targets CP-CML LSC *in vitro* and in a murine model of CML. LDE225 combined with nilotinib represents a promising novel strategy for eradicating the LSC population in CP-CML patients.

0522

ENHANCED EXPRESSION OF THE HEDGEHOG PATHWAY TARGET GENE PTCH1 PREDICTS INFERIOR RESPONSE TO IMATINIB IN CHRONIC MYELOID LEUKEMIA

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Background. Tyrosine kinase inhibitors (TKI) for chronic myeloid leukemia have yielded great success. However, BCR-ABL1-positive leukemic stem cells (LSCs) can persist despite lifelong TKI therapy and are believed to be a source of disease relapse and eventual progression. Recent studies indicate that activity of the Hedgehog (Hh) signaling pathway is crucial for survival of LSCs in CML. The level of Hh activity can be quantified via expression of its downstream target genes GLI1 and PTCH1. *Aims.* The aim of this study was to investigate the range of expression of PTCH1 in CML patients at diagnosis and to compare expression of PTCH1, and thus the level of Hh activity, to clinical outcome. *Methods.* Real-time quantitative PCR was used to measure PTCH1 expression in relation to a housekeeping gene (GUSB). RQ-PCR was performed on cDNA samples from peripheral blood granulocytes taken from 85 unselected CML patients at the time of diagnosis. Imatinib was given as a first line therapy for patients in chronic phase. A second cohort of 31 CML patients was identified for validation of clinical associations. Probabilities of OS, PFS and EFS were calculated using the Kaplan-Meier method. A receiver operating characteristic (ROC) curve method was used to calculate a threshold for poor response. *Results.* The median level of PTCH1 expression relative to GUSB was 3x10-4 (range 0 to 3.76x10-1). Importantly, the 59 patients with low expression of PTCH1 at diagnosis (calculated as on or below the 59th percentile of the expression range) had a superior seven year probability of CCyR, MMR, EFS, PFS and OS than the 26 patients with high expres-

sion; namely 94.9% vs 65.4% (p=0.007), 72.0% vs 23.7%, (p=0.0007), 84.1% vs 39.8 (p< 0.0001), 93.1 vs 53.2 (p< 0.0001) and 97.4% vs 58.9% (p< 0.0001). The expression of PTCH1 considered as a continuous variable was also predictive for the achievement of CCyR (RR= 0.68, p=0.01), MMR 0.49, p=0.004), EFS (RR= 0.56, p<0.0001), PFS (RR= 0.67, p=0.001) and OS (RR= 0.76, p=0.006). Expression of PTCH1 was an independent predictor for OS, PFS and CCyR and its predictive value was independent of Sokal score. The CD34 count in the bone trephine and the percentage of blast in peripheral blood at diagnosis was also comparable between patients with low (3.9% and 2.8%) and high (4.9% and 2.7%) PTCH1 expression (p=0.72 and p=0.88 respectively). The predictive value of PTCH1 expression was validated in an independent cohort of 31 patients. *Summary.* Our data suggest that PTCH1 has significant potential in predicting response to imatinib therapy in CML. The inter-patient variability of Hh activity in granulocytes may reflect similarly variable activity of this pathway in CML HSCs. This is supported by the observation that BCR-ABL1 increases Hh signaling in progenitor cells and stem cells to a comparable degree relative to normal counterparts. Variability in Hh pathway activity in CML HSCs therefore represents a plausible mechanism by which the CML clone in some patients may escape the inhibitory effects of imatinib to become a source of frank relapse.

0523

CANCEROUS INHIBITOR OF PP2A (CIP2A) INHIBITS PP2A AND STABILISES PIM1 AND C-MYC IN CML LEADING TO BLAST CRISIS

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Background. BCR-ABL1 tyrosine kinase activity induces and maintains chronic myeloid leukaemia (CML), but the molecular factors that contribute to disease progression are not well understood. PP2A is a phosphatase which regulates cell proliferation, differentiation and survival. Disease progression in CML is associated with inhibition of PP2A activity. In MNC and CD34+ cells taken at diagnosis from patients destined to progress to blast crisis (BC), we have shown that CIP2A functionally inhibits PP2A, and that levels of CIP2A detected in diagnostic samples can prospectively predict disease progression. We have also shown that 1) CIP2A is not suppressed by imatinib treatment and 2) CIP2A levels rise as patients progress into BC, further suppressing PP2A activity. As CIP2A levels rise BCR-ABL1 tyrosine kinase activity increases. *Aim.* The aim of this study was to investigate the mechanism by which CIP2A predisposes patients to disease progression. *Methods.* CIP2A, PP2A, pY^{307}-PP2A and PIM1 proteins were assessed by flow cytometry in 31 newly diagnosed chronic phase patients. c-Myc and pS^{62}-c-Myc were assessed by ELISA. CIP2A siRNA was transfected into K562 cells. Results were analysed in patients stratified according to their eventual clinical outcome - cytogenetic responders (CCR), Non-responders (No-CCR) and blast crisis (BC). *Results.* Phosphorylation of c-Myc on serine 62 stabilises c-Myc from degradation. MNC from chronic phase patients destined to progress to BC demonstrate significantly higher pS^{62}-Myc and c-Myc levels than in those patients who do not progress (p=0.04, p=0.002 respectively) suggesting high c-Myc levels indicate a high risk of disease progression. PP2A is the major phosphatase that dephosphorylates and destabilises Myc, so high levels of Myc support the observation of PP2A activity inhibition. siRNA knockdown of CIP2A restored PP2A activity and decreased pS^{62}-Myc levels and BCR-ABL1 tyrosine kinase activity. PIM1 phosphorylates and stabilises c-Myc; PIM1 is also a target for PP2A. PIM1 was elevated in CD34+ cells from patients destined to progress to BC compared to CCR and No-CCR patients. Analysis undertaken in K562 cells revealed that activation of PP2A, either by addition of the PP2A activators forskolin and FTY720 or by inhibition of CIP2A by imatinib, also decreased PIM1 levels. These data further support PP2A activity suppression in CD34+ cells of patients at high risk of developing BC. *Conclusion.* We show here firstly that pY^{307}-PP2A (inactivity), PIM1 and pS^{62}-Myc levels correspond with high levels of CIP2A and secondly that CIP2A siRNA knockdown restores PP2A function, and decreases BCR-ABL1 and c-Myc activities. Our data suggest that CIP2A predisposes patients to disease progression by inhibiting PP2A leading to the stabilisation of PIM1 and stabilisation and increased activity of c-Myc. C-Myc via its role in cell cycle promotion and increased cellular proliferation may then contribute to disease progression by promoting aneuploidy as a result of deregulated cell division and increased mismatch repair.

0524

THE PROPORTION OF LEUKEMIC STEM CELLS (PH+ CD34+CD38-) IN BONE MARROW OF NEWLY DIAGNOSED PATIENTS WITH CML CORRELATES WITH EARLY CYTOGENETIC AND MOLECULAR RESPONSE TO IMATINIB OR DASATINIB

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Background. In vitro studies have suggested that CML stem cells are resistant to bcr-abl inhibitors. However, no prospective clinical studies have evaluated their effect on leukemic stem cells in patients. In addition, the prognostic value of leukemic stem cell burden at diagnosis is unknown. *Aims.* To analyze the proportion of Ph^+ putative leukemia stem cells in CML patients at diagnosis and during imatinib or dasatinib treatment (TKI therapy), and correlate the leukemic stem cell burden with disease eradication kinetics and early treatment response. *Patients and Methods.* 42 newly diagnosed chronic phase CML patients within the Nordic countries were enrolled in a Phase II study (NordCML006) comparing the effect of dasatinib 100 mg (n=21) to imatinib 400 mg (n=21) at the stem cell level. Ph+ candidate leukemic stem cells were analyzed at 0, 1, 3, and 6 months after start of therapy. After pre-selection of CD34+ cells from BM aspirates, the cells were fractionated into CD38 positive ($CD34^+CD38^+$) and negative ($CD34^+CD38^-$) pools with sorting flow cytometer. The proportion of Ph^+ cells in different fractions was determined by interphase FISH for *BCR-ABL1*. *Results.* Measurement of Ph+ stem cells was feasible in most patients at all time-points. The median percentage of Ph^+ cells at diagnosis was significantly lower in $CD34^+CD38^-$ fraction when compared to $CD34^+CD38^+$ fraction or to unfractionated BM (79%, range 0.6-100%; 96%, 50-100%; and 96%, 57-100%, respectively, p=0.0001, n=41). During TKI therapy, the proportion of Ph^+ cells decreased rapidly in both stem cell fractions. At 1 month, the median proportion of Ph^+ cells was 14% and 56% in $CD34^+CD38^-$ and $CD34^+CD38^+$ fractions, respectively compared to 71% in whole BM (p=0.0002, n=36). At 3 month, the respective numbers were 0.18%, 0.19% and 0.80% (p=0.03, n=27).The proportion of Ph^+ cells in the $CD34^+CD38^{neg}$ fraction at diagnosis correlated with high leukocyte count (r=0.50, p<0.001), enlarged spleen (r=0.43, p=0.0055), high blood blast percentage (r=0.57, p=0.0001) and low hemoglobin concentration (r=-0.44, p=0.004). Pre-treatment leukemic stem cell burden also correlated with cytogenetic response during TKI therapy at 1, 3 and 6 months time-points (r=0.67, p<0.0001; r=0.52, p=0.0017; r=0.43, p=0.015, respectively). Patients who achieved MMR at 6 months time-point (n=17) had significantly lower proportion of Ph^+ $CD34^+CD38^-$ cells at diagnosis compared to patients without MMR (n=21) at 6 months (70% vs. 89%, p=0.03). Grade ≥2 hematological adverse effects were more common during first 3 months of therapy in patients with high leukemic stem cell burden at diagnosis (55% vs. 19%, p=0.02). *Conclusions.* Our results indicate that the proportion of Ph^+ stem cells at diagnosis is a key biological marker and correlates with cytogenetic and molecular response as well as hematological toxicity within first 6 months of treatment. Successful TKI therapy rapidly eliminated most Ph^+ cells from the stem cell compartments *in vivo*. The effect of therapy by the treatment arm will be analyzed when all patients have reached the primary endpoint at 6 months time-point.

Platelets and bleeding disorders

0525

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE II TRIAL ON THE EFFICACY, SAFETY AND TOLERABILITY OF E5501 (AKR501) IN SUBJECTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (CITP)

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Background. Thrombopoietin (TPO) receptor agonists mimic TPO, the endogenous platelet production regulator, and have demonstrated efficacy in randomized controlled trials in chronic immune thrombocytopenia (cITP) patients who relapsed after first- and/or second-line agents. *Aims.* E5501 (previously AKR501) is a novel, orally-active, once-a-day TPO agonist which increased platelet counts in healthy volunteers. This study investigated the efficacy and safety of E5501 in subjects with cITP. *Methods.* This was a Phase II, multicenter, randomized, double-blind, placebo-controlled, dose-ranging, parallel group 4-week study (501-CL-003). Subjects had cITP that was refractory to, or had relapsed after, at least one prior therapy, and had a baseline platelet count of <30 x10^9/L, or <50 x10^9/L if they were on stable corticosteroid therapy. Sixty-four subjects were enrolled and randomized in the ratio 3:3:3:3:1 respectively to E5501 (2.5, 5, 10 or 20 mg) or placebo, administered orally, once daily for 28 days. Response was assessed by weekly platelet count and the primary endpoint was responder rate at Day 28, with responders defined as having a platelet count ≥50 x10^9/L that had risen by ≥20 x10^9/L above baseline. *Results.* A dose-dependent increase in responder rate at Day 28 was observed among subjects receiving E5501 (Table 1). Responder rate at Day 28 was significantly higher in the E5501 20 mg group (80.0%) than the placebo group (0%; p=0.0036) and the E5501 2.5 mg group (13.3%; p=0.0007). Median platelet counts at Day 28, and change above baseline, increased dose dependently in subjects receiving E5501 (Table 2). In the E5501 20 mg group, 93.3% of subjects achieved early response (by Day 7; Table 3). For subjects achieving early response with E5501 20 mg, 76.9% maintained platelet response up to Day 28. None of the subjects receiving E5501 2.5 mg achieved and maintained response. None of the 5 placebo-treated subjects demonstrated response at any point. A similar proportion of treatment-emergent adverse events (TEAEs) occurred in all dose groups. Most TEAEs were mild and transient, with fatigue (20.3%), headache (20.3%) and epistaxis (15.3%) the only TEAEs that occurred in ≥10% of E5501-treated subjects. Overall few AEs led to dose interruption or treatment discontinuation in the study, and there was no clear dose relationship in the incidence of these events. Two subjects (1 each in the 5-mg and 10-mg dose groups) withdrew due to a drug-related AE (musculoskeletal chest pain [5 mg]; myocardial infarction, transient ischemic attack, and retinal artery occlusion [10 mg]); and 2 subjects [20 mg] withdrew due to increased platelet counts (≥500,000/mm^3). Three subjects reported 3 additional serious TEAEs unrelated to study drug: 1 pneumonia by the previously mentioned subject in the 10 mg dose group and 2 occurred in 2 subjects in the 2.5-mg dose group. *Conclusion.* E5501 was effective in increasing platelet counts in subjects with cITP at doses of 5, 10, and 20 mg daily, was well tolerated and had an acceptable safety profile. These data support continued development of E5501 as an effective treatment in patients with cITP.

Table 1.

Responders at Day 28

Population category	Placebo	E5501 2.5 mg	E5501 5 mg	E5501 10 mg	E5501 20 mg	Trend Test P value
Responder, number (%)						
Yes	0	2 (13.3)	8 (53.3)	7 (50.0)	12 (80.0)	
No	5 (100)	13 (86.7)	7 (46.7)	7 (50.0)	3 (20.0)	
Total	5	15	15	14	15	
P values (Fisher's Exact test)						
vs. Placebo		1	0.0547	0.106	0.0036	
vs. E5501 2.5 mg			0.0502	0.0502	0.0007	
vs. E5501 5 mg				1	0.2451	
vs. E5501 10 mg					0.1281	
Exact Cochran-Armitage trend test						0.000123
Exact logistic trend test						0.000135

Table 2.

Median platelet counts at Day 28

	Placebo	E5501 2.5 mg	E5501 5 mg	E5501 10 mg	E5501 20 mg
Baseline median platelet counts, x10^9/L (N)	19 (5)	18 (15)	27 (15)	22.5 (14)	22 (15)
Median platelet counts at Day 28, x10^9/L (N)	20 (5)	19 (13)	37 (13)	54 (12)	95 (13)
Median change in platelet counts above Baseline, x10^9/L	-2	0	7	39	76

Table 3.

Responder rate by visit, LOCF method

	Placebo		E5501 2.5 mg		E5501 5 mg		E5501 10 mg		E5501 20 mg	
	Total N Analyzed	N (%) Responded	Total N Analyzed	N (%) Responded	Total N Analyzed	N (%) Responded	Total N Analyzed	N (%) Responded	Total N Analyzed	N (%) Responded
Full Analysis Population										
Day 7	5	0	15	1 (6.7)	15	10 (66.7)	14	9 (64.3)	15	14 (93.3)
Day 14	5	0	15	3 (20.0)	15	9 (60.0)	14	11 (78.6)	15	14 (93.3)
Day 21	5	0	15	2 (13.3)	15	9 (60.0)	14	7 (50.0)	15	13 (86.7)
Day 28	5	0	15	2 (13.3)	15	8 (53.3)	14	7 (50.0)	15	12 (80.0)
Per Protocol Population										
Day 7	5	0	13	1 (7.7)	13	8 (61.5)	12	9 (75.0)	12	11 (91.7)
Day 14	5	0	13	3 (23.1)	13	7 (53.8)	12	11 (91.7)	12	11 (91.7)
Day 21	5	0	13	2 (15.4)	13	7 (53.8)	12	7 (58.3)	12	10 (83.3)
Day 28	5	0	13	2 (15.4)	13	6 (46.2)	12	7 (58.3)	12	9 (75.0)

LOCF=last observation carried forward

0526

A FAMILY WITH TYPE 2M VWD WITH NORMAL VWF:RCO BUT REDUCED VWF:CB DUE TO A M1761K MUTATION IN THE A3 DOMAIN

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Type 2 VWD is characterised by a qualitative defect in VWF and is diagnosed by demonstration of a discrepancy between circulating plasma levels of VWF and its functional activity. Type 2 VWD is subdivided into types 2A, 2B, 2M and 2N. Type 2A and 2M variants show decreased platelet binding but type 2A VWD is also associated with an absence of high molecular weight multimers. Type 2B variants have increased affinity for platelet glycoprotein 1b. Type 2N VWD refers to variants with a decreased affinity for F8. A 17-year-old presented with menorrhagia, Fe deficiency, epistaxis and bruising (bleeding score 5). Both her sisters and mother had menorrhagia and bruising, one sister had nose bleeds and the mother had a PPH (bleeding scores 2, 3 and 4). The principal detectable abnormality was defective collagen binding. Genetic analysis showed the mutation c.5282 T>A, p.M1761K in the A3 domain of VWF. All have the same mutation, normal RIPA and normal multimers. The table shows the results including VWF:CB measured by two different kits. Four previous mutations with this phenotype have been described S1731T, W1745C, S1783A, H1786D. Our novel mutation was picked up by the Corgenix assay (equine type III collagen) but not by the Technozym assay (pepsin-digested human type III collagen) which demonstrates that this sub-type of type 2M VWD will be missed if VWF:CB assays are not performed and that different assays may differ in their sensitivities. The 1994 definition of type 2M VWD depended on "decreased platelet-dependent function"which defined patients with VWF GPIb binding site defects who have reduced VWF:RCo. This family would have remained unclassified. In the recently updated ISTH SSC VWD classification isolated collagen binding defects are included within the 2M subgroup. Impairment of VWF-dependent platelet adhesion to the endothelium in type 2M VWD occurs in the presence of a full range of VWF multimers. This is a key characteristic of the 2M subtype. There are 3 important differences with the 2M group. 1. 2M mutations are clustered in the A1 domain (residues 1260-1471) of VWF, whereas these mutations af-

fecting collagen binding are found in A3. 2. 2M is characterised by impaired binding to platelet GpIbα, demonstrated by reduced ratio of RCo:Ag. 3. A clinically important feature of the 2M phenotype is it tends to be associated with poor functional response to DDAVP. VWF secreted in response to DDVAP continued to show a qualitative defect in collagen binding demonstrated by persistence of the abnormal CB:Ag ratio. In addition to measuring GP1bα-dependent function using riscocetin cofactor activity (VWF:RCo), it is recommended that collagen binding activity (VWF:CB) is analysed in the subclassification of type 2 VWD. Mutations in the A3 domain of VWF causing reduction in collagen binding, independent of multimer composition may cause clinically significant bleeding symptoms.

Table 1.

0527

SUBCUTANEOUS INJECTIONS OF LOW-DOSE ANTI-CD20 VELTUZUMAB FOR PATIENTS WITH RELAPSED IMMUNE THROMBOCYTOPENIA (ITP)

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Background. Subcutaneous (SC) injections of veltuzumab, a 2nd-generation humanized anti-CD20 monoclonal antibody with structure-function differences from rituximab, may offer potential benefits to both patients and the healthcare system. *Aims.* A multicenter, phase I/II study to evaluate SC veltuzumab in adults with primary ITP who failed ≥1 standard therapy and presented with platelets ≤30K/μL, but without major bleeding. *Methods.* All patients received 2 doses of veltuzumab 2 weeks apart (without steroids), initially administered IV, but then by SC injection after a higher concentration formulation became available. By recent international working group categories, efficacy was evaluated separately for patients with newly-diagnosed or persistent disease (≤1 year duration) compared to patients with chronic disease (>1 year), with best responses (on at least 2 occasions, one week apart) classified as complete (CR, >150K/μL), partial (PR, 50-150K/μL), or minor (MR, 30-50K/μL). Adverse events (AEs) and safety laboratories were evaluated by NCI CTC v3 toxicity grades. Other evaluations included circulating B-cell levels (CD19), veltuzumab serum levels, and human anti-veltuzumab antibody (HAHA) titers. *Results.* Of 36 patients now entered, 7 received IV veltuzumab doses of 80 (N=3), 120 (N=3), or 200 mg (N=1), and 29 received SC doses of 80 (N=9), 160 (N=5), or 320 mg (N=15). One patient had a Grade 3 infusion reaction after receiving ~100 mg veltuzumab at first IV dose. Otherwise veltuzumab was well tolerated with a limited number of AEs (All Grade 1-2 transient infusion/injection reactions) and no other safety issues. Of 8 patients (5 female/3 male; median 55-years old) with ITP ≤1 year and treated with steroids and/or immunoglobulins, 7 (88%) achieved an objective response (OR: CR+PR+MR), including 3 (38%) CRs. Of 28 patients (16 female/12 male; median 53-years old) with ITP > 1 year (13 with 5 - 31 years) and additional therapies [splenectomy (N=8), azathioprine (N=8), rituximab (N=6), tpo-receptor agonists (N=5), chemotherapy (N=4)], 17 (61%) still achieved ORs, including CRs in 4 (14%) patients with ITP for 1.4, 2.0, 2.4 and 25 years. ORs and CRs occurred with both SC and IV administrations, and across all dose levels. The CRs were durable, with median relapse-free survival currently 1.2 years (0.3 - 1.9 yr) and 5/7 CRs still continuing. Most PRs and MRs relapsed before 6 months, and of 7 patients then retreated, most obtained responses comparable to their initial response. B cells were depleted rapidly with both IV and SC dosing with recovery starting 12 to 16 months after treatment. Compared to IV dosing, SC veltuzumab had slower release over several days with lower serum levels, but comparable availability/exposure. Four patients developed low-level HAHA titers of uncertain clinical significance. *Conclusions.* Low-dose SC veltuzumab was convenient, well tolerated and with promising activity in relapsed ITP. With only 2 SC doses, patients with limited disease duration of ≤1 year achieved high rates of objective responses [88%], including 38% durable CR's. In patients with longer-standing disease, CRs occurred less frequently, but there was activity [61% ORs], and more extended dosing SC regimens may be required in this more refractory population.

0528

BASELINE CHARACTERISTICS FOR PREDICTING INHIBITOR ERADICATION AFTER FIRST LINE IMMUNOSUPPRESSION IN ACQUIRED HAEMOPHILIA A: ANALYSIS OF EUROPEAN ACQUIRED HAEMOPHILIA REGISTRY (EACH2)

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Background. Acquired haemophilia A (AHA) is an autoimmune disease characterised by development of an autoantibody to factor VIII (FVIII). Immunosuppression to eradicate the inhibitor should start as soon as the diagnosis has been made to reduce the time patients are at risk of bleeding. If presenting characteristics could be used to predict the likelihood of inhibitor eradication it might be possible to improve treatment. To increase knowledge of AHA the European Acquired Haemophilia Registry was established and collected data on 501 patients from 12 countries. *Aim.* Investigate whether presenting characteristics could be used to predict inhibitor eradication. *Methods.* A subset of 240 patients was eligible for analysis. The patients included were from countries that had no restrictions on entry and on whom outcome data were available. Inhibitor eradication was defined as FVIII >70IU/dL and inhibitor negative. The baseline model included age, gender and underlying aetiology (autoimmune, pregnancy, malignancy or idiopathic). To investigate the potential clinical utility of presenting FVIII level and inhibitor titre; survival c-index derived from a Cox model, net reclassificaction improvement (NRI) and integrated discrimination improvement (IDI) were calculated. The IDI is a measure of how much a parameter (FVIII, inhibitor titre or both) improves the overall predictive value of the model for inhibitor eradication compared to the baseline model, whereas the NRI measures the improvement in prediction according to pre-specified cut offs of risk. *Results.* 156/240 (65%) patients achieved remission after first line immunosuppression after a median (IQR) 35 (19-77) days. The underlying aetiology and gender were not significantly associated with time to inhibitor eradication on Cox regression modelling but age was borderline significant: hazard ratio (HR) (95% CI) 1.02 (1.0-1.03), P<0.03. Using Cox regression modelling, an inhibitor titre <16 BU/mL (the median inhibitor titre of the cohort) HR 1.57 (1.10-2.23), P<0.02 and higher FVIII level at presentation (analysed as a continuous variable) HR 1.06 (1.02-1.101), P<0.001 were both associated with faster inhibitor eradiation. Compared to the baseline model (age, gender and aetiology), adding

inhibitor titre <16 BU/mL resulted in a survival c-index (95% CI) of 0.62 (0.58-0.67), P=0.0006. The NRI for inhibitor titre <16BU/mL was 15.75%, P=0.14 and the IDI was 3.38%, P<0.005. FVIII level at presentation resulted in survival c-index 0.61 (0.56-0.66), P<0.02, NRI 16.48%, P=0.08 and IDI of 2.94%, P<0.005. Combining both inhibitor titre <16BU/mL and FVIII level gave a survival c-index 0.64 (0.59-0.69), P<0.001, NRI 16.48%, P=0.06 and IDI 4.56%, P<0.001 for the time to inhibitor eradication. *Conclusions.* These data are the first demonstration that inhibitor titre and FVIII level at diagnosis provide time dependent prognostic information about the likelihood of inhibitor eradication. The results may be useful for improving the understanding of the biology of AHA. Whether this information will be clinically useful is not known. It is possible, however, that patients presenting with high titre inhibitors and low FVIII levels may benefit from more intensive immunosuppressive regimens but this will required further study. The results further highlight the value of high quality registry data for the investigation of rare diseases.

0529

INCIDENCE AND DETERMINANT OF BLEEDING IN DIFFERENT TYPES OF VON WILLEBRAND DISEASE: RESULTS OF A PROSPECTIVE MULTICENTER COHORT STUDY ON 797 ITALIAN PATIENTS

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Background. von Willebrand disease (VWD) is the most common inherited bleeding disorder and is due to quantitative and/or qualitative defects of von Willebrand factor (VWF). Despite the improved knowledge of this disorder, no data on the incidence and determinants of bleeding requiring specific treatments are available up to now. Aims and design of the study: To determine the incidence and determinants of bleeding requiring therapy with DDAVP and/or VWF/FVIII concentrates in patients with VWD, a national registry (RENAWI) was organized to collect detailed retrospective information. Patients included in RENAWI were then followed up for one year and prospective data on number, type and management of bleeding episodes were analyzed. *Methods.* All patients were diagnosed following recommendations of the ISTH-SSC-SC on VWF, with bleeding score (BS) calculated at enrollment. Diagnosis of VWD was confirmed by the coordinating center using multimeric analysis in plasma and mutations of VWF gene. For different risk categories, the incidence of bleeding (mucosal and non-mucosal) was calculated. Bleeding-free survival was computed with the Kaplan-Meier method, and a Cox's proportional hazard model was builded to calculate the risk of bleeding [expressed as hazard ratio (HR)] associated with different determinants, adjusted for the effect of the other variables. *Results.* In the prospective study based on 797/1,234 (65%) cases of the registry (VWD1=57%, VWD2A=8%, VWD2B=7%; VWD2M=21%; VWD3=6%), 147/797 (18%) were treated in a year for 318 bleeding episodes and 87 minor or major surgeries. At univariate analysis, the determinants significantly associated with a high risk of bleeding were: BS>10 [HR=6.72 (95%CI: 3.74-12.07)], bleeding time>20 min [HR=5.66 (3.19-10.02)], VWF:RCo<10 U/dL [HR=3.26 (1.76-6.04)], and FVIII:C<20 U/dL (HR=4.20 (2.43-7.26)]. Including all the variables in a Cox's proportional hazard model, BSS was the most significant determinant of bleeding [HR=5.26 (2.64-10.44)]. The probability of being free from bleeding at one year was significantly lower in VWD3 (49%) than in VWD1 (95%) and VWD2 (90%) patients. Those with both VWF:RCo>30 U/dL and FVIII:C>40 U/dL always showed BS<5 and the lowest incidence of bleeding. A total of 292 DDAVP injections were used to manage bleeding and surgeries in VWD1 (65%) and VWD2 (35%) patients, and 452 administrations of VWF/FVIII concentrates were used to treat bleeding and to manage surgeries in VWD3 (75%), VWD2 (34%) and VWD1 (15%). *Conclusions.* This prospective study confirms that BS is an important clinical predictive factor for bleeding and for the need of treatment. In cases with VWF:RCo>30 U/dL and FVIII:C>40 U/dL bleedings are very rare, in agreement with their relatively low BS.

Improving outcome in chronic lymphocytic leukemia

0530

RITUXIMAB THERAPY OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS IN REMISSION

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The application of the combination of rituximab with a fludarabine-containing chemotherapy regimen in recent years has significantly improved the results of overall and disease-free survival of patients with chronic lymphocytic leukemia (CLL). *Aim.* To study the efficiency of maintenance with rituximab after induction chemotherapy or immuno-chemotherapy in CLL patients. *Materials and Methods.* The study included 213 patients in remission. The age of patients ranged from 34 to 76 years (median, 59 years). Remission was induced after a RFC program in 117 patients and FC in 96 patients. Complete remission (CR) was observed in 121 (57%) patients, and partial remission (PR) in 92 (43%). The patients were randomly assigned to either observation (133 patients) or supporting rituximab therapy in the form of 4 weekly injections (375 mg/m2) every 6 months over 2 years (60 patients). *Results.* The patients treated with a RFC regimen with subsequent rituximab maintenance had a significantly lower frequency of relapse and death compared to the observation group ($\chi2$=10.749, p=0.001 and $\chi2$=5.877, p=0.015, respectively). Analyzing these indicators in patients treated with an FC regimen we also see the advantage of maintenance therapy in relation to the observation group ($\chi2$=49.896, p=0.0001 and $\chi2$=9.985, p=0.002, respectively). Comparative analysis of progression free survival (PFS) of CLL patients who received various regimens revealed a significant difference. Thus, in patients who completed the program followed by RFC and supporting rituximab therapy, the median PFS was achieved, while in patients without supporting rituximab therapy it was 42 months (p=0.009). The related indicators of patients receiving the combination of FC with further supporting rituximab therapy differed significantly: their median PFS was not achieved in contrast to patients in the monitoring group, whose PFS was 24 months (p=0.001). During the period of supporting rituximab therapy no additional toxicity was observed. *Conclusion.* The results of our study confirm the role of rituximab therapy in CLL remission maintenance.

0531

FLUDARABINE PLUS RITUXIMAB CHEMOIMMUNOTHERAPY FOLLOWED BY A CONSOLIDATION AND MAINTENANCE PLAN WITH RITUXIMAB IMPROVES OUTCOME IN CHRONIC LYMPHOCYTIC LEUKEMIA

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The treatment target of chronic lymphocytic leukemia (CLL) is the attainment of an optimal disease control combining chemotherapy with monoclonal antibodies. This approach may produce complete molecular remissions and longer response duration (RD), remaining often a minimal residual disease (MRD). We treated in first line 138 CLL symptomatic patients (pts), after informed consent, median age 63 years, with six monthly courses of intravenous (25 mg/sqm) or oral (30-40 mg/sqm) fludarabine and then, after a median time of 30 days, with four weekly doses (375 mg/sqm) of rituximab (rtx). Fourteen pts had a modified low Rai stage, 121 an intermediate stage and 3 a high stage. We defined as high risk pts having at least two of these markers: unmutated IgVH, CD38>30%, ZAP-70>20%, intermediate/unfavorable cytogenetics (trisomy 12 or del11q or del17p). For MRD flow cytometric study, the threshold was set at >1% CD19+CD5+CD79b+/- bone marrow (BM) CLL cells. Based on NCI criteria 106/138 (77%) pts

achieved a complete remission (CR), 26/138 (19%) a partial remission (PR) and 6/138 (4%) stable disease or progression. Phenotypic CR (CD19+CD5+CD79b- BM cells <1%) was achieved in 80/138 (58%) CLL pts. Interestingly, MRD+ pts showed a significant shorter overall survival (OS) in comparison with MRD- pts (24% vs 72% at 16 years, P=0.00016). During the induction and consolidation/maintenance, 13 pts underwent grade 2-3 (WHO) infective lung toxicity and 2 pts progressed towards Richter's syndrome. Hematologic toxicity was mild including mainly neutropenia (grade 3 and/or 4 in 60 pts) and thrombocytopenia (grade 3 and/or 4 in 8 pts). Fifty-seven pts (43%) either in CR with B-CLL BM cells >1% (MRD+, n=15 pts) or in CR MRD negative, but developing MRD positivity within 2 years after induction (n=24 pts) or in PR (n=18 pts), underwent consolidation and maintenance therapy with four monthly cycles of rtx at 375 mg/sqm followed by twelve monthly low doses of rtx (150 mg/sqm). The median follow-up duration was 59 months. Noteworthy, both persistently MRD negative (>2 years) pts (n=52) and pts undergoing consolidation/maintenance therapy (n=57) showed a longer RD vs MRD+ not consolidated pts (n=22) [76% vs 57% vs 0% at 5 years; P<0.0001]. Equally, OS was shorter in MRD+ not consolidated pts in comparison with the other two subsets (0% vs 61% vs 97% at 15 years; P=0.03). Moreover, ZAP-70+ or unmutated IgVH pts revealed shorter RD (17% vs 53% at 16 years, P=0.001; 16% vs 53% at 6.5 years, P<0.0001). Importantly, within the high risk subset (n=59), pts in persistent phenotypic CR (n=20) and consolidated pts (n=20) showed a longer RD (90% vs 61% vs 0% at 2.5 years, P=0.0009) vs MRD+ not consolidated pts (n=13). In multivariate analysis, consolidation/maintenance (P<0.0001) and biologic risk classes (P=0.001 and P<0.0001) were confirmed as independent prognosticators with regard to RD and OS. Therefore, persistent MRD negativity and/or rituximab consolidation/maintenance therapy improve RD and OS in CLL, also within the high risk subset, and important biological markers such as ZAP-70 and IgVH mutational status retain their prognostic impact with regard to the clinical outcome.

0532

A PHASE II STUDY OF CHLORAMBUCIL+RITUXIMAB (CLB-R) FOLLOWED BY R MAINTENANCE VS OBSERVATION IN ELDERLY PATIENTS WITH PREVIOUSLY UNTREATED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): INDUCTION PHASE RESULTS

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Background. The addition of rituximab to fludarabine-cyclophosphamide (R-FC) significantly improves outcome in CLL patients (pts). Myelotoxicity and immunosuppression represent an important limitation for the use of R-FC in elderly patients who frequently show impaired performance status and multiple comorbidities. *Aims.* This study was designed to determine whether the CLB-R combination is a feasible and beneficial first-line treatment for elderly CLL patients and to define the role of maintenance with R. *Methods.* Between October 2008 and January 2010, 97 elderly patients with untreated CLL requiring therapy according to the IWCLL criteria were enrolled. Written informed consent was obtained from all patients. CLB was administered every 28 days up to 8 courses at a dose of 8 mg/m^2/day on days 1-7 combined with 375 mg/m^2 R for cycle 3 and 500 mg/m^2 for cycles 4-8. Responsive patients were subsequently randomized to receive R maintenance every 2 months for 2 years or clinical observation. At baseline, blood samples were analyzed for FISH, IGHV mutational status, p53 mutation, and Zap-70 and CD38 expression. Minimal residual disease (MRD) was evaluated by flow cytometry and PCR on CR patients. The primary endpoint was the overall response rate (ORR) at the end of the induction phase on an intention-to-treat (ITT) population (all enrolled patients who received at least 1 R dose). *Results.* Eighty-five patients from 19 Italian centers were evaluable on an ITT basis. Median age was 70.0 years (range 61-84). Overall, 52.9% of patients were ≥70 years. One or more comorbidities were recorded at baseline in 47.1% of cases. Binet's stage was A in 25.9% of cases, B in 57.6% and C in 16.5%. Trisomy 12, deletion 13q, deletion 11q and deletion 17 were found in 26.5%, 48.2%, 19.3% and 4.8% of cases, respectively. p53 mutations were recorded in 4.8% of patients. Fifty-eight percent were IGHV unmutated, 41% CD38+ and 75.9% Zap-70+. The ORR was 81.2% (69 pts). CR, confirmed by CT scan, was found in 16.5% of pts (14 pts), CRi in 2.4% (2 pts), nPR 2.4% (2 pts) and PR in 60% (51 pts). In the 14 CR cases, MRD evaluated by flow cytometry in blood and marrow was negative in 2; no patient was PCR negative. A treatment failure was recorded in 18.8% of cases (16 pts): PD 3.5% (3 pts), SD 4.7% (4 pts) and lack of response assessment 10.6% due to early treatment withdrawal (9 pts: investigator's decision, 2; treatment-related AEs, 4; treatment-unrelated AEs, 3). Twenty SAEs were recorded in 17 patients: 5 (4 pts) related to CLB only, 3 (3 pts: pleural effusion, 1; anemia, 1; neutropenia, 1) related to CLB-R and 12 (12 pts) treatment-unrelated. The most common hematologic toxicity was neutropenia (grade III-IV: 13.5% of patients, 7.4% of episodes and 2.9% of cycles). The median number of administered CLB-R cycles in patients <80 years was 6. A dose reduction of CLB was required in 7.8% of cycles, mainly for myelotoxicity. *Conclusions.* This study shows that R-CLB is an active and well tolerated front-line regimen for elderly CLL patients.

0533

NCRN CLL207 STUDY OF ALEMTUZUMAB CONSOLIDATION IN CLL: FINAL RESPONSES ASSESSMENT, EARLY FOLLOW-UP AND IMPACT ON PROGRESSION FREE SURVIVAL

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Background. Remission duration in chronic lymphocytic leukaemia (CLL) depends on the level of minimal residual disease (MRD). Conversion from MRD positive to negative should prolong remissions and survival. Several small studies have reported alemtuzumab consolidation following chemotherapy but with concerns over toxicity; primarily due to immune suppression and infections. Dose and interval between prior chemotherapy and alemtuzumab appear critical. *Aims.* Phase II trial designed to assess the efficacy and toxicity of alemtuzumab consolidation post-chemotherapy. *Methods.* The alemtuzumab was administered at 30mg subcutaneously 3 times a week for 6 weeks followed by marrow assessment. MRD negative patients and non-responders stopped therapy; MRD positive patients with a significant response continued therapy for a further 6 weeks. MRD was assessed in blood and marrow using multi-parameter flow cytometry with a 0.01% detection limit. All patients received PCP prophylaxis and aciclovir as well as CMV-PCR monitoring. *Results.* 47 patients re-

ceived alemtuzumab (median age 58yrs [40-77] and 35 [74.5%] males). There was a median of 2 prior therapies (range 1 to 4) with 46 patients receiving fludarabine combinations and 9 rituximab-containing combinations. There were a total of 22 SAE's in 17(36.2%) patients with 2(4.3%) treatment related deaths (EBV-LPD and parainfluenza). G-CSF was given when the neutrophil count fell below 1 x 10^9/l, and 27(58%) patients required G-CSF during or after alemtuzumab. Positive CMV PCRs were detected in 21(45%) patients, all of whom were successfully treated with pre-emptive antiviral therapy. 13/23(56%) patients in partial remission achieved a CR three months after alemtuzumab. 39/47 (83%) patients had MRD negative marrows at the end of alemtuzumab, 7 (15%) remained MRD positive and 1 (2%) was not evaluable. Six months after alemtuzumab, 18/39(46%) MRD negative patients became MRD positive in the blood (although all except 2 with low CLL levels (<0.1x10^9/l), and 20/39(51.0%) patients remained MRD negative. Of these, 15/16 (94%) remained MRD negative at 12 months. Therefore MRD negativity in blood at 6 months predicts for persistent MRD negativity better than the marrow assessment at the end of therapy. 6/9 patients receiving 12 weeks of alemtuzumab became MRD negative, but only 1(17%) responder remained MRD negative in the blood at 6 months. In contrast, of the 33 MRD negative patients after 6 weeks of treatment, 19(58%) remained MRD negative at 6 months. The 24 month PFS after start of alemtuzumab consolidation is 82% for all patients and 85% for those who achieved MRD negativity. Of the 18 MRD negative patients 6 months after alemtuzumab, after median follow-up of 21 months from treatment, only one patient has progressed clinically at 38 months. *Conclusion.* Consolidation with alemtuzumab is associated with largely manageable mainly infectious toxicities. 43% of patients were MRD negative 6 months following alemtuzumab consolidation which justifies the continued investigation of this approach in CLL primarily within a clinical trial setting with appropriate monitoring of patients. To this end we plan a randomized Phase III trial of consolidation with alemtuzumab compared to observation (the CLARET study).

0534

PHASE-II STUDY OF NAVITOCLAX (ABT-263) SAFETY AND EFFICACY IN PATIENTS WITH RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): INTERIM RESULTS

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Background. Despite available, effective treatments, most patients with CLL will experience multiple relapses and become refractory to standard therapy. Bcl-2 is universally over-expressed in CLL cells, thus suppression of Bcl-2 is an attractive therapeutic target. Navitoclax, a novel BH3 mimetic, binds with high affinity (Ki ≤1nM) and inhibits multiple anti-apoptotic Bcl-2 family proteins. Our earlier phase-I trial established that single-agent navitoclax 250mg/day achieved predicted pharmacokinetic (PK) parameters, was well tolerated and provided a signal of activity in previously treated patients with relapsed/refractory CLL, justifying phase-II evaluation at this dose. *Methods.* This multicenter international trial assessed safety, efficacy, and PK of oral navitoclax in patients with relapsed/refractory CLL, ECOG status ≤1, platelets ≥75,000/mm^3, who had received ≤5 prior regimens. Patients from the phase-I study were excluded. Following a 7-day lead-in at 100mg/day, navitoclax was dosed at 250mg/day on a 21-day cycle (C) until PD or intolerable toxicity. Preliminary efficacy endpoints included tumor response (NCI-WG 1996) and PFS. Disease was assessed at end of C2 and C4, every 4 cycles through C20, and every 8 subsequent cycles. Adverse events (AE) were graded by NCI CTCAE V3. *Results.* Thirty-one patients, median age 70y (range 44-82), were enrolled; 7/19 with available data were fludarabine-refractory. Median (range) number of prior therapies was 2.5 (1-6). So far, 13 patients have cytogenetic data; 9 were high-risk (5 with 11q-, 2 with 17p-, and 2 with both); 4 had neither deletion. Median time on study is 5.6 months (3.8-14.5+) with 10 patients still receiving drug. Twenty-six patients are evaluable for response (5 too early); 10 (38%) PR, 14 (54%) SD, 2 (8%) PD. Median PFS [95% CI] for 29 patients was 8.7 months [6.0, not reached]. By serial CT 12 (46%) patients had >50% nodal regression. Of 27 patients with baseline lymphocytosis, 24 (89%) had ≥50% reduction (median reduction 78.4%). Seventeen patients (55%) had bulky disease (adenopathy >5 cm); of the 13 evaluable all showed anti-tumor activity: 6 with PR and 7 with SD. PK analysis of trough concentrations suggested consistent exposure across cycles. The most common navitoclax-related AEs (all grades) were diarrhea (57%) and nausea (43%), both most likely attributable to the formulation; Grade 3/4 AEs included thrombocytopenia (27%) and neutropenia (17%). One patient each had a serious, navitoclax-related AE: pyrexia (Grade 1/2), tumor-lysis syndrome (Grade 3), dizziness (Grade 1/2). Three patients had AEs leading to discontinuation, and 9 leading to dose reduction, mainly thrombocytopenia. Nineteen patients discontinued: 6 due to PD, 6 due to AEs, 5 withdrew consent, and 2 due to other reasons (lack of response, investigator decision based on low-trending platelets). Two of 4 patients with 17p- achieved PR; patients with 11q- (n=5) appeared to have favorable outcome vs patients with 17p- (n=4) or with normal cytogenetics (n=4) (PFS 183 days vs not reached, p=0.0376). *Conclusions.* These data confirm that navitoclax has an acceptable safety profile at 250mg/day and significant anti-tumor activity in patients with heavily pre-treated CLL, including those with 17p- and other high-risk cytogenetic characteristics. Updated results will be presented.

Red cells

0535

THE CGMP PATHWAY AS A DRUG TARGET FOR THE REDUCTION OF VASO-OCCLUSION IN SICKLE CELL ANEMIA MICE: ACUTE EFFECTS OF HYDROXYUREA AND A PHOSPHODIESTERASE 9 INHIBITOR

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Background. Modulation in the levels of the second messenger cyclic guanosine monophosphate (cGMP) downstream in the nitric oxide (NO) pathway, represents a possible therapeutic approach for sickle cell disease (SCD). Up-regulation of cGMP-dependent signaling may induce γ-globin production in erythroid-lineage cells and *in vitro* experiments demonstrate a reduction in the adhesive properties of leukocytes following activation of this pathway. Evidence indicates that hydroxyurea (HU) may act as a donor of NO *in vivo*; in addition, recent data suggest that the cGMP degrading enzyme, phosphodiesterase 9 (PDE9), may be highly expressed in hematopoietic cells, possibly providing a cell-specific drug target. *Aim.* Since leukocyte adhesion to the vessel wall plays a crucial role in vaso-occlusion initiation, we investigated the effects of the acute administration of HU and a PDE9-inhibiting agent, BAY73-6691, on a humanized model of sickle cell vaso-occlusion. *Methods.* Fully chimeric male sickle cell mice (SCD mice), produced by transplanting the bone marrow of Berkeley SCD mice to lethally irradiated C57BL/6 animals, were utilized for intravital microscopy at 3-5 months post-transplantation. An inflammatory process was induced in mice by TNF-α injection (0.5 μg i.p.); mice were concurrently treated with BAY73-6691 (3 mg/Kg i.v.) or HU (100 mg/Kg i.v.) or both drugs or vehicle. At 2.5h after TNF-α administration, venules of the cremaster muscle were videotaped continuously for 1 min. Leukocyte rolling, adhesion and extravasation were monitored and analyzed for 45 minutes after surgery. *Results.* SCD mice (N=4) treated with hydroxyurea and BAY73-6691 demonstrated increased leukocyte rolling (36.9±8.2; 14.1±3.9 leuk/min, for HU+BAY73-6691 and vehicle control, respectively, $p<0.05$). Reduced leukocyte adhesion to the vessel wall was observed in the treated groups (3.7±0.4; 3.7±0.7; 1.6±0.1; 6.0±0.3 leuk/100μm, for HU; BAY73-6691; HU+BAY73-6691 and vehicle control, respectively, $p<0.0001$; $n \geq 4$). Cell extravasation was also decreased in all three groups (2.0±0.2; 1.1±0.1; 1.3±0.2; 3.2±0.4 leuk/100x50μm^2, for HU; BAY73-6691; HU+BAY73-6691 and vehicle control respectively, $p<0.0001$; $n\geq4$). Additionally, administration of BAY73-6691 or HU+BAY73-6691 decreased RBC-leukocyte interactions (0.3±0.06; 0.2±0.09; 0.7±0.14 RBC-leukocyte interactions/min, for BAY73-6691; HU+BAY73-6691 and vehicle control respectively, $p<0.05$). Surprisingly, the combination of drugs significantly prolonged the survival of SCD mice after TNF-α ($p<0.05$; $n\geq5$). In C57BL/6 mice, where HU and BAY73-6691 also reversed TNF-α-induced alterations in leukocyte parameters, ODQ (guanylate cyclase inhibitor; 15mg/Kg i.v.) was able to revert the effects of HU on leukocyte adhesion (3.2±0.2; 9.4±0.7 leuk/100μm, for HU and ODQ+HU, respectively, $p<0.0001$; $n\geq4$). Similarly, when C57BL/6 mice were treated with KT5823 (protein-kinase-G inhibitor; 1mg/Kg i.v.) and BAY73-6691, the reduction in leukocyte adhesion was reversed (3.3±0.3; 8.8±0.8 leuk/100μm, for BAY73-6691 and KT5823+BAY73-6691, respectively, $p<0.0001$; $n\geq4$). *Conclusions.* These results suggest that drugs that target the NO/cGMP pathway may reduce vaso-occlusive processes and increase survival, at least in SCD mice. Importantly, HU, thought to have NO donating properties, when administered acutely, was seen to significantly alter leukocyte properties in the SCD mouse, demonstrating that this drug could have immediate beneficial effects that are independent of its fetal hemoglobin-elevating properties. Furthermore BAY73-6691, a tissue-specific drug, when combined with HU amplifies the beneficial effects observed on the vaso-occlusion process.

0536

RELATIONSHIPS BETWEEN PLASMA NON-TRANSFERRIN-BOUND IRON AND MARKERS OF IRON OVERLOAD, ANAEMIA AND INEFFECTIVE ERYTHROPOIESIS IN NON-TRANSFUSION-DEPENDENT THALASSAEMIA SYNDROMES

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Background. Patients with forms of non-transfusion-dependent thalassaemia (NTDT), such as β-thalassaemia intermedia, milder forms of haemoglobin (Hb)E/β-thalassaemia and HbH α-thalassaemia, have little or no requirement for transfusions, but may still develop iron overload as a consequence of increased gastrointestinal iron absorption secondary to ineffective erythropoiesis. Factors determining the distribution of iron in NTDT are not completely understood. The prospective, randomized, double-blind, placebo-controlled Phase II clinical trial of deferasirox (THALASSA) enrolled 166 patients with NTDT and iron overload. Laboratory parameters prior to treatment initiation are analyzed. *Aims.* To assess the interrelationships between the key components of the NTDT phenotype, namely anaemia, ineffective erythropoiesis and iron overload. *Methods.* Patients aged ≥10 years with NTDT, liver iron concentration (LIC) ≥5 mg Fe/g dry weight (dw) and serum ferritin (SF) levels >300 ng/mL were enrolled into the study. Exclusion criteria included: anticipated requirements for regular transfusion during the study period, transfusion within 6 months or chelation therapy within 1 month prior to study entry, HbS variants of thalassaemia and impaired renal or liver function. Correlations were assessed by simple linear regression model. *Results.* Of 166 patients randomized, mean age was 32.1 ± 12.0 years and 53.6% were male. Most had β-thalassaemia intermedia (57.2%) or HbE/β-thalassaemia (29.5%), while the remaining 13.3% had HbH α-thalassaemia. 53.0% of patients were splenectomized. Patients generally had received little or no transfusion on a regular basis; 21 patients (12.7%) were not transfused at all. The randomized patients were anaemic (median Hb 8.1, range 4.5-14.0 g/dL) and iron-overloaded; the latter being reflected by increased SF (median 992, range 304-6419 ng/mL), LIC (median 12.1, range 2.6-49.1 mg Fe/g dw), transferrin saturation (TSAT; median 90.8, range 24-100%) and non-transferrin-bound iron (NTBI; median 2.2, range -3.2 to 8.5 μmol/L). There was a significant correlation between SF and LIC, which confirmed previous reports (Taher *et al*. *Haematologica* 2008). The patients showed increased levels of serum erythropoietin (EPO; median 101.0, range 18.3-3405.0 U/L), soluble transferrin receptor (sTfR; median 28.8, range 8.3-64.3 mg/L) and growth differentiation factor 15 (GDF15; median 9179, range 689-53,730 ng/L); the latter two are markers of ineffective erythropoiesis. sTfR showed a significant inverse correlation with Hb and a significant positive correlation with EPO, while GDF15 correlated significantly with sTfR and EPO. NTBI correlated significantly with TSAT, SF and LIC. A weaker but significant correlation was also found between NTBI and sTfR. Statistics for all correlations are presented in the Table. Interrelationships of underlying disease, age and previous therapy with the indices of iron metabolism and erythropoiesis will also be analyzed. *Conclusions.* Non-transfused or infrequently transfused NTDT patients develop significant

Table 1.

Statistics for correlation analyses

Correlation	*R*	*P*-value
SF and LIC	0.64	<0.0001
NTBI and TSAT	0.84	<0.0001
NTBI and SF	0.39	<0.0001
NTBI and LIC	0.35	<0.0001
NTBI and sTfR	0.20	0.0133
sTfR and Hb	–0.48	<0.0001
sTfR and EPO	0.24	0.0021
GDF15 and sTfR	0.32	<0.0001
GDF15 and EPO	0.47	<0.0001

iron overload as assessed by LIC and SF in parallel with increments in TSAT and NTBI, the latter of which is the source of potentially toxic free iron. Increased levels of NTBI result from both significant iron overload and ineffective erythropoiesis, two key components of the NTDT phenotype.

0537

RISK OF ACUTE ISCHAEMIC STROKE (AIS) IN CHILDREN WITH SICKLE CELL DISEASE (SCD) SCREENED WITH TRANSCRANIAL DOPPLER (TCD) PRE- AND POST-STOP PROTOCOL IMPLEMENTATION

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Background. The STOP trial, published in 1998, showed that regular transfusion of children with SCD and abnormal TCD velocities reduced the risk of AIS by about 90%. *Aims.* To assess the long-term results of TCD screening +/- early transfusion of children with abnormal TCD in a non-trial setting. *Methods.* We compared two cohorts of children followed in our network. Cohort 1 children were first screened with TCD between 1991 and 2000. Recomendations for transfusion were made in a joint haematology/neurology specialist clinic on the basis of symptoms and signs of ischaemia. Cohort 2 children were first screened after 1/1/2001 and were routinely offered transfusion for primary AIS prophylaxis if found to have abnormal TCD. All children had a diagnosis of HbSS or HbS/beta thalassaemia. Those with previous stroke were excluded. Scans were done by PT,FK, BK and CA, using non-imaging equipment. Classification of abnormal scan required Vmax>200cms/sec on two occasions. Stroke was diagnosed by standard clinical criteria and confirmed by cerebral MRI. Follow-up for both cohorts continued either until censorship date of 28/2/2011, death or transfer to a different adult clinic at age 16. Kaplan Meier survivorship and Cox proportional Hazards modelling were used for data analysis. *Results.* 537 children had a total of 1548 scans. Cohort 1 consisted of 81, and Cohort 2 of 456 children. Average age at first scan was 7.2 years and did not differ between the two cohorts. The majority of children continued adult follow-up in our unit after the age of 16. Doppler categories at first scan were: Standard 78%; Conditional 12%; Abnormal 3%; Low velocity/asymmetric 1%; inadequate 6%. There was a significant difference in doppler categorization at first scan between the two cohorts, with relatively more conditional scans and less abnormal in Cohort 2. During follow-up, a total of 41 (7.6%) children developed abnormal Doppler. The risk of abnormal Doppler at 5,10, and 15 yrs of age was 2%, 8% and 12%. 5 (6.2%) in Cohort 1 developed abnormal TCD, and only one was transfused prospectively compared to 36 (7.9%) in cohort 2, the majority progressing from conditional to abnormal. 35 of these were transfused prospectively. There were a total of 11 (2%) AIS events, 5 (6%) in Cohort 1 and 6 (1.3%) in Cohort 2. The probability of remaining free of AIS at ages 5,10,15 and 20 years for Cohort 1 100%; 96.3%; 93.7% and 91.9%. For Cohort 2 they were 99.8%; 99.4%; 98.3%; 93.6% (Figure). There was a trend to reduced risk of AIS in Cohort 2, although this did not reach statistical significance (p=0.09) due to the small numbers with AIS. *Conclusions.* We showed a lower percentage developing abnormal TCD than previously described. Transfusion is generally accepted by parents of children with abnormal TCD. AIS rate is only 1.7% at age 15 in those managed according to the STOP protocol, but is not completely prevented. There is some evidence for an advantage in prospective transfusion over transfusion based on clinical assessment in these children.

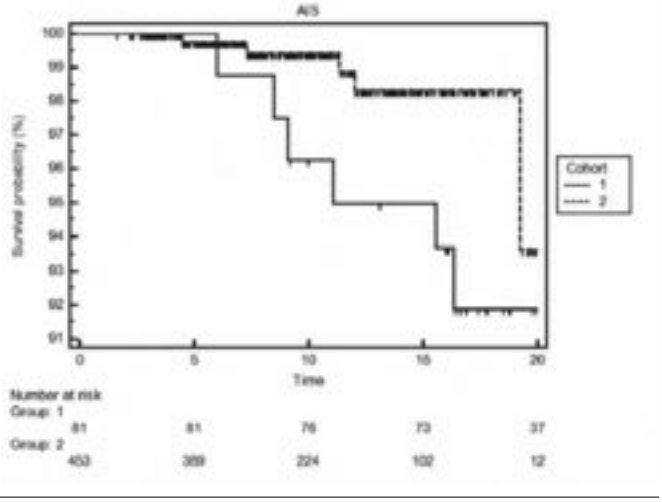

Figure 1. Risk of acute ischaemic stroke.

0538

HFE MUTATIONS ASSOCIATED WITH HIGH LEVEL SPORT PERFORMANCE

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Background. Iron is essential to erythropoiesis and muscular metabolism. High levels sport is associated with a lower iron biodisponibility due to malabsorption, sequestration, or loss excess (sweat, microhemorrhage, hemolysis) that may impair athlete performance. Recently, the increase of hepcidin synthesis during sport training has been shown to lower iron bioavailability. Strong experimental evidence suggests that hemochromatosis associated to HFE mutation is due to a decrease in hepcidin production. Therefore, HFE mutations may counterbalance the decrease of iron avaibility and as such may improve performance in high level athletes. *Aim.* To assess HFE mutations frequency in elite athletes and its impact on sport performance. *Methods.* We performed a prevalence study of HFE gene mutations in sportsmen of four top level French teams, practicing aerobic (Group 1, n=95: nordic ski, rowing), anaerobic (Group 2, n=34: judo), and non energetic (Group 3, n=41: petanque), compared to control subjects, matched by geographic origin, age, and gender (n=219). High level performance can be assessed through the titles and podiums collected during international competitions (continental or world championships and olympic games). In group 1, we compared athletes, who reached the three best places (international podium group: IPG, n=17) to those who did not succeed to reach this level (no international podium group, NPG, n=60). According to the Necker hospital Ethics committee, sport and control subjects have been screened for 18 haemochromatosis linked gene mutation. Test strip containing allele-specific oligonucleotide probes immobilized are realized according to haemochromatosis strip assay kit protocol (Haemochromatosis StripAssay Atm, Vienna Lab Diagnostics GmbH). We used logistic regressions to test the relative frequency of the mutations in athletes compared to the control group, and expressed as odds-ratio. *Results.* Among HFE mutations, H63D was found in 106 cases (27.0%), C282Y in 28 cases (7.2%), S65C in 11 cases (2.8%) and H63H in 1 case only (0.3%). Among them, 92% were heterozygotic. In energetic sports, the frequency of any mutation was superior to the control (Group 1: OR1=1.97, p=0.008, Group 2: OR2=3.85, p=0.003). In contrast, a non significant difference was found in the non energetic Group 3: OR3=1.12, p=NS). In the international podium group, the frequency of any mutation was even larger: among Group 1 athletes, the HFE mutation frequency was 13.3 higher in the subgroup with international titles as compared to the NPG (p=0.0001). The higher frequency of HFE mutations was found in both genders, with a tendency toward higher OR in women (OR1: 3.33 vs 1.37, OR2: 3.92 vs 2.51, ORIPG=17.3 vs 12.6, for women and men respectively). *Conclusion.* This study demonstrates that HFE frequency is significantly larger in French athletes of high energetic sports and strongly correlated with international top performance. During evolution, these genotypes altering a major protein

of iron, red cell and muscle metabolism may have been selected, in the heterozygotic form, due to their large impact on phenotypes ie. energy performance under extreme physiological constraint. Genetic studies in high level sport might reveal such associations.

0539

GLOBIN GENE EXPRESSION IS CORRELATED WITH G PROTEIN-RELATED GENES DURING ERYTHROID DIFFERENTIATION

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The pattern of changes in human globin gene expression during development has been well studied. To study the contributions of other genes and pathways to these processes, we have used oligonucleotide microarray and real-time quantitative PCR technologies to examine gene expression modulation in human erythroid progenitor cells of various tissues during ontogeny. Human hematopoietic CD34+ progenitor cells were isolated from fetal liver (FL), cord blood (CB), adult bone marrow (BM), peripheral blood (PB) and G-CSF stimulated mobilized PB (mPB), and then differentiated *in vitro* into erythroid progenitor cells (EPC) by cocktail of cytokines including erythropoietin. We found that cell growth capacity was most abundant in FL and CB-derived cells. The EPC were sorted as 100% CD71+. During ontogeny, beta-globin gene expression reached maximum levels in cells of adult blood origin (176 fmol per microgram of RNA). For the period of early *in vitro* erythropoiesis culture, gamma-globin gene expression was consistently up-regulated in CB-derived cells (60 fmol). In microarray studies, a total of 3917 genes were persistently expressed in mPB, 3844 in CB, 1770 in BM, 1755 in FL and 1325 genes in PB-derived EPC. A total of 994 common genes were identified in majority of samples of EPC derived from previously mentioned tissues through ontogeny. The common genes related to the hematological system development were further assessed. During gamma-globin induction, we identified G protein-related genes that were activated via the JAK-STAT (SOCS1, HSD3B1), MAP kinase (SERBP1, JUN, MAX) and NO/cGMP (PRPF18) signaling pathways. This study correlates in EPC changes in the expression of globin genes with variations in other genes expression during ontogeny, with the accent on G protein-coupled receptor signaling pathways.

Acute myeloid leukemia - Biology

0540

KNOCK IN OF HUMAN NPM1 MUTATION A BLOCKS MEGAKARYOCYTIC DIFFERENTIATION IN A MOUSE MODEL

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Background. NPM1 mutation (NPMc+), the most frequent genetic alteration in Acute Myeloid Leukemia (AML), accounts for about 30% of all AMLs. Despite the progress in the diagnosis and in the clinical and biological characterization of NPM1-mutated AML, little is known about NPMc+ role in leukemogenesis *in vivo*. Recently, a transgenic mouse model showed NPMc+ was implicated in abnormal myelopoiesis but not progression towards leukemia (Cheng, Sportoletti *et al*., Blood 2010). Thus, more sophisticated gene-targeting approaches are needed to model human NPMc+ AML in mice and to study NPMc+ role in leukemia development *in vivo*. *Aims.* To investigate the *in vivo* role of NPMc+ in hematopoiesis and leukemia we generated a knock-in mouse model that conditionally expresses human NPMc+ cDNA. *Methods.* The model was generated by targeted insertion of human NPMc+ cDNA into the Rosa26 locus via homologous recombination in embryonic stem cells. NPMc+ mutants were crossed with Mx1-Cre transgenic mice to restrict expression of the conditional allele in hematopoietic progenitors. In progeny 1) RT-PCR examined NPMc+ transgene expression; 2) Western blot on cellular fractions determined NPMc+ protein localization using an anti-NPMc+ antibody 3) FACS analysis determined phenotype in bone marrow (BM) cells. *Results.* In NPMc+Mx1Cre+ mice RT-PCR detected NPMc+ transgene mRNA in peripheral blood, spleen and BM cells. After Cre induction transgene expression was maintained for 1-year (latest observation). Western blot detected NPMc+ protein in peripheral blood, spleen and BM cells and demonstrated the NPMc+ mutant localized in the BM cell cytoplasm. Strikingly, compared with NPMc+MxCre- controls, platelet counts were significantly lower while white blood cells counts and hemoglobin levels were not. Platelets were one-half of the control count in mutants harboring one conditional allele and one-quarter in homozygous NPMc+ knock-in mice. To investigate whether the low platelet count was due to abnormal hematopoiesis, 2 months after Cre induction we analyzed BM cells from NPMc+MX1Cre+ mice. Compared with controls, CD41+ cells were double in number ($p<0.05$) while serum TPO levels were identical. Lin-Kit+Sca-1-CD150+CD41+ megakaryocytic progenitors (MkPs) were increased 2-fold ($p<0.001$), suggesting NPMc+ expression leads to an expansion of immature megakaryocytes. Interestingly, immunohistochemistry on BM trephines from patients with NPMc+ AML detected megakaryocyte expansion in some. No significant differences emerged in total BM cellularity, in Lin-Kit+Sca-1+ cells or Lin-Kit+Sca-1-CD41-CD150+FcγR-CD105lo erythromegakaryocytic progenitor cells. The splenic CD41+ cell count and the spleen/body weight ratio were higher ($p<0.05$ for both). Since miR-10a down-regulation was recently hypothesized to unblock target genes involved in megakaryocytic differentiation, we evaluated miR-10a expression in BM cells. Compared with controls, mi-R10a was significantly up-regulated, suggesting the increase in MkPs was related to a differentiation blockage. MiR-10a up-regulation was associated with increased expression of HOXB4 and HOXB5 genes in which cluster miR-10a is embedded. Similar findings have already been described in human NPMc+ AML. There was no leukemic evolution after 1 year follow-up. *Summary.* These results demonstrate that NPMc+ expression impedes megakaryocyte maturation by blocking differentiation. This new mouse model is expected to aid understanding of the molecular and genetic background to NPMc+ AML.

0541

DNMT3A MUTATIONS IN ACUTE MYELOID LEUKEMIA: RESULTS ON 687 PATIENTS TREATED WITHIN THE AML HD98A STUDY OF THE AML STUDY GROUP (AMLSG)

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Background. Alteration of DNA methylation is a hallmark of epigenetic modification in human cancers. A gene family of *DNA methyltransferases* (*DNMT*), *DNMT3A*, *DNMT3B* and *DNMT1B*, catalysing the addition of a methyl group to the cytosine residue of CpG dinucleotides affect promoter methylation status and therefore gene expression. Using a next generation sequencing approach a frameshift mutation of *DNMT3A* has been detected in an AML case. Subsequent sequencing analysis in an independent cohort of 288 AML patients (pts) revealed *DNMT3A* mutations in 22% of the pts; *DNMT3A* mutations were associated with intermediate-risk cytogenetics and poor outcome. *Aims.* To evaluate frequency and clinical impact of *DNMT3A* mutations in younger (16 to 60 years of age) adult AML pts who were treated within the AML HD98A study. *Methods.* *DNMT3A* mutation screening was performed in 687 AML (de novo AML, n=631; s-AML, n=22; t-AML, n=31) using a DNA-based PCR assay for all coding exons (1 to 23) followed by direct sequencing. The median follow-up was 6.46 years. *Results.* *DNMT3A* mutations were found with an overall incidence of 18% (125/687), with two AML exhibiting two mutations. 109 mutations were located in the MTase domain clustering at amino acid R882 (77%). All mutations were heterozygous. *DNMT3A* sequence alterations included 7 frameshift, 3 nonsense and 117 missense mutations. Pts with *DNMT3A* mutations were significantly older (P=.009), had higher white blood cell and platelet counts (P<.001, P<.001, respectively) and higher LDH serum levels (P=.002). There was no correlation with respect to type of AML (P=.21). DNMT3A mutations were significantly associated with cytogenetically normal AML (CN-AML, P<.001), while an inverse correlation was found for AML with t(8;21), inv(16), t(15;17), t(11q23), and complex karyotype (P=.02; P=.009; P<.001; P=.005 and P=.006, respectively). Correlations with other molecular markers (*NPM1*, *CEBPA*, activating FLT3, *IDH1/2* and *TET2* mutation) revealed a significant association with *NPM1* (P<.001), *FLT3*-ITD (P=.01) and *IDH1/2* (P<.001) mutations and in trend an inverse correlation with *CEBPA* mutations (P=.08). *DNMT3A* mutation status did not impact cumulative incidence of relapse (CIR) and overall survival (OS) in the whole AML cohort (P=.49; P=.28, respectively) as well as in the subgroup of CN-AML (P=.78; P=.65, respectively). We next performed subgroup analysis in CN-AML according to the *NPM1*/*FLT3*-ITD genotypes. *DNMT3A* mutations had no impact in pts with the genotype *NPM1*-mutated/*FLT3*-ITD-negative [CIR (P=.99); OS (P=.56)], while in pts with all other genotypes (*NPM1*-wildtype/*FLT3*-ITD-positive, *NPM1*-wildtype/*FLT3*-ITD-negative, *NPM1*-mutated/*FLT3*-ITD-positive) *DNMT3A* mutations were associated with inferior OS and in trend inferior CIR (P=.01, and P=.06, respectively). *Conclusions.* We confirm that *DNMT3A* mutations are frequent genetic aberrations in AML, associated with normal karyotype, and *NPM1*, *FLT3*-ITD and *IDH* mutations. Our data suggest a negative prognostic impact in molecular high-risk CN-AML, however, these data need to be validated in larger patient cohorts.

0542

MODELING RUNX1 BIALLELIC MUTATIONS ASSOCIATED WITH AML-M0 AND AML-FPD IN MICE REVEALS IMPORTANCE OF RESIDUAL RUNX1 FUNCTION

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Disruption of the RUNX1 gene is one of the most common aberrations found in myeloid leukemia. The most common disruptions are chromosomal translocations, but intragenic mutations are found at a high incidence in minimally differentiated AML (AML-M0) and in AML secondary to familial platelet disorder (AML-FPD). The majority of these mutations impact the conserved Runt domain, specifically disrupting the DNA-binding (DB) interface, while other mutations are predicted to be null alleles, due to complete deletion or frameshifts leading to premature stop codons. Interestingly, although monoallelic RUNX1 germline mutations have been reported in 27/28 families reported with FDP, recent evidence demonstrates that patients developing AML have acquired mutations in the second RUNX1 allele. Similarly, the AML-M0 subtype also shows a high incidence of biallelic RUNX1 mutations (circa 25%). In both cases, trisomy 13 (correlating with high FLT3 expression) or FLT3 activating mutations (FLT3-ITD) are common secondary mutations. Earlier work by us and others have shown that either functional inactivation of the Runx1 gene or expression of DB-RUNX1 mutants leads to increased self-renewal capacity of myeloid progenitors *in vitro*. We thus predicted that either null or DB mutations may disrupt a critical 'gatekeeper' function of this gene, permitting the accumulation of secondary mutations (e.g. FLT3 activation) that lead to an overt leukemia. In a study to test our hypothesis, constitutively active FLT3-ITD alone or together with RUNX1-DB was introduced into hematopoietic progenitors from Runx1 deficient, heterozygous, or wildtype mice. These studies showed that in the C57Bl/6 mouse background, activated FLT3-ITD most readily induces a T-cell thymoma. Neither Runx1 inactivation nor coexpression of RUNX1-DB shifted the disease spectrum to myeloid neoplasia. However, together, FLT3-ITD, Runx1 deletion, and RUNX1-DB led to a rapid and fatal myeloid disorder with left-shifted myelopoiesis. These results support a tumor suppressor function for wildtype RUNX1 - but indicate that RUNX1 DB mutants have retained important Runx1 oncogenic activity, which cooperates with loss of wild-type Runx1 in disease progression. Important signaling pathways differentially regulated by wildtype and DB RUNX1 will be discussed. These results help explain the significance of the specific mutations in the DNA-binding interface and the high incidence of biallelic mutations in AML-M0 and AML-FPD.

0543

OVEREXPRESSION OF SET IS A RECURRENT EVENT ASSOCIATED WITH POOR OUTCOME THAT CONTRIBUTES TO PP2A INHIBITION IN ACUTE MYELOID LEUKEMIA

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Protein phosphatase 2A (PP2A) is a tumor suppressor reported as a potential therapeutic target in chronic and acute leukemias. The protein SET (I2PP2A/TAF-Iβ), a potent PP2A inhibitor, has been implicated in many cell processes and signaling pathways. Moreover, SET has been described as an oncogene that is overexpressed in several neoplasms, including chronic myeloid leukemia, where it correlates with the activity of BCR/ABL, leading to PP2A inhibition. We previously described how SETBP1 in AML cells protects SET from protease cleavage, leading to PP2A inhibition, Moreover, we observed a recurrent PP2A inactivation in AML, and noted that PP2A activation impairs cell proliferation in AML cells. In addition, we postulated that SET deregulation could be one mechanism contributing to the inhibition of PP2A in AML. In order to study whether SET was deregulated in AML, we first analyzed its levels in 13 AML cell lines, observing SET overexpression at both mRNA and protein levels. Prevalence of SET overexpression in AML patients at diagnosis was 28% (60/214), and was associated with SETBP1 ($p<0.01$) and EVI1 overexpression ($p=0.02$). Interestingly, patients with SET overexpression had worse overall survival ($p<0.01$) and event free survival ($p<0.01$). We next confirmed SET overexpression at protein level in a series of 16 patients with AML at diagnosis. We found increased levels of SET protein in 9 out of 16 cases (56.2%), although only 6 of these cases had SET overexpression by real-time RT-PCR (QRT-PCR), which indicates that SET overexpression is a recurrent event in AML that could be underestimated by QRT-PCR. It has been demonstrated that SET upregulation, and the resulting PP2A inhibition, is critical in BCR/ABL-positive cells to fulfill its tumorigenic potential. Analysis by MTS assay showed that ectopic expression of SET restores proliferation in AML cells ectopically expressing PP2A. When we investigated the molecular mechanisms involved in SET deregulation in AML, we observed that activation of PP2A leads to reduced SET levels. Therefore, we postulated that the inhibited status of PP2A could contribute to deregulate SET in AML cells. Moreover, analysis of the SET proximal promoter identified hypothetical binding-sites for transcription factors such as AP-1, GATA1, and EVI1. In our series of AML patients, SET and EVI1 overexpression were associated, suggesting that EVI1 could regulate SET. ChIP showed that EVI1 binds the SET promoter; however, we detected no differences in the luciferase assay, sug-

gesting that EVI1 could regulate SET indirectly. In summary, we demonstrate that SET overexpression is a recurrent molecular event associated with poor outcome in AML, which promotes cell proliferation and restores the reduced cell viability induced after PP2A overexpression. Moreover, PP2A activation status could be involved in the regulation of SET. Altogether, SET overexpression could differentiate a subgroup of patients with poor prognosis who could be treated with PP2A activators in future clinical trials. e-mail address: icristobal@alumni.unav.es

0544

SLEEPING BEAUTY DRIVEN LEUKAEMOGENESIS FOLLOWS AN ACCELERATED DARWINIAN-LIKE EVOLUTION IN A MOUSE MODEL OF NPM1C+ ACUTE MYELOID LEUKAEMIA

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Heterozygous somatic mutations in NPM1, the gene for Nucleophosmin, are the commonest group of mutations in acute myeloid leukaemia (AML). We recently described a humanised mouse model of these mutations, in which one third of mice with conditional activation of a humanised Npm1c knock-in allele, Npm1flox-cA, developed late-onset AML. The same model was subjected to insertional mutagenesis with Sleeping Beauty, through the mobilisation of 80 copies of the novel transposon GrOnc, from a resident locus on mouse chromosome 19. Rapid onset AML developed in 80% of these mice in association with recurrent insertions in many known and novel leukaemia genes including Csf2, Flt3 and Nup98 (Vassiliou *et al*, Nature Genetics 2011 - In Press). Here we report the findings from a similar but novel model mobilising 15 copies of GrOnc from mouse chromosome 16 (Figure 1a). Common integration sites (CISs) from the analysis of 40 leukaemias showed a striking overlap with CISs identified in the aforementioned study, confirming the strong cooperativity of such insertions with Npm1c (Figure 1b). To understand the molecular basis of leukaemogenesis in this model, fortnightly blood samples were taken from 12 of these mice from the time of activation of the SB transposase (via Mx1-Cre) to the onset of frank leukaemia. Additionally, single-cell derived leukaemic blast colonies were generated and analysed. Leukaemia onset was sudden and could not be predicted in advance from FBC parameters (Figure 1c). A subset of transposon integrations were found to occur early and persist over several months during leukaemia development (Figure 1d). Large numbers of transposon integrations (50-200) were identified within each tumour, however only a small number of these were common to multiple single cell colonies generated from the same tumour. Similarly only a few integrations persisted on serial transplantation of tumour cells. These results reveal that transposon mobilisation continues throughout leukaemia evolution leading to the development of multiple sub-clones within these neoplasms. Our data suggest that only a subset of the integrations identified in leukaemia samples behave as "driver"mutations, whilst most insertions are "passengers". Continued mobilisation of transposons from these passenger sites occurs without loss of proliferative potential. By contrast, mobilisation of driver insertions in host cells is immediately selected against. Our findings validate the critical pathways able to cooperate with Npm1c in leukaemogenesis, whilst also giving important novel insights into the way Sleeping Beauty operates in carcinogenesis and highlighting critical differences to retroviral mutagenesis. We are currently applying these insights to improve recognition of leukaemic "drivers"and to develop novel applications, such as the identification of genetic pathways able to overcome inhibition of leukaemic growth by anti-leukaemic drugs.

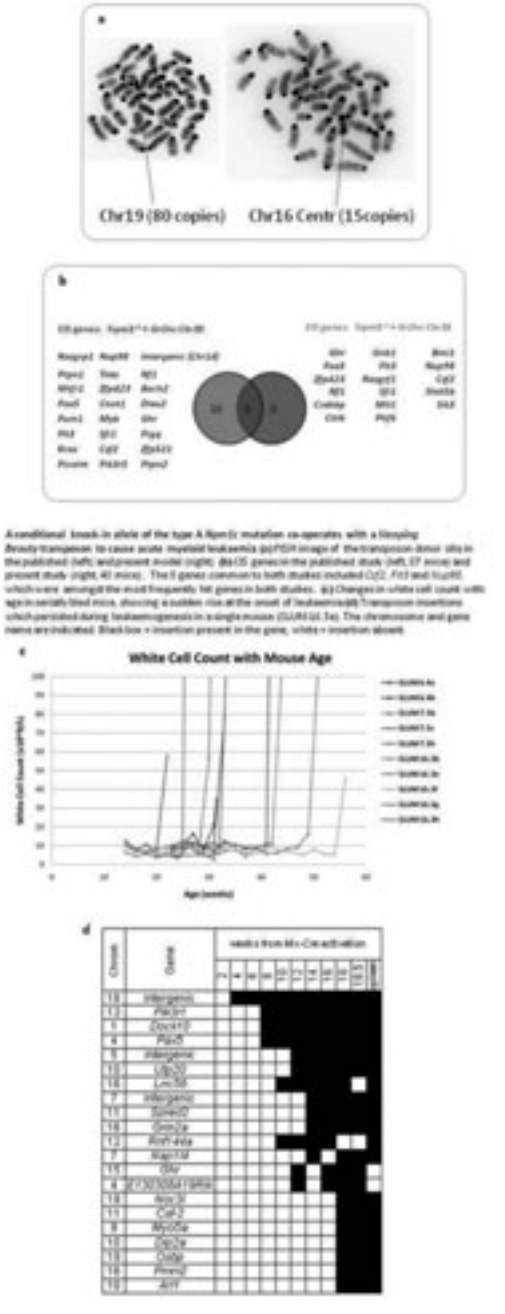

Figure 1.

Stem cell transplantation - Clinical 1

0545

TREATMENT OUTCOMES IN MULTIPLE SCLEROSIS PATIENTS AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION: ANALYSIS OF 191 PATIENTS

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During the last decade high-dose immunosuppressive therapy with autologous hematopoietic stem cell transplantation (HDIT+AHSCT) has been used with increasing frequency as a therapeutic option for MS patients. The major treatment outcomes for MS patients are disease-progressive free period and improvement of patient's quality of life (QoL). We aimed to study safety and treatment outcomes in MS patients after HDIT+AHSCT. 191 patients with MS (secondary progressive - 84, primary progressive - 28, progressive-relapsing - 5 and relapsing-remitting - 74) from 6 clinical centers were included in this study. Mean age - 34.0, range: 17-55; male/female - 78/113. BEAM or BEAM-like conditioning regimens were used in 182 patients, and Fludara - in 9 patients. Median EDSS at base-line was 4.5 (range 1.5 - 8.5). The median follow-up duration was 29 months (range 1.5 - 128). QoL was assessed using the generic questionnaire SF-36. QoL assessment and neurological examination were performed at baseline, at discharge, at 3, 6, 9, 12 months, and every 6 months thereafter. For treatment outcomes clinical response and QoL response rates were evaluated at 6, 12, 24 months after HDIT+AHSCT and at longer-term follow-up. QoL treatment response was classified as improvement, stabilization or worsening. In the vast majority of cases the mobilization and transplantation procedures were well tolerated. One case of death at early posttransplant period was registered - a female patient died on day +8 from sepsis with multiple organ failure. The efficacy analysis was performed in 121 patients who had follow-up for at least 12 months (median follow-up duration - 36.5 months). At long-term follow-up overall treatment response in terms of neurological improvement (n=49) or stabilization (n=46) was observed in 79% of patients. 26 (21%) patients progressed at different time-points after AHSCT. One patient died after disease progression at 3 years posttransplant. QoL treatment response was evaluated in 73 patients. At 6 months after HDIT+AHSCT significant improvement of all the scales of SF-36 except pain and role emotional functioning as compared with base-line was demonstrated ($p<0.05$). At longer term follow-up further QoL improvement was found. At 6 months post-transplant QoL improvement was achieved in 52% of patients, QoL stabilization - in 36%, and QoL worsening - in 12% of patients. At 12 months post-transplant the vast majority of patients (92%) were either stable or improved. At long-term follow-up QoL improvement was registered in 44%, QoL stabilization - in 40% of patients, and QoL worsening - in 16% of patients. This study provides ample evidence in support of HDCT+AHSCT efficacy in MS patients both in terms of clinical and QoL response rates. Further studies should be done to investigate clinical and patient-reported outcomes of HDCT+ASCT in MS patients to better define treatment results.

0546

HIGH-DOSE RITUXIMAB IN THE CONDITIONING REGIMEN BEFORE ALLOGENEIC STEM CELL TRANSPLANTATION REDUCES THE INCIDENCE OF ACUTE GVHD IN B-CELL LYMPHOMAS

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Background. Allogeneic stem cell transplantation (alloSCT) with a reduced-intensity conditioning (RIC) is an effective salvage therapy for relapsed lymphomas. However, in the last GITMO RIC-alloSCT trial we observed an incidence of grade II-IV acute graft-versus-host disease (GVHD) of 35% (Leukemia 2007). The present GITMO study is a prospective multicenter phase II trial designed for patients affected by CD20 positive lymphomas. It increases the thiotepa dose of 20%, and incorporates high-dose Rituximab (R) in a RIC regimen to improve the outcome and possibly modulate the incidence of acute GVHD. *Aims.* Primary end-point was 1-year progression-free survival; secondary endpoints were non-relapse mortality and incidence of acute and chronic GVHD. *Methods.* Fifty-two patients were enrolled so far in the study and 34 are evaluable for preliminary analysis. Treatment plan consisted of high-dose R (500 mg/m2 on day -6) followed by a RIC regimen containing thiotepa (12 mg/kg), fludarabine (60 mg/kg) and cyclophosphamide (60 mg/kg). Graft-versus-host disease (GVHD) prophylaxis included cyclosporine and mini-methotrexate; ATG was added to the patients allografted from class I antigen mismatched sibling or unrelated donors. Histopathological subtypes included aggressive (n=11 diffuse large B-cell lymphomas, n=5 mantle cell lymphomas) and indolent lymphomas (n=10 follicular lymphomas, n=8 small lymphocytic/chronic lymphocytic leukemia). Patients were allografted from matched related siblings (n=23) or alternative donors (n=11). All the patients had chemosensitive disease (38% in complete remission) and 16 (47%) failed a previous autoSCT. *Results.* The median follow-up is 1 year (range, 180-1000 days). The cumulative incidence (CI) of non-relapse mortality (NRM) was 9% at 1 year. In total only 5 of 34 patients had acute GVHD (n=4 grade II, n=1 grade III) with an estimated CI of 17% at 100 days. Only 25 patients are evaluable for chronic GVHD with an estimated CI of 41% at 1 year (n=8 limited, n=2 extensive). Infections after engraftment requiring hospitalization or intravenous treatment occurred in 15 patients (44%). Preliminary data on immune-reconstitution showed absence of circulating CD19 B cells at 6 months after allograft. The CI of relapse was 19% and 31% at 6 months and 1 year, respectively. In the indolent and aggressive groups, OS estimates were 88% (95%CI, 60% to 97%) and 59%(95CI, 31% to 78%) and PFS estimates were 63% (95%CI, 32% to 83%) and 55% (95% CI, 28% to 75%), respectively. *Conclusions.* The present data suggest that the administration of high-dose R is feasible and causes an unexpected reduction of the incidence of acute GVHD (only 1 case had GVHD grade III) without increasing the NRM and the incidence of severe infections complications. Complete data evaluating the effects of R on immune reconstitution are ongoing.

0547

IMMUNE-ABLATIVE REGIMEN FOLLOWED BY AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR NEWLY-DIAGNOSED TYPE I DIABETIS

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Background. Patients with Type one diabetis mellitus (DM) is mostly juvenile, insulin-dependent and associated with auto-immune-mediated in nature. *Aim.* To determine the safety and efficacy of immune-ablative regimen followed by autologous hematopoietic stem cell transplantation (Auto-HSCT) in newly-onset type 1 DM patients. *Methods.* We conducted a prospective clincial trial in newly-diagnosed type 1 DM patients(NCT00807651). All patients received cyclophosphamide (200mg/kg) and ATG (4.5mg/kg) followed by infusion of autologous mobilized peripheral hematopoietic stem cells. Monitoring of serum hemoglobin A1c, C-peptide levels and anti-glutamic acid decarboxylase antibody (GAD) titers was carried out before and after auto-HSCT. *Results.* A total of 18 patients were enrolled with a median average age of 18 (range, 15~23). The median follow-up was 414 days (range, 140~750). Among these patients, 12 (67%) patients achieved full stop of insulin with a median of 6 weeks (range 2~21) after HSCT. Four cases eventually resumed the insulin therapy all triggered by mild illness (common cold). With the last follow-up, 44.4% (8/18) remained free of insulin therapy, and the other patients acheived reduction of insulin dose on an average of 67.3% ± 22.4%. All 18 patients achieved a singificant decrease of GAD level and among them 6 (33.3%) became negative. Fasting C peptide and postprandial 2 hour C peptide levels increased significantly after HSCT and the C peptide area under the curve (AUCC) increased remarkably and can maintain for more than 1 year. During the transplantation treatment process, all patients had varying degrees of gastrointestinal reactions, hair loss, fever and bone marrow suppression. Five patients must received supportive blood transfusion. No severe adverse event involving the heart, liver, kidney and other or-

gans was documented. *Conclusion.* AutoHSCT for the treatment of newly-onset type 1 diabetes is feasible and effecitve. Large scale clinical trial with long-term followed-up are warranted.

0548

PLERIXAFOR (MOZOBIL) AND G-CSF FOR FRONT-LINE PERIPHERAL BLOOD STEM CELL MOBILISATION FOR AUTOLOGOUS TRANSPLANTATION IN LYMPHOMA OR MULTIPLE MYELOMA: PRELIMINARY RESULTS FROM THE EU PREDICT STUDY

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Background. In Europe, plerixafor + G CSF is approved for mobilisation of haematopoietic stem cells (HSC) for autologous HSC transplantation (auto-HSCT) in patients with lymphoma and multiple myeloma (MM) whose cells mobilise poorly. *Aims.* We report preliminary results from the PREDICT study, a multicentre, open label, single arm study conducted in Europe to further assess the safety and efficacy of plerixafor + G-CSF for front-line mobilisation in patients with lymphoma or MM. *Methods.* Adult patients with non Hodgkin's lymphoma (NHL), Hodgkin's disease (HD), or MM who required auto-HSCT, in first or second complete or partial response were eligible; those with prior allogeneic or >1 prior auto-HSCT were excluded. All patients provided informed consent. Patients received G CSF (10µg/kg/day) subcutaneously (SQ) for 4 days; on the evening of Day 4 they received plerixafor (0.24 mg/kg SQ). Patients underwent apheresis on Day 5 after an AM dose of G CSF, 10-11 hours after plerixafor. Plerixafor, G CSF and apheresis were continued for up to 5 days. The primary study objective was to confirm the safety of mobilisation with plerixafor. Secondary objectives included assessment of efficacy (apheresis yield; time to engraftment). *Results.* 118 patients (MM=90; NHL=25; HD=3) were mobilized with plerixafor + G-CSF (Table 1; data for HD patients not included). Treatment-emergent plerixafor-related adverse events (AEs) occurring from the first G-CSF dose up to 30 days after the last plerixafor dose or the first dose of myeloablative chemotherapy, whichever occurred first (period 1) were reported in 18 pts. Most AEs occurred within 1 hour post-injection, were grade 1 or 2 in severity and included gastrointestinal disorders or injection-site reactions. There were three grade 3 related AEs: myocardial infarction (MI; occurred 19 days after first plerixafor dose; n=1), injection-site reaction (n=1) and asymptomatic leucocytosis (n=1; baseline WBC=8.9x 10^9/L; WBC on apheresis Day1=71.4x10^9/L). 3 patients reported drug-related serious AEs in period 1: disease recurrence, hypomagnesaemia (occurred 2 days post-apheresis) and the abovementioned MI. At a median follow-up of 1 year, there were 4 unrelated deaths due to relapse (n=1), liver failure likely due to disease relapse (n=1), cardiorespiratory failure (n=1) and sepsis (n=1). The minimum cell yield (≥2x10^6 CD34+ cells/kg) was harvested in 98% MM and 80% NHL patients in a median of 1 apheresis. The optimum cell dose (≥5x10^6 CD34+ cells/kg) was harvested in 92% MM and 48% NHL patients (few NHL patients close to collecting 5x10^6 cells elected not to complete all protocol-specified aphereses). Eighty-four (93%) MM and 19 (76%) NHL patients were transplanted. All patients with available data (75 MM and 17 NHL patients) engrafted; median time to neutrophil and platelet engraftment was 14 and 18 days in MM patients and 17 and 19 days in NHL patients. *Conclusions.* In this prospective, multi-center European study, mobilisation with plerixafor + G-CSF allowed the majority of patients with MM or NHL to undergo transplant with minimal toxicity, providing further data supporting the safety and efficacy of plerixafor + G-CSF for front-line mobilisation in patients with NHL or MM.

Table 1. Patient characteristics and outcomes.

	MM (n=90)	NHL (n=25)
Median age (range)	60 (39-71)	58 (29-68)
Gender, male (%)	48 (53)	18 (71)
No. of patients who had undergone prior auto-HSCT (%)	5 (5.5)	0 (0)
Median CD34+ cells/kg x 10^6 collected (range)	7.6 (1.5-24)	5.2 (0.2-16.7)
No. of patients collecting ≥2 x 10^6 CD34+ cells/kg (%)	88 (98)	20 (80)
Median days to collect ≥2 x 10^6 CD34+ cells/kg (range)	1 (1-3)	1 (1-3)
No. of patients collecting ≥5 x 10^6 CD34+ cells/kg (%)	83 (92)	12 (48)
Median days to collect ≥5 x 10^6 CD34+ cells/kg (range)	1 (1-4)	3 (1-3)
No. of patients proceeding to transplant (%)	84 (93)*	19 (76)†
Median CD34+ cells/kg x 10^6 transplanted (range)	3.9 (0.8-9.2)	3.5 (2.0-7.8)
Median days to neutrophil engraftment (range)‡	14 (9-61)	17 (10-60)
Median days to platelet engraftment (range)‡	18 (3-61)	19 (11-33)
Patients with plerixafor-related adverse events§, n (%)	13 (14)	5 (20)
Patients with serious adverse events§, n (%)	2 (2)	1 (4)

*4 patients collected the minimal dose but did not proceed to transplant due to myocardial infarction, death, disease relapse, or patient was disease-free and transplant was not done
†Of the 6 patients not proceeding to transplant, 5 did not collect the minimal cell dose and 1 patient had borderline cell yield and was not transplanted per site standard operating procedure
‡Based on data available from 75 MM and 17 NHL
§Occurring from first dose of G-CSF up to 30 days after last dose of plerixafor or first dose of myeloablative chemotherapy, whichever occurs first

0549

AZACITIDINE CAN PREVENT OR DELAY RELAPSE OF PATIENTS WITH MDS OR AML AND MINIMAL RESIDUAL DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: FINAL RESULTS OF THE RELAZA1 TRIAL

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Background. Relapse is one of the major challenges in the care of patients (pts) with MDS or AML undergoing allogeneic hematopoietic stem cell transplantation (HSCT). However, relapse can be predicted by monitoring minimal residual disease (MRD). In case of CD34-expression on the leukemic clone, a sensitive chimerism analysis in sorted CD34+ peripheral blood (PB) cells correlates with MRD levels. If CD34+ donor cells drop below 80%, relapse is almost inevitable within a median of 60 days even in the presence of interventions like immediate cessation of immunosuppression or the administration of DLI. However, both approaches often result in clinically significant GVHD. *Aims.* The aim of this prospective phase II clinical trial was to evaluate the efficacy of azacitidine (AZA) to treat MRD as defined by a decreasing CD34-donor chimerism, and thus prevent hematological relapse in pts with CD34+ AML or MDS after allogeneic HSCT. *Methods.* A total of 59 pts with CD34+ MDS (n=5) or AML (n=54) were prospectively screened after HSCT for a decreasing chimerism of donor CD34+ cells. In case of increasing MRD heralding imminent relapse, AZA was given at a dose of 75mg/m^2/d s.c. day 1-7. A total of 4 cycles every 28 days were allowed. Pts showing either insufficient CD34-chimerism response, i.e. an increase but below 80%, or again decreased below the cut-off were eligible for a second treatment phase. *Results.* At a median of 169 days after HSCT, 20 out of 59 pts screened entered the treatment phase of the study with a median of 25% (range 0-79%) CD34+ donor cells in the PB. However, a complete overall and T-cell donor chimerism in the PB as well as less than 5% marrow blasts were documented in all pts before AZA treatment. Median age of the treated population was 58 years (20 - 74 years). Two out of 20 pts had already undergone a 2nd transplant because of relapse. During preemptive AZA treatment reversible neutropenia grade 3/4 occurred in 80% of the pts whereas thrombocytopenia grade 3/4 was observed in 65% of them. Four out of 20 pts (20%) relapsed during the first 4 cycles (between cycle 2 and 4). Of the remaining pts, ten (50%) showed a complete clearance of MRD which was sustained in four of them. In an additional six pts (30%) MRD was stable during the first 4 cycles in the absence of hematological relapse. The latter as well as those pts with later drop of CD34+ DC <80% after initial response were eligible for subsequent AZA cycles. In fact, 11 pts (55%) received a median of 4 (range 1-11) additional cycles. Overall, hematological relapse occurred in a total of 13 pts (65%) but was prolonged by a median of 6 months compared to our previous experiences with DLI or cessation of immunosuppression only. *Summary/Conclusions.* Preemptive treatment of MRD with AZA can prevent or at least delay hematological relapse in pts with advanced MDS or AML and MRD after allogeneic HSCT.

POSTER SESSION II

Acute lymphoblastic leukemia - Clinical

0550

IN ACUTE LYMPHOBLASTIC LEUKEMIA AN MRD-GUIDED STRATEGY IMPROVES RISK STRATIFICATION, ABROGATES REMISSION MORTALITY IN MRD-NEGATIVE GROUP AND CONFIRMS THE ROLE OF TRANSPLANTATION IN MRD-POSITIVE GROUP

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Background/Aims. Clinical risk stratification fails to predict relapse in many patients with ALL (acute lymphoblastic leukemia), causing undertreatment with chemotherapy and overtreatment with stem cell transplantation (SCT) in trials with a risk-oriented design. We used MRD (minimal residual disease) as leading predictive factor for relapse and key decisional tool for allocation to SCT or chemotherapy (Blood 2009;113:4153). Here we provide the final study update in a larger cohort of patients, excluding Ph+/t(4;11)+ ALL (eligible to SCT regardless of MRD). *Methods.* Details of induction/consolidation and molecular MRD study were reported. Risk class was defined standard (SR-B: pre-B, WBC <30; SR-T: cortical T, WBC <100) or high (HR, others). Bone marrow MRD was assessed after chemotherapy blocks 3,5,7 (weeks 10,16,22) using 1 or 2 patient-specific probe(s) with sensitivity >10-4. MRD negativity (Mneg) was defined by w16 levels <10-4 and negative w22. Postconsolidation was maintenance in Mneg, SCT (family-related/unrelated) in Mpos, autologous stem cell-supported hypercycles (H/C) plus maintenance in Mpos without donor, or was driven by risk class when missing MRD analysis. *Results.* 237 out of 278 patients (median age 35 years [range 16-68], SR 138, HR 129, unknown 11) entered CR (85%), and 179 (75.5%) had at least one sensitive probe. 178 completed consolidation (75.1%) while 94 did not because of relapse (15.6%), early SCT (6.7%) and toxicity. Median and 5-year survival rates of CR patients were 3.1 years and 45%. 134 patients completed the MRD study (75.2%). 75 (56%) were MRDneg with no difference among SR-B (59%), HR-B (51%), SR-T (64%), HR-T (47%). MRD analysis reclassified 40% of SR patients (30/75 Mpos) and 50% of HR patients (28/56 Mneg). Most MRD-studied patients received the planned chemotherapy (69/75 Mneg, 92%) and SCT or H/C (42/59 Mpos, 71%). By treatment intention, incidence of relapse (any site), treatment-related mortality (TRM), and 5-year survival and disease-free survival (DFS) rates were 33%, 0%, 76%, 65% in Mneg patients, compared to 58%, 19%, 33%, 22% in Mpos patients (all P's=0.0000). In Mneg group, relapse rate was unrelated to number of probes (one vs. two, P=0.46) and initial risk classification (SR vs. HR, P=0.53). In Mpos group, relapse rate was lower in patients receiving H/C therapy (10/17, 58%) or allogeneic SCT (8/25, 32%, P=0.026 vs. H/C) compared to others (16/17, 94%, P=0.000). Moreover, 5-year survival was improved after H/C or SCT, from 12% in 17 untreated patients to 41% (n=42, P=0.005), and finally allogeneic SCT proved superior to H/C therapy (survival 49% vs. 20% [P=0.06], DFS 42% vs. 14% [P=0.032]). *Conclusions.* The simplified two-step approach chosen for MRD analysis in this study (w16 and w22, from middle to end of consolidation) led to refine risk classification in 40-50% of patients, identified the majority of those curable by chemotherapy without TRM and independently of risk class and probe number (Mneg), and documented the substantial relevance of allogeneic SCT against chemoresistant ALL (MRDpos). Being at moderate risk of relapse, MRDneg patients must be monitored closely during maintenance.

0551

DESIGN AND VALIDATION OF A PROGNOSTIC INDEX SCORE FOR PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) BASED ON CLINICAL AND EPIGENETIC DATA

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Introduction. Despite major improvements achieved in the treatment of childhood ALL, significant challenges still remain. Traditional risk factors have proven to be important in predicting patient outcome, but more than half of dismal events still occur in non high risk patients. If identified early, these patients may benefit from an intensified treatment. However, current strategies fail to identify these patients and indicate the need for new prognostic markers. *Material and Methods.* We analyzed 245 patients with de novo ALL divided into training and validation cohorts. The training cohort consisted of 165 patients from the PETHEMA Spanish group. The validation cohort comprised 80 patients from SHOP Spanish group trials. Protocols were approved by the IRB of the involved institutions and written informed consent was provided. We studied the methylation profile of 50 genes (36 cancer-related genes and 14 microRNAs) belonging to pathways involved in cell transformation in the training cohort by MSP (Methylation-specific PCR) in order to design a Prognosis Index Score (PIS). Univariate and multivariable analyses were performed using the Cox proportion hazards model. The multivariable analyses were undertaken with both forward and backward stepwise procedures for identifying the independent prognostic variables. The model results were used to define levels of risk for survival. *Results.* Promoter methylation of 27 genes was found in at least 20% of the patients, and these genes were selected for further analysis. In the univariate analysis, methylation of 16 genes was associated ($P \leq 0.1$) with shorter OS. In multivariate analysis, methylation of 6/16 genes remained as independent predictors of OS (hsa-mir-124-1, hsa-mir-196-2, Wnt5a, Reprimo, Wif1 and Lats1). ALL patients were classified into two different methylation phenotype (MP) groups: MP-negative (no methylated genes) and MP-positive (at least, one methylated gene). In addition, univariate analysis revealed four clinical variables (WBC count, presence of TEL-AML1, age at diagnosis and immunological phenotype) and the MP that were significantly associated with OS. However, multivariate analysis showed that only three of these variables were independently associated with survival (age, MP and immunophenotype) and they were used to design a model to predict an individual patient's risk of OS. The index score was defined as the sum of the number of risk factors present with each risk factor receiving a value of 1 except for MP status, which was scored as 2 for MP-positive patients. Patients could receive a score from 0 to 4 and they could be grouped as low risk (score 0-1, n=46), intermediate risk (score 2, n=72), high risk (score 3, n=39) or very high risk (score 4, n=8). The mean survivals for these groups were 202.6, 150.2, 80.3 and 33.3 months, respectively, (P<0.0001). These four groups also showed distinctive differences in, relapse rate, mortality rate and DFS. Results were validated in an independent cohort of 80 patients. *Conclusion.* We have created a new prognostic score for ALL children that integrates for the first time, traditional parameters and epigenetic data. Our scoring system stratifies patients into 4 groups at very different risk of death after treatment.

0552

CLINICAL ACTIVITY OF THE ANTI-CD19 BITE BLINATUMOMAB IN PATIENTS WITH RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): INTERIM RESULTS OF A PHASE II STUDY

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Background. Relapsed/refractory B-precursor ALL is an aggressive malignant disease with a dismal prognosis and therapy is an unmet medical need. 80-90% of adults with relapsed ALL die from their disease or adverse effects of therapy. CD19 is the most frequently expressed B-cell differentiation antigen and can be targeted by blinatumomab, a member of a novel class of T-cell engaging, bispecific single-chain antibodies called BiTE antibodies. We initiated an open-label multi-centre exploratory Phase 2 study in collaboration with the German Multicenter Study Group for Adult Lymphoblastic Leukemia (GMALL) in order to determine the efficacy and safety of blinatumomab in adult patients with relapsed/refractory B-precursor ALL. *Methods.* The primary endpoint is complete remission (CR) rate with hematological recovery. Secondary end-points are minimal residual disease (MRD) response rate (defined by an MRD level below the quantitative detection limit of 10e-4), time to hematological relapse and overall survival. Eligible patients must have B-precursor ALL relapsed after at least induction and consolidation or primary refractory disease. Prior allogeneic HSCT is permitted. Patients with Ph-positive ALL have to be ineligible for tyrosine kinase inhibitors. Blinatumomab is administered as a 4-week continuous intravenous infusion followed by a 2-week treatment-free period. Responders may receive up to 3 additional cycles. The first cohort of five evaluable patients received a dose of 15 μg/m²/d. A second cohort receives 5 μg/m²/d for the first 7 days of the first cycle followed by 15 μg/m²/d for the remaining 3 weeks of the cycle and the following cycles. A risk-benefit assessment will determine which of the dose levels will be evaluated in the second stage enrolling 10 additional patients. *Results.* Seven patients have been treated in the first cohort. Their age ranged from 18 to 77 years. Four of the five evaluable patients had a reduction of bone marrow blasts < 5% within the first cycle two with CR and two with a complete remission with only partial hematologic recovery (CRh*). To date, three also have an elimination of MRD below quantitative detection limit within the first 2 cycles. One responder had an extra-medullary relapse during the third cycle of treatment. The most common adverse events were fever and chills. Two patients were not evaluable for response assessment as they had to permanently discontinue treatment without completion of the first cycle due to adverse events. One non evaluable patient with high leukemic burden had a completely reversible SAE of cytokine release syndrome (CRS). Subsequent patients with high leukemia burden were managed by pre-treatment with dexamethasone and/or cyclophosphamide, and no further treatment discontinuations due to CRS were observed. The second non evaluable patient had treatment discontinued due to a completely reversible CNS event of encephalopathy and disorientation. Despite a limited course of treatment, this patient showed an elimination of MRD below quantitative detection limit. Recruitment of the second cohort receiving a low initial dose of blinatumumab is ongoing, *Conclusion.* These initial data show that blinatumomab elicits pronounced anti-leukemic activity in patients with relapsed/refractory ALL and support further evaluation in this patient population.

0553

CD20 EXPRESSION IN PHILADELPHIA-NEGATIVE B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA DOES NOT SHOW SIGNIFICANT IMPACT ON OUTCOME: RESULTS OF NORTHERN ITALY LEUKEMIA GROUP ALL 09-2000 PROTOCOL

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Background. The prognostic significance of CD20 expression in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) has been investigated in children and adults and its role is still under debate. The first study addressing the prognostic relevance of CD20 in adults was carried out by Thomas *et al* (Blood 2009); CD20 positivity (i.e. more than 20% of positive ALL cells) was associated with worse disease-free survival (DFS) and overall survival (OS), and this effect appeared unrelated to age. The French group (Maury *et al*, Haematologica 2010) documented a significant prognostic impact of CD20 only in patients with white blood cell (WBC) count above 30 x 10⁹/L. Conversely, Chang *et al* (Haematologica 2010) did not detect any prognostic relevance of CD20. *Aims.* The aim of our study was to correlate CD20 expression with clinical-biological characteristics and outcome in Philadelphia-negative (Ph-) BCP-ALL patients prospectively treated within the multicenter NILG 09-2000 study (Bassan *et al*, Blood 2009), designed to orientate post-remission strategy upon minimal residual disease (MRD) assessment. *Methods.* Immunophenotyping was performed according to the general recommendations from European Group for the Immunological characterization of Leukemias (EGIL). Phenotypic data were expressed as the percentage of CD20 positive cells on whole leukemic population; we considered 20% as the threshold for positivity. *Results*. From March 2000 to September 2008, 172 Ph- BCP-ALL were enrolled in the study. Median age was 37 years (range 16-68); median WBC was 10 x 10⁹/L (0.5-730). According to EGIL classification, BCP-ALL diagnoses were B-I 50, B-II 96, and B-III 26. Fifty-two (30.2%) patients resulted CD20-positive. The CD20-positive group showed

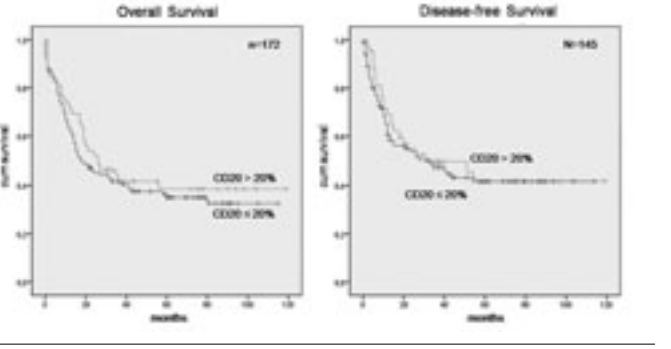

Figure 1.

higher frequency of B-II phenotype (77.0% vs. 33.3%; p<0.0001), and higher incidence of splenomegaly (53.8% vs 35.0%, p=0.067) and hepatomegaly (46.2% vs 25.8%, p=0.014), while no other difference was detected with regard to demographic and diagnostic characteristics. As regards treatment, no difference emerged between the two groups with respect to complete remission rate, MRD response, DFS and OS (Figure 1). Moreover, we searched for an impact of CD20 expression within specific patient and disease subgroups: age, WBC count and EGIL classification were not associated with CD20 positivity and CD20 expression did not affect outcome in any of these subsets. Exclusion of t(4;11)+ ALL from analysis did not alter these results. *Summary/conclusions*. Our study failed to demonstrate a prognostic significance for CD20 expression in BCP-ALL. The discrepancy between our data and others might be related to differences in study design and therapeutic strategy, herein MRD-oriented, that could have resulted in abrogation of the pejorative prognostic effect by CD20. Nonetheless, independently of these considerations, CD20 antigen remains a useful therapeutic target to improve outcome further in CD20+ ALL.

0554

MINIMAL RESIDUAL DISEASE IN PERIPHERAL BLOOD AT DAY 15 PREDICTS PROGNOSIS OF CHILDHOOD B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA AND REFINES RISK STRATIFICATION BASED ON BONE MARROW

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Background. Most minimal residual disease (MRD)-directed treatment interventions in current treatment protocols for acute lymphoblastic leukemia are based on bone marrow testing, which is a consequence of previous reports showing the superiority of bone marrow (BM) over peripheral blood (PB) as an investigational material. Those studies typically did not explore the prognostic impact of peripheral blood involvement and lacked samples from very early time points of induction treatment. *Aims.* To compare MRD levels in BM vs. PB at early time points of treatment and to evaluate their impact on prognosis. *Design and Methods.* In this study, we analyzed 398 pairs of simultaneously taken blood and marrow follow-up samples from 95 children with B-cell precursor acute lymphoblastic leukemia during ALL IC-BFM 2002 treatment using immunoglobulin and T-cell receptor gene rearrangement testing at diagnosis (n=93), day 8 (n=83) and day 15 (d15, n=78) of induction, the end of induction phase 1 - day 33 (n=53), preconsolidation - week 12 (n=47), prior to maintenance therapy (n=6), and at the end of maintenance therapy (n=38). *Results.* Also at early treatment time points, we confirmed the previously published poor correlation between MRD in BM and PB, with BM-MRD being higher than PB-MRD in most samples (BM/PB: median 7.9, range 0.04-8 293). Higher PB involvement at diagnosis was associated with higher WBC (p=0.003), enlargement of the spleen (p=0.0004) and of the liver (p=0.05). No obvious difference has been observed between PB-MRD levels regarding immunophenotype or genetic subtype including Ikaros gene status, except for the fact that hyperdiploid leukemias had a trend towards lower d15 PB-MRD values than other patients excluding TEL/AML1 cases (p=0.057). At day 15, PB-MRD lower than 10-4 was achieved in 45% of patients and was associated with an excellent five-year relapse-free survival (100% vs. 69±7 %; p=0.0003). PB-MRD subgroups (high-risk, HR:≥10-2, intermediate-risk, IR:<10-2 and ≥10-4, standard-risk, SR:<10-4) correlated with d15 BM-MRD based stratification proposed for BFM protocols (HR:≥10-1, IR:<10-1 and ≥10-3, SR:<10-3), but the risk groups did not match completely. Together, day 15 BM- and PB-MRD identified a larger low-risk group with no relapse (49% of patients) than BM alone. However, a larger study would be needed to assess if PB-MRD could also improve the identification of HR patients, if combined with BM testing. No other treatment time point was predictive of outcome regarding PB, except for a trend at day 8 (p=0.057). *Conclusions.* PB-MRD at d15 identified a large group of patients with an excellent prognosis on BFM-based protocol. When combined with BM-MRD, it could further refine the risk group stratification based on d15, which is currently limited mainly to flow cytometric MRD testing.

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0555

ONLY ADULTS UNDER 35 YEARS TRANSPLANTED FOR RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA WERE RESCUED - POPULATION-BASED STUDY IN SWEDEN 2003-2007

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Background. Results of adult acute lymphoblastic leukemia (ALL) treatment have improved in recent years. In spite of this, ALL relapse remains challenging as only a minority of patients are cured. *Aims.* The aim of this population-based study was to assess results of reinduction treatment and allogeneic stem cell transplantation (SCT) in Swedish patients diagnosed with first ALL relapse 2003-2007. *Methods.* 76 patients with ALL relapse (Burkitt leukemia excluded) 2003-2007 were prospectively reported to The Swedish Adult Acute Leukemia Registry. Informed consent was obtained from all patients. Missing data were complemented retrospectively. The national guidelines recommended retreatment with initial therapy (ABCDV) for late relapses and two alternatives for early relapses, FLAG-Asp (Fludarabine 30 mg/m2 i.v., Ara-C 2000 mg/m2 i.v. on days 1-5 and PEG-Asparaginase 500 E/m2 i.v. on days 2 and 16) and MEA (Mitoxantrone 12 mg/m2 i.v., Etoposide 100 mg/m2 i.v. and Ara-C 1000 mg/m2 b.i.d. i.v. on days 1-4). For most relapsing patients not transplanted in first remission (CR1) the aim was to perform allogeneic SCT in second remission (CR2). *Results.* For all relapsing patients median age at initial diagnosis was 40 years (range 15-65). Median time from diagnosis to relapse was 13 months (2-116). Median overall survival (OS) after relapse was 8 months (0.5-96). OS at 1 year was 38%, 2 years 25% and 5 years 14%. Five patients (7%) given palliative treatment at relapse were excluded from further analyses. 37/71 patients (52%) given intensive salvage treatment achieved CR2 after the first reinduction course. Additionally, 13 patients reached CR2 after a second course. MEA and FLAG-Asp gave CR2 in 6/9 (67%) and 10/16 (63%) patients respectively. Three patients died of toxicity / infection after reinduction without reaching CR2. Allogeneic SCT in CR2 was performed in 29 patients (sibling-14, unrelated-14, cord blood-1) of whom 19 were under 35 years at diagnosis. Multivariate analysis for OS after relapse (Table1)

Table 1.

	All patients (N=66), missing values: site of relapse (N=5)			*Patients not transplanted in CR1 (N=54)*		
	N	HR-OS (95%CI)		N	HR-OS (95%CI)	
Age at diagnosis *35-65 vs 15-35 years*	37/29	3.6 (1.8-7.2)	**p<0.001**	28/26	3.8 (1.7-8.6)	**P=0.001**
Time diagnosis to relapse *<18 vs >18 months*	35/31	2.2 (1.2-4.0)	**p=0.012**	29/25	3.4 (1.6-7.6)	**P=0.002**
Site of relapse *Extramedullary +/- BM vs sole BM*	14/52	0.6 (0.3-1.2)	p=0.157	10/44	0.3 (0.1-0.8)	**p=0.017**
Treatment after relapse						
Allo-SCT in CR2	29	1.0	**p<0.001**	29	1.0	**p<0.001**
No allo-SCT in CR2	19	1.5 (0.7-2.9)	p=0.267	10	3.1 (1.2-7.5)	**p=0.015**
CR2 not achieved	18	5.6 (2.7-11.4)	**p<0.001**	15	4.9 (2.2-10.8)	**p<0.001**

identified age over 35 years and time to relapse <18 months as significant negative prognostic factors. For patients not transplanted in CR1, treatment with allogeneic SCT in CR2 was favourable compared to CR2 achievement without subsequent transplantation. Unexpectedly, sole bone marrow relapse was a negative factor compared to extramedullary +/- bone marrow relapse. Eleven patients, all under 35 years at diagnosis, are still alive at median 63 months (46-96) after relapse. Ten of these received allogeneic and one autologous SCT in CR2. All 10 patients over 35 years who received allogeneic SCT in CR2 died, as did all patients treated solely with chemotherapy. *Summary/Conclusions.* Both MEA and FLAG-Asp appear effective as reinduction therapies. Of our patients under 35 years treated with allogeneic SCT in CR2, 10/19 (53%) have sustained survival. However, in older and most of the early relapsing patients, outcome was poor. Prevention of relapses is paramount, and new salvage treatments are urgently needed. piotr.kozlowski@orebroll.se

0556

ACUTE LYMPHOBLASTIC LEUKEMIA IN INFANTS TREATED BY STANDARD CHEMOTHERAPY ALONE OR IN COMBINATION WITH ATRA

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Background. Infants' ALL still remain a disease with poor outcome due to the early recurrence and treatment-related mortality. Recently developed ATRA containing MLL-Baby protocol-ATRA(+) treatment approach-is intended to overcome high early relapses rate without additional treatment toxicity. *Aims.* To perform comparative analysis of treatment results in patients from ATRA(+) group and patients treated by standard chemotherapy alone-ATRA(-) treatment approach. *Methods.* From July 2003 till November 2010 ninety-nine infants younger 365 days with ALL were non-randomly allocated either to ATRA(+) schedule-66 pts. or to ATRA(-) schedule-standard chemotherapy, mainly ALL-MB protocol, - 33 pts. Treatment approaches have been chosen under the decision of treating clinics. Both ATRA(-) and ATRA(+) groups of pts. were similar by initial characteristics: median age 6 (range 1-11) and 6 (range 0-11) months (p=0.99); sex-m/f ratio 12/21 and 24/42 (p=0.82); WBC - 96 (range 0.7-940) and 70 (range 1.6-2058) per µl (p=0.99); initial CNS involvement: 4 and 14 pts. (p=0,4), immunophenotype: BCPI - in 30.8% pts. and 52.3% pts.(p=0.1), BCPII - 34.6% pts. and 29.2% pts. (p=0,8), BCPIII - 26.9% pts. and 13.8% pts. (p=0.23); biphenotype: 0 and 1,5% pts. (p=0.63); AUL: 0 and 1.5% pts. (p=0.63); TIII - 3,8% pts. and 0 (p=0.63); TIV - 3.8% pts. and 1,5% pts. (p=0.91)respectively. MLL rearrangements were detected in 45 (68.2%) among 66 pts. allocated to ATRA(+) and in 15 (53.6%) among 28 examined pts. allocated to ATRA(-) regimen. More than half of MLL-positive pts. had t(4;11): 23 out of 45 pts. from ATRA(+) group and 8 out of 15 pts. from ATRA(-)group, respectively. *Results.* We did not observe any significant difference in induction deaths: 5 (15.1%) vs. 5(7.6%), p=0.43 in ATRA(-) and ATRA(+) groups; CR rates: 28(84.9%) out of 33 pts. vs. 59(89.4%) out of 66 pts., p=0.74; remission deaths: 0 vs. 6(10.1%), p=0.83 respectively; but proportion of relapses remains different: 16(57.1%)from 28 pts. in ATRA(-)group vs. 11(18.6%) out of 59 pts. in ATRA(+)group, p=0.0001. Probability of RFS - 0.74±0.06 vs. 0.37±0.09, p=0.008; cumulative incidence of relapse - 0.62±0.01 vs. 0.25±0.004, p=0.01 in ATRA(-) and ATRA(+) groups correspondingly. Univariant analysis identified the following parameters to have a significant negative impact on EFS-age younger 6 months (p=0,0001); MLL rearrangements (p=0,018) and treatment without ATRA (p=0,04). Multivariant Cox regression analysis confirmed the significant negative value on EFS age younger 6 months with Hazard Ratio 2,550(95%CI 1.272-5.112) p=0,008 and ATRA(-) treatment apptroach-HR 2.017 (95%CI 1.072-3.796) p=0.03. *Conclusion.* Our data demonstrates that ATRA based approach could be treatment of choice in infants with ALL.

0557

NEW FIRST LINE CHEMOTHERAPY PROGRAM WITH LINEAGE-TARGETED METHOTREXATE INFUSIONS IS FEASIBLE AND IMPROVES THE EARLY MINIMAL RESIDUAL DISEASE RESPONSE AND SURVIVAL IN ACUTE T-LYMPHOBLASTIC LEUKEMIA

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Background/Aims. In acute lymphoblastic leukemia (ALL) an early reduction of minimal residual disease (MRD) <10-4 confers a significant survival advantage. Since 2008 we adopted a T-ALL regimen including methotrexate (MTX) infusions at 5 g/m2. Obtaining a high rate of early molecular remissions was a major study endpoint, to improve survival also in comparison to an historical control series. *Methods.* Program N-10 consisted of 5 standard chemotherapy blocks alternating with 3 MTX blocks at 5 g/m2 (24-h infusion, folinic acid rescue 6-hourly from h 42 to a level <0.25 micromol/L; age >55 years: MTX 1.5 g/m2). This schedule targeted an *in vivo* MTX concentration of ~65-70 micromol/L, in keeping with the concept of lineage-targeted MTX developed at St. Jude's Hospital. A concurrent molecular evaluation of bone marrow MRD was performed to optimize risk stratification and related therapeutic decisions. Early treatment consisted of pre-phase (PDN/CY), cycle 1 (VCR/IDR/DEX/ASP), cycle 2 (VCR/IDR/CY/DEX/AraC/6MP) and cycle 3 (MTX/HD-AraC), plus day +30 and +70 MRD analysis. Cycles 4 and 6 were like 2, 5 and 7 like 3 (no. 5 with ASP instead of HD-AraC), and 8 like 1. Risk classes were standard (SR: thymic CD1a+ and WBC <100; CR cycle 1) and high (HR: others). Allo-SCT was prescribed to HR patients, and to SR patients with MRD >10-4 on day +70 and/or positive at later time-points. Auto-SCT followed by maintenance could be used as an alternative. SR MRD negative patients were submitted to maintenance. *Results.* All 24 evaluable patients (median age 40 years [range 17-65], SR 10, HR 14) entered CR (100%), 23 after N-10 induction and one refractory to cycle 1 after Clofarabine/AraC. Twenty patients are alive in CR1 (83%), 4 relapsed (17%) and one died in CR after SCT. With a maximum follow-up of 3 years, 2-year overall and disease-free survival are 74%. The associated MRD response was highly favorable. Day +30 MRD was negative in 62.5% (5/8) and 33% (3/9) of evaluable SR and HR patients, respectively, and <10-4 in 2 other patients, for a major postinduction response of 75% in SR and 44% in HR. Post-MTX day +70 MRD was negative in 80% (8/10) and 67% (6/9) of SR and HR patients, respectively, and <10-4 in 2 other patients, for a major post-consolidation response of 90% in SR and 78% in HR. MTX plasma determinations at 8-h and 24-h from start of infusion were available from 37 5 g/m2 blocks administered to 19 patients, and were generally close to stated therapeutic target (8-h, mean: 78.1 [21.6]; median: 75 [44-120]; 24-h, mean: 76.9 [22.5]; median 84 [27.6-124]). Extrahematologic grade III-IV toxicity was occasional (liver: 5.4%, gastrointestinal 8%, metabolic 2.7%) and transient in nature. *Conclusions.* Protocol N-10, introducing for the first time in adult ALL 5 g/m2 MTX infusions, was feasible and highly active in adult T-ALL. Compared to the results from a previous study (protocol N-9: 84 patients, CR 85%), day +70 MRD response and 2-year survival are being improved (MRD negative 74% vs 58%; survival 74% vs. 41%, P=.046).

0558

CURE RATES AND TOXICITY VARY ACCORDING TO AGE < VS. > 55 YEARS IN B-ALL AND BURKITT LYMPHOMA TREATED WITH THE GERMAN CHEMOTHERAPY PLUS RITUXIMAB PROTOCOL: ITALIAN STUDY ON OVER 100 PATIENTS

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Background. Mature B-ALL (acute lymphoblastic leukemia) and Burkitt lymphoma are characterized by high cell proliferation, aggressive clinical behavior and poor prognosis unless a highly specific treatment is used. The German Multicenter Study Group for Adult ALL (GMALL) recently introduced a short intensive chemotherapy program in combination with rituximab to improve results in B-ALL and Burkitt lymphoma (Hoelzer *et al*, ASH 2007, abstr 518). The Northern Italy Leukemia Group (NILG) adopted the same protocol to treat >100 patients since 2002. *Aims.* To evaluate efficacy, toxicity and long-term results obtained with this regimen in a prospective cohort of unselected patients with B-ALL and Burkitt lymphoma. *Methods.* Treatment consisted of six chemotherapy courses (4 courses in stage I-II disease without mediastinal or extranodal involvement) plus Rituximab (R) 375 mg/m2 for 6-8 total doses, and local radiotherapy (mediastinal or CNS involvement, or residual tumor). Treatment plan was as follow: prednisone-cyclophosphamide prephase —> R+course A (dexamethasone, vincristine, ifosphamide, HD-methotrexate, teniposide [or etoposide], Ara-C,intrathecal therapy) —> R+course B (dexamethasone, vincristine, cyclophosphamide, HD-methotrexate, adriamycin, intrathecal therapy) —> R+course C(dexamethasone, vindesine, HD-methotrexate, etoposide, HD-Ara-C) —> R+A —> R+B —> R+C. Patients aged >55 years received only courses A and B but not C and lower dose methotrexate (0.5 instead of 1.5 g/m2). *Results.* Between December 2002 and June 2010, 106 patients were enrolled. Fifty patients had B-ALL and 56 Burkitt lymphoma (stage III-IV 28%, bulky 47%, extranodal involvement 64%). Median age was 47 years (range 17-78), 60% were male, 31% were >55 years, 16 (15%) were HIV+, 35% had an ECOG PS > 2, and 79% an elevated LDH. Eighty-three patients (78%) achieved CR, 8 had refractory disease and 15 died early (10 by infection, 3 hemorrhage, 2 other) (Table). No statistically significant difference in CR rate was observed between HIV negative and positive patients (80% vs. 69%, p=0.3). Sixty-eight patients (64%) received the whole treatment program. At a median follow-up of 3 years, 65 patients (61%) are alive in CR1, 20 (19%) died of treatment complications (TRM) and 11 developed recurrent disease (3 BM, 1 CNS, 1 BM + CNS, 6 nodal). Projected 5-year OS and DFS were 62% and 75%, respectively, with significant differences in favor of patients aged <55 years (Table). Other clinical indicators that significantly affected OS and DFS were an elevated serum LDH (P=0.03) and an ECOG PS > 2 (P=0.003). *Conclusions.* The short intensive German chemotherapy/rituximab regimen was confirmed effective for the management of adult patients with B-ALL and Burkitt lymphoma up to an age of 55 years (75% projected alive at 5 years). In the older age group, the lower cure rate (37% at 5 years) was equally related to TRM (mainly caused by infection) and progressive disease.

Table 1.

	Age (years)	no.	CR no. (%)	OS (5 years)	DFS (5 years)	Failures, no.
B-ALL	≤55	32	27 (84)	75% (P=0.001)	85% (P=0.01)	disease, 7 TRM, 2
	>55	18	12 (67)	33%	50%	disease, 4 TRM, 8
Burkitt lymphoma	≤55	41	33 (80)	76% (P=0.03)	83% (P=0.09)	disease, 4 TRM, 7 secondary AML, 1
	>55	15	11 (73)	40%	55%	disease, 4 TRM, 3 late death, 1

0559

RAPID ADVERSE OUTCOME IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA AND A MIXED 3 REPEATS/ 4 REPEATS IN THE ENHANCER REGION OF THE 5'-UNTRANSLATED REGION OF THE THYMYDILATE SYNTHASE GENE

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Background. Several cohort studies have investigated the relationship between thymidylate synthase (TS) genotype, expression and survival in children with cancer. A high level of TS expression is correlated with poor prognosis in paediatric patients with acute lymphoblastic leukemia (ALL). While polymorphic tandem repeats in the TS enhancer region (TSER) of the 5'Untranslated Region (5' UTR) has been shown to impact on TS expression, it is reported that the TSER3R/TSER3R genotype increases the resistance to methotrexate (MTX) and is associated with a higher risk of relapse after chemotherapy. *Aim.* The goal of this preliminary study was to compare the TSER genotype prevalence in a cohort of Belgian and Vietnamese ALL children. *Material and Methods.* Blood Samples from Belgian (n=114) and Vietnamese (n=115) ALL children were collected in Saint-Luc Hospital (UCL, Brussels, Belgium) and Blood Transfusion - Hematology Hospital (PNT Medical University, Ho Chi Minh city, Vietnam) in accordance with a protocol approved by the local ethics committees. Genomic DNA was obtained from whole blood. It was extracted with BioRobot EZ1 (Qiagen, Hilden, Germany) using a DNA (EZ1 DNA Blood 350 µl/kit (48) and EZ1 DNA buffy coat card) according to the manufacturer's instructions. Purified DNA was quantified using NanoDrop ND1000® spectrophotometer (Nanodrop Technologies, Inc. Wilmington, DE, US) and stored at -20°c prior to subsequent molecular analysis. DNA samples were amplified by polymerase chain reaction for the TSER. The DNA fragments were run in a 2.5% agarose gel with ethidium bromide and visualized on a UV transilluminator. Sequencing analysis was performed in the validation phase as well as as a confirmation in case of unusual genotype (3R, 4R). DNAs from anonymous African patients (n=50) were used as a control for interethnic differences in the prevalence of TSER genotypes. *Results.* The distribution of TS genotype in the patient and DNA control groups is given in Table 1. Interethnic differences are highly significant (Fisher exact test, p<0.0001). Two leukemic children respectively of African and Vietnamese ancestry were found with the rare 3R/4R TS genotype. Both had a poor therapeutic outcome after chemotherapy: a 15-year-old African girl was indeed unsuccessfully treated with the FRALLE-2000 group T chemotherapy after presenting with an undifferentiated acute leukemia. The patient died during the consolidation phase of ELAM-02 chemotherapy; a 18-month-old Vietnamese boy who presented with ALL-L2 was unsuccessfully treated with FRALLE 2000 group B1 chemotherapy (including MTX). Extramedullary (Central Nervous System) relapse was diagnosed 29 days after starting the intensification phase. Up to now, the patient has undergone 4 successive relapses and has never achieved remission. *Conclusions.* The 3R/4R TS genotype was found in one Vietnamese and one African ALL children but not in the Caucasian cohort. Nevertheless, this genotype seems to be associated with a very bad outcome and chemoresistance in carriers. Further investigations are now pursued on Vietnamese and African cohort to assess their TSER genotypic status and response to therapy.

Table 1.

	TSER genotypes in ALL children		
	Vietnamese	Belgian	African DNA controles
	n=115	n=114	n=50
2R/2R	1 (0,87%)	26 (22,8%)	8 (16,0%)
2R/3R	34 (29,57%)	48 (42,1%)	21 (42,0%)
3R/3R	79 (68,69%)	39 (34,2%)	21 (42,0%)
3R/4R	1 (0,87%)	1 (0,9%)	0 (0%)

0560

CD20+ PROGNOSTIC SIGNIFICANCE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background. The prognostic significance of CD20 expression in Acute Lymphoblastic Leukemia (ALL) blasts is still a matter of debate in adult ALL patients. These patients' outcome has been considered up to now to be variously affected both by non homogeneous chemotherapy approaches and by different biological parameters. *Aim.* Aim of our study was to evaluate the prognostic impact of CD20 expression in 119 adult ALL patients (<60 yy), diagnosed according to the FAB/WHO classification and homogeneously treated between 1996 and 2009. *Patients and Methods.* Cut-off for CD20+ expression was 20%. All patients were treated according to the 0496 Gimema Protocol (Prednisone, Vincristine, Daunolastine, Asparaginase). Patients median age was 32 years (r 15-60). *Results*. The median age of patients expressing CD20 (CD20+: 54 pts) was higher than of CD20- patients (42 vs 26, p=0.039), while there were no differences regarding white blood cells, platelets, percentage of peripheral and bone marrow blasts cells, recurrent genetic abnormalities (t(9;22), t(4;11), t(8;14)). CD20+ patients showed a lower incidence of myeloid antigen expression (CD13 and/or CD33, p.0.04), but this was not confirmed in CD20+Ph- patients. There was no correlation between Ph+ (40 pts) and CD20+ and the outcome was independent of CD20 expression. In the Ph- group (85 pts), Disease free survival (DFS, p=.9) and Overall survival (OS, p=.24) did not seem to be affected by CD20 expression. *Conclusions.* Taken together, our data seem to suggest that CD20+ ALL adult patients are older and have a lower expression of myeloid antigens, but these data are uncorrelated with OS and DFS. Although our patients sample was a homogeneous study cohort for age and treatmen, further large case series are needed to evaluate the true prognostic impact of CD20 expression in adult ALL patients and the role of CD20 antibodies therapy.

0561

EFFECT OF PREVENTIVE ANTITHROMBOTIC MEASURES ON THROMBOTIC RISK IN ADULT PATIENTS TREATED FOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Treatment of acute lymphoblastic leukemia (ALL) is frequently complicated by venous thromboembolism (VTE). The reported incidence varies from 2% to 37%. The highest VTE risk arises in the first treatment weeks, while the value of preventive measures is not clear yet and standardized prevention protocols are lacking. *Aims.* To assess the effect of various preventive antithrombotic protocols on the VTE risk in adult patients during treatment for ALL. *Methods.* 240 patients aged 16-59 years with newly diagnosed ALL were treated on the same anti-leukemic protocol, containing L-asparaginase in the first induction cycle, in a Dutch-Belgian multicenter study from 1999 to 2005. All VTE complications during protocol were recorded. VTE prophylaxis differed between centers (no prophylaxis, frozen plasma (FP), antithrombin (AT)). We retrospectively analyzed the various preventive antithrombotic protocols during the first ALL induction cycle to assess their effect on the risk of VTE, using available patient records. Informed consent was obtained for this analysis. *Results.* 36 of 240 patients (15.0%; 95% CI 10.5-19.5) experienced VTE during protocol (10 sagittal sinus, 20 upper limb (90% central venous catheter-related), 4 deep-vein thromboses of the leg, 2 pulmonary embolisms). In 25 patients VTE occurred during the first induction cycle. Prophylactic FP compared to no VTE prevention reduced the VTE risk in the first induction cycle by nearly three-quarters (RR 0.3; 95% CI 0.1-0.5; see Table). Since prophylactic AT was only rarely given in two centers, its effect could not be properly assessed. Low-molecular-weight heparin (LMWH) was not used as regular VTE prophylaxis during this study. *Conclusions.* The reduced VTE risk with prophylactic FP during adult ALL induction therapy could be explained by a FP-induced increase of AT, in contrast with previous studies that showed a negligible benefit of FP, a skewed ratio of FP protocols between centers, or another more obscure manner of FP-induced anticoagulation. Moreover, this was a retrospective quasi-randomized (by treatment center) observation. The effect of FP or other preventive VTE measures during adult ALL treatment should be confirmed by a randomized controlled study.

Table 1. Frozen plasma protocols.

ALL treatment centers with frozen plasma protocols		
Treatment center	Total number of included patients	Number of patients with VTE in first induction cycle
Protocol FP +		
1	38	1
2	30	3
3	26	1
4	22	2
5	18	1
6	14	1
7	13	2
8	10	2
9	8	0
10	7	1
11	5	0
12	3	0
13	3	0
14	2	0
Total FP +	199	14
Protocol FP -		
15	21	5
16	20	6
Total FP -	41	11

ALL = acute lymphoblastic leukemia; FP + = with frozen plasma; FP - = no frozen plasma given; VTE = venous thromboembolism

0562

MIXED PHENOTYPE ACUTE LEUKEMIA (MPAL) ACCORDING TO THE WHO 2008 CLASSIFICATION-REPORT OF 17 CASES

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Background. Biphenotypic acute leukemias are rare leukemias and account less than 4% of acute leukemias, characterized by coexpression of lymphoid and myeloid markers on the same leukemic cells. The diagnostic criteria until now were based on the scoring sistem proposed by EGIL, that was adopted by the WHO 2001 classification. The most recent edition of the WHO classification (2008) has established and published new criteria for the diagnosis of BAL, which is now termed mixed phenotype acute leukemia (MPAL).The WHO definition of MPAL is based on the expression of strictly specific T lymphoid (cytoplasmic CD3) and myeloid (MPO) antigens, shown by either flow cytometry or cytochemistry, and/or evidence of monocytic differentiation; for B -cell lineage strong expression of CD 19 together with another B-cell associated marker or, in cases with weak CD 19, the expression of at least three B- lineage markers. *Aim.* to describe clinical features and treatment response in 17 cases of MPAL. *Methods.* We studied 896 adult patients with de novo acute leukemia, selected from clinical archives (1999-2010), and described clinical features, morphology and cytochemistry according to the French-American-British (FAB) criteria, immunophenotypic characteristics (by flow cytometry and immunocytochemistry), and cytogenetics by conventional karyotypic studies and molecular analysis in 17 MPAL. Patients were treated according to the national protocols for ALL or AML. *Results.* The final dg of MPAL fulfilled 17/896 (1,9%) patients. There were 10 male and 7 female, median age 45 (range 18-61). Morphology was consistent with ALL (65%) and AML (35%). Immunophenotyping disclosed B/myeloid variant (11/896, 1,2%pts.),T/myeloid (5/896, 0,6%pts),and rare B/T (1/896, 0,1%pts.)variant. All studied patients expressed HLA-DR and/or CD 34 Ags. Cytogenetics were available in all patients and evidenced t(9;22)(12%), in 1pts. associated to complex karyotypic changes, complex (23,5%),abberant (23,5%) or normal (35%)karyotypes. 12% patients had not metaphases. 5 patients received ALL therapy, 10 patients

AML therapy, two patients a combination of therapy. ALL treatment induced a response in 40% patients, AML treatment in 40% patients; one patient responded to the combination of therapy. 15 (88 %) patients died, 7(41%) of resistant/relapsed disease.Two patients (12%) received high dose therapy for AML followed by BMT and PBSCT, obtained CR and still surviving. Overall median survival was 13,6 months and 12% of the patients are alive (1 and 11 years). *Conclusions.* Our results confirm a poor prognosis of MPAL.There are no uniform criteria about type of treatment in this rare disease. Adults patients should be considered for stem cell transplantation in first remission.

0563

PROGNOSTIC SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE DETECTED BY PCR FOR FUSION GENE TRANSCRIPTS IN INFANTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED BY MLL-BABY PROTOCOL

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Background. Prognostic significance of minimal residual disease (MRD) in acute lymphoblastic leukemia (ALL) was shown within several treatment regimens. Majority of infants with ALL carry *MLL* rearrangements, so in this group MRD monitoring by detection of fusion gene transcripts (FGt) could be fast, easy and cost-effective approach. MLL-Baby treatment protocol is successfully applied for infant ALL within Russian Federation and Republic of Belarus (L. Fechina *et al*, ASH 2007 #2828). In this treatment approach conventional chemotherapy is augmented by administration of all-trans retinoic acid (ATRA). *Aim.* To evaluate the prognostic significance of MRD detected by PCR for FGt in *MLL*-rearranged infant ALL, enrolled into MLL-Baby study. *Methods.* 23 infants with defined FGt who had at least 4 available follow-up samples were included in the current study. Median of follow-up period was 30 months (range 7-90 months). Presence of *MLL* rearrangements was detected by nested reverse-transcriptase PCR (RT-PCR), FISH and confirmed by long-distance inverse PCR (C. Meyer *et al*, 2005). MRD detection in bone marrow (BM) was performed by both real-time quantitative PCR and qualitative nested RT-PCR as previously described (N. Palisgaard *et al*., 1998, J. Gabert *et al*, 2003). MRD-negativity was defined as absence of FGt in both assays with sensitivity 1E-05. Among 23 infants there were 13 *MLL-AF4*-positive patients, 4 *MLL-MLLT10*-positive patients, 3 *MLL-EPS15*-positive patients, 2 *MLL-MLLT1*-positive patients and one *MLL-MLLT3*-positive patient. BM samples were obtained at the time of diagnostics, on day 15 of remission induction (time point 1 (TP 1)), at the end of remission induction (TP2) and after each ATRA course (TP3-TP9). Event-free survival (EFS) was calculated. Informed consent was obtained in all cases. *Results.* According to the qualitative MRD results patients were divided into MRD-positive and MRD-negative categories. All pts were MRD-positive at TP1. At TP2 3 patients became MRD-negative. At TP3 other 5 patients converted to MRD-negativity. By TP4 18 patients were MRD-negative, while FGt was detected in 5 patients. 2 patients became MRD-negative before protocol II (at TP9), while 3 patients never achieved MRD-negativity. Retrospectively all patients were referred to 3 groups in respect of MRD status at TP3 and TP4. The MRD-low risk group included 8 patients who were MRD-negative at both TPs. The MRD-intermediate risk group consisted of 10 patients who were MRD-positive at TP3 but MRD-negative at TP4. 5 patients remaining MRD-positive at TP4 were referred to MRD-high risk group. Outcomes in these three groups were significantly different. EFS was 1.00 in MRD-low risk group, 0.70±0.14 in MRD-intermediate risk group and 0.20±0.17 in MRD-high risk group (log-rank p for trend 0.002). *Conclusions.* MRD monitoring by FGt measurements has significant prognostic value in infants with *MLL*-rearranged ALL treated by MLL-Baby protocol. Consideration of MRD status at TP3 and TP4 allows dividing patients into 3 groups with different outcomes. These data could be implemented into the patients' stratification in MLL-Baby trial, when more patients will available for the analysis.

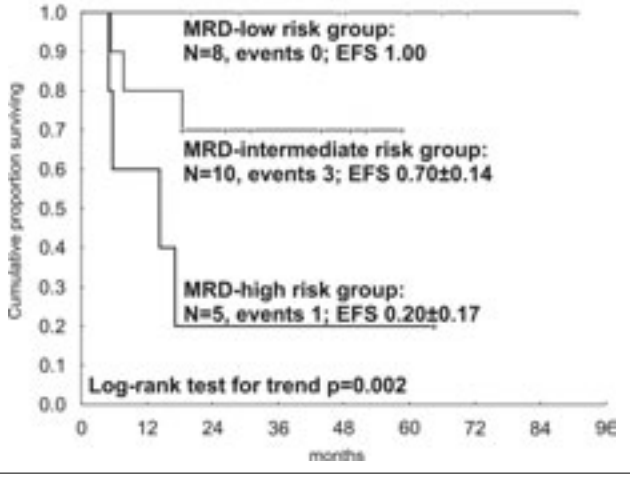

Figure 1. Event-free survival for MRD-defined risk group.

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THYROID DYSFUNCTION IN LONG-TERM SURVIVORS OF ACUTE LYMPHOBLASTIC LEUKEMIA: EXPERIENCE OF A SINGLE ITALIAN PEDIATRIC INSTITUTION

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Background. The risk of thyroid dysfunction increases in childhood survivors of acute lymphoblastic leukemia (ALL) treated with cranial or craniospinal radiotherapy, due to disruption of the hypothalamic-pituitary axis or direct injury to the thyroid gland. There are few reports in the literature on the contribution of chemotherapy to the development of central hypothyroidism in childhood cancer survivors. Previous studies, with small numbers of survivors and limited follow-up post-treatment, have reported no significant thyroid dysfunction in childhood ALL survivors treated with chemotherapy alone. *Aim.* We retrospectively evaluated the incidence of thyroid dysfunction in 280 childhood long survivors with ALL treated according to the protocols of the Italian Association of Pediatric Hemato-Oncology (AIEOP). *Methods.* From June 1986 to January 2011, two hundred and eighty patients (145 males and 135 females) survivors of ALL were followed in a single pediatric Hemato-Oncology Institution. Two hundred and thirty-eight children were treated with chemotherapy alone and 42 received also cranial/craniospinal radiotherapy. All patients annually underwent thyroid echography and blood measurement of free FT4, FT3, TSH, anti-thyroperoxidase and anti-thyroglobulin antibodies. All thyroid nodules exceeding 1,5 centimetres were aspirated and cytologically analyzed. *Results.* In our series of 280 patients, the mean age at outcome assessment was 7 years (range, 4 to 30 years) and the average follow-up duration from completion of therapy to last survey was 8.6 years (range, 1 to 24 years). No patients treated with radiotherapy experienced thyreopathy. Nine (2 males and 7 females) out of 238 patients (3,7%), treated with chemotherapy alone, reported thyroid dysfunction. Five survivors 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 neoplasms. 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 only one patient, familiar anamnesis was positive for thyreopathy. The mean time to thyroid dysfunc-

Table 1.

Clinical characteristics of patients

Pt	Sex	Age at diagnosis (yrs, mos)	Age at stop therapy (yrs, mos)	Thyreopathy	Thyroid antibodies	Thyroid function	Time to event from stop therapy (yrs)	Age at event (yrs, mos)	Need for therapy	Familiarity
1	F	8 yrs	10 yrs	Thyroid nodules	Yes	Normal	5 yrs	15 yrs	No	No
2	F	6 yrs 5 mos	8 yrs 5mos	Hypothyroidism	Yes	Altered	1 yrs	9 yrs 5 mos	Yes	No
3	F	2 yrs 5 mos	4 yrs 5 mos	Thyroid nodule	No	Normal	3 yrs	7 yrs 5 mos	No	Yes
4	F	9 yrs 5 mos	11 yrs 5 mos	Thyroid nodule	No	Normal	4 yrs	15 yrs 5 mos	No	Adopted
5	F	2 yrs 9 mos	4 yrs 9 mos	Thyroid nodules	No	Normal	12 yrs	16 yrs 9 mos	No	No
6	F	4 yrs 9 mos	6 yrs 9 mos	Increase of TSH	No	Normal	1 yrs	7 yrs 9 mos	No	No
7	M	4 yrs 10 mos	6 yrs 10 mos	Thyroid nodules	No	Normal	4 yrs	10 yrs 10 mos	No	No
8	M	2 yrs 10 mos	4 yrs 10 mos	Increase of TSH	No	Normal	2 yrs	6 yrs 10 mos	No	No
9	F	11 yrs	13 yrs	Hypothyroidism	Yes	Altered	9 yrs	22 yrs	Yes	No

Abbreviations: Pt: patient; yrs: years; mos: months

tion after stop-therapy was 4.5 years (range, 1 to12 years); the mean age at the event was 12.3 years (range, 6.9 to 22 years). Table I resumes the clinical characteristics of survivors with thyreopathy. *Conclusion.* In our experience, the incidence of thyroid dysfunction in long survival pediatric ALL treated with chemotherapy alone results comparable to that reported in literature after cranial or craniospinal radiotherapy. 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 thyreopathy incidence in this group of patients. We believe that childhood leukemic survivors require lifelong surveillance after completion of chemotherapy, for an early recognition and prompt treatment of late thyreopathy.

0565

USE OF CLOFARABINE IN CHILDREN WITH RELAPSED/REFRACTORY ACUTE LEAUKAEMIAS; THE LARGEST SINGLE UK CENTRE EXPERIENCE

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Acute leukaemia is the most common cause of malignancy in children less than 18 years. Advances in effective treatment options have improved outcomes in recent years such that 5-year survival rates now approach 85% for ALL and 50-60% for AML. Despite these advances, approximately 20% of children with ALL experience relapse, which remains the leading cause of treatment failure (Pui & Evans, 2006). Prognosis remains poor for children who experience relapses or are refractory to front line therapy. The safety and efficacy of the combination clofarabine/cyclophosphamide/etoposide was assessed retrospectively in children with relapsed or refractory acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML) or non-Hodgkin's Lymphoama (T-NHL). In this single centre experience we describe 14 children (10 males, 4 females) (median age 14 years, range 3-18 years) with either relapsed/refractory ALL (n=8), AML(n = 5) or T-NHL (n =1), who received clofarabine in combination with cyclophosphamide and etoposide .7 children received doses of clofarabine 40 mg/m2, cyclophosphamide 440mg/m2 and etoposide 100mg/m2 for 5 days,(doses as per CLO218 Hijiya *et al*) and 7 received doses of clofarabine 40 mg/m2, cyclophosphamide 300mg/m2, etoposide 150mg/m2 for 5 days, 3 along with dexamethasone. (Doses from High Risk modification arm of MRCUKALLR3). Informed consent was obtained from all families. Of the 8 children with underlying ALL, 7 (87.5%) achieved complete morphological remission. 1 who had bulky extramedullary disease achieved good partial response. 5 of the 8 children have received a haemopoietic stem cell transplant (HSCT) (62.5%). 2 children are currently awaiting HSCT. 1 child could not receive HSCT due to invasive fungal infection. 1 child, with T-NHL, achieved remission and received HSCT. Of the 5 children with underlying had AML, 3(60%) achieved morphological remission and received HSCT. 1 child died soon after chemotherapy due to multiorgan failure, and hence response could not be asessed.1 child received this combination chemotherapy for post HSCT relapse, and did not respond. The most common adverse events were febrile neutropenia, mucositis and reversible liver toxicity; no case of liver veno-occlusive disease was reported. Heavily pre-treated children had more side effects. These data suggest that the clofarabine/cyclophosphamide/etoposide regimen is reasonably well tolerated and can induce clinical response in a relevant proportion of children with refractory/multiple relapsed ALL and AML. Good supportive care with prophylactic antifungal and anti-PCP agents is important. (pnemocystis carinii).

0566

PROGNOSTIC IMPACT OF CD20 EXPRESSION IN ADULTS WITH DE NOVO PRECURSOR B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. In the last decades, we observed an improvement in treatment outcome of adult *de novo* acute lymphoblastic leukemia (ALL) brought mainly by intensive chemotherapy and increased use of allogeneic hematopoietic stem cell transplant (HSCT). The addiction of tyrosine kinase inhibitors to chemotherapy, for patients with Philadelphia-chromossome (Ph) positive ALL, significantly improved outcome. However, the incidence of relapse is still high in these patients. CD20 is a cell surface marker expressed in the majority of mature B-ALL blast cells, but expressed in only 40% of precursor B-cell ALL blast cells. Recently, a few studies have been published suggesting a worse outcome associated with CD20 expression in adult ALL. *Aim.* To evaluate the prognostic impact of CD20 expression in adults diagnosed with de novo precursor B-cell ALL. *Methods.* From 1997 to 2009, we diagnosed 147 new cases of ALL and lymphoblastic lymphoma. Fifty-four patients met criteria of inclusion in this report (diagnosis of Burkitt-type ALL, T-cell ALL and lymphoblastic lymphoma were excluded). Statistical analysis was performed with SPSS®Statistics v18. *Results.* Fifty-four patients were diagnosed with precursor B-cell ALL (57.4% males; median age of 49 years, range 14-79) and were treated in first-line with Hyper-CVAD (n=44; 7 of these associated Imatinib for Ph-positive ALL), Linker Protocol (n=2), BF12 (n=2; younger patients presenting with complex karyotype), ALL-BFM90 (n=2; less than 21 years old) and vincristine plus dexamethasone (n=4; above 70 years old). Allogeneic HSCT was performed in 15 patients. Nineteen patients expressed CD20 at a level of at least 20%. Distribution of pretreatment characteristics such as age, gender, performance status, leukocyte count, FAB subtype, LDH, CNS involvement at diagnosis and presence of Philadelphia chromosome was similar by CD20 status. First-line therapy was equality distributed between both groups. Complete response rate was similar irrespective of CD20 status (85.3% in CD20-negative vs. 84.2% in CD20-positive). There was a higher incidence of disease recurrence in the CD20-negative group (51.4% vs. 36.9%, p=0.3) and CD20-positive patients had better overall survival (OS, 39.4 months vs. 22 months, p=0.51) and disease-free survival (DFS, 72 months vs. 26.3 months, p=0.41), but this did not reach statistical significance. The median time to relapse was 11.7 months in the CD20-negative group and 16.9 months in the CD20-positive group (p=0.7). Analyzing only the subset of patients treated with Hyper-CVAD, we obtained similar results. Multivariate analysis of pretreatment characteristics was also performed; the only independent prognostic factor was the presence of Philadelphia chromosome which was associated with a worse outcome (p=0.006). There was no difference in median OS or DFS in both CD20 positive and negative Philadelphia ALL, although at 3 years, CD20-positive patients had better OS (29% vs. 9%, p=0.53) and DFS (33% vs. 13%, p=0.52). *Conclusions.* Although none of the results was statistically significant, we observed a trend towards a better outcome associated with the presence of CD20 in adults with *de novo* precursor B-cell ALL, even in Philadelphia positive patients. Further investigation is needed to clarify the role of CD20 as a prognostic factor in adult ALL.

0567

ASSESSMENT OF ENDOCRINOLOGIC AND CARDIOLOGIC LATE EFFECTS AMONG SURVIVORS OF CHILDHOOD LEUKEMIA

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Background. Survival rates for childhood acute leukemia have significantly improved and late effects of therapy have been important in follow-up of survivors. *Aims.* The objective of this study is to identify the endocrinologic and cardiologic late effects of acute leukemia patients treated in our pediatric hematology unit. *Methods.* Patients treated for leukemia with BFM protocols after at least five years of diagnosis were included in the study. Endocrinologic late effecs (growth failure, obesity, insulin resistance, dyslipidemia, thyroid gland disorders such as hypothyroidism, osteopenia/osteoporosis, pubertal disorders) and cardiologic late effects (cardiac toxicity, hypertension) were evaluated. The study group was evaluated with anthropometric measurements, body mass index, laboratory testing of fasting glucose, insulin, serum lipids and thyroid functions. Pubertal stage was determined by using the Tanner criteria. Bone mineral densities were measured by DEXA (dual-energy X-ray absorptiometry). Blood pressures were noted. Evaluation of cardiac systolic and diastolic functions were performed using standard M-mode echocardiography and tissue doppler imaging. *Results.* Of 43 acute leukemia survivors with a median age of 15 (range; 7-30 years), 23 (54%) were females and 20 (46%) were males. Five (12%) of the patients had acute myeloid leukemia and 38 (88%) of them had acute lymphoblastic leukemia. They had been off therapy for an average of eight years (range; 5-17 years, SD 3.4 years). At least one adverse event occured in 25 (58%) of the 43 survivors, with 10 of them (23%) having multiple problems. Six (14%) of the survivors were obese and 10 (23%) of them were overweight. Subjects who were overweigth or obese at the time of diagnosis and at the end of therapy were more likely to be overweight or obese at last follow-up. Overweight and obesity were more frequently determined in patients who were younger than six years of age at the the time of diagnosis. Insulin resistance was observed in nine (20%) subjects. Insulin resistance was more frequently seen in

subjects who are overweight or obese and who have family history of type 2 DM. Hyperlipidemia was detected in eight (18%) of the 43 survivors. Premature telarche was detected in one (2%) subject. None of the patients had short stature. Hypothyroidism was observed in one (2%) survivor. Two (5%) survivors had osteopenia. Avascular necrosis of femur head occurred in two (5%) survivors. Cardiovascular abnormalities occurred in one (2%) of the subjects with hypertension and cardiac diastolic dysfunction. No statistically significant difference was determined for the distribution of late effects between subjects who received cranial radiotherapy or not. *Conclusions.* In our study at least one adverse event occured in most of the cases. Acute leukemia survivors should be followed up for the endocrinologic and cardiologic late effects with concerning sex, age at diagnosis and contents of the therapy.

0568

THE PROGNOSTIC IMPACT OF METHYLATED P15 AND P73 GENES IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Aberrant methylation of promoter-associated CpG islands is an epigenetic modification of DNA that plays an important role in leukemia pathogenesis. This phenomenon is frequently observed in ALL and results in the functional inactivation of its associated genes. The aim of this study was to investigate the frequency and prognostic impact of methylated p15 and p73 genes in adult acute lymphoblastic leukemia patients. *Patients and Method.* The study included 51 newly diagnosed adult ALL patients who presented to the Medical Oncology Department of the National Cancer Institute - Cairo University in the period from January 2008 to May 2009. Written consent was obtained from all patients. Eligible patients were adult up to the age of 50 years with adequate organ function and performance status. Mature B phenotype cases were excluded. Risk stratification based on age, initial total leucocytes count, immunophenotyping, cytogenetic and complete remission rate at 4 weeks were performed at diagnosis. The treatment plan was risk adapted, standard versus high and very high risks with more intensified treatment for the high and very high risk patients. Methylation-specific polymerase chain reaction was used to analyze methylation of the p15 and p73 genes. *Results.* We included 30 males and 21females, their median age was 23years. Precursor B phenotype was detected in 37 patients while T phenotype in 14 cases. Overall risk stratification showed 18 standard (35.3%), 27 high (52.9%) and 6 very high (11.8%) risk patients. The methylation frequencies of p15 and p73 at diagnosis were 41.2% and 27.5%, respectively. Concomitant methylation was detected in 14%. The CR rate was 80.4%. No association was encountered between CR rate and methylation of a single gene, however, concomitant methylation of p15 and p73 was associated with significant lower rate of CR compared to patients without methylation (57% versus 90%), p=0.008.The median survival of the standard risk patients was significantly longer than those of the high and very high risk group (15.8 versus 7 months respectively, p = 0.03). The p15 methylation status did not affect the overall survival but the p73 methylation was associated with poorer overall survival and the difference was near significant (p = 0.059). The survival benefit was significant for patient without methylation compared to patients with methylation of p15, p73 or both genes (p = 0.047). At 12 months, The LFS of the whole group was 67.4%. The leukemia free survival was not affected by the methylation status of a single gene p15 or p73. But it was worse in patients who have methylation of p15, p73 or both genes when compared to patients without methylation (p = 0.08). In conclusion, aberrant p73 promoter methylation is a potential prognostic factor. P15 methylation is frequent in Egyptian adult ALL patients, its concomitant methylation with p73 is of poor prognostic significance. Identification of these molecular targets results in improved risk assessment and accordingly selection of appropriate therapy.

0569

APPLICABILITY OF NG2 FOR FLOW CYTOMETRIC MINIMAL RESIDUAL DISEASE MONITORING IN INFANTS WITH MLL-REARRANGED ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. In spite of great progress in flow cytometric minimal residual disease (MRD) detection in ALL patients in last two decades, new leukemia-associated markers searching is still a demanding task. Neuroglycan-2 (NG2), known to be expressed by leukemic blasts in patients with *MLL*-rearranged ALL, could be a valuable tumor-specific marker for MRD monitoring. Applicability of any leukemia-associated antigen for MRD detection depends on heterogeneity of its expression by tumor cells' population and on its expression modulation during treatment. *Aim.* We analyzed NG2 applicability for MRD monitoring in *MLL*-rearranged infants' ALL. *Methods.* NG2 expression and percentage of NG2-positive cells were assessed in 65 bone marrow samples from 15 infants with *MLL*-rearranged ALL: 12 samples were obtained at diagnosis, 15 - during remission induction, 18 - at post-induction follow-up, 6 - in relapses and 14 - during relapses treatment. MRD detection was performed by multicolor flow cytometry. NG2 expression was assessed by 7.1-PE antibody (Beckman Coulter, US). For comparison reasons mean fluorescence intensity (MFI) values were converted to molecular equivalent of soluble fluorochrome (MESF) units. *Results.* At the time of initial diagnostics NG2-positive cells' percentage varied from 20.60% to 95.80% (median 63.75%). Only in two patients nearly all leukemic blasts expressed NG2. NG2 MESF values at diagnosis were significantly higher comparing to post induction follow-up, relapses and relapses during treatment (p=0.0245, p=0.0032, p=0.0001 respectively). No difference in MESF values in diagnosis and remission induction was found (p=0.7551). NG2 expression in remission induction samples was also significantly higher than in later time-points and relapses (p=0.0053, p=0.004, p=0.0084 respectively). Distribution of NG2-positive cells number in tumor cells' population presented the similar differences. NG2 was heterogeneously expressed by leukemic cells' population (range 0.00%-96.80%) also in the follow-up samples. MRD values detected by standard approach were significantly higher than number of residual cells calculated according to NG2-positivity (p<0.0001). We also found no correlation between NG2 expression level and normalized copy numbers of fusion genes, measured by quantitative real-time PCR. Thus, due to leukemic population heterogeneity and significant treatment-induced downexpression NG2 cannot be applied for MRD gating, although it could be useful for previously gated cells' population description, especially in samples with low MRD-positivity. *Conclusion.* Due to leukemic population heterogeneity and significant treatment-related downexpression NG2 cannot be used as a single marker for MRD detection in infants with *MLL*-rearranged ALL. Nevertheless NG2 could be helpful as tumor-specific marker in combination with other antigens.

0570

EVALUATION OF ESSENTIAL STEPS TO PROMOTE ADHERENCE TO CYTOTOXIC DRUGS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Drug adherence is important for successful therapy in children with Acute Lymphoblastic Leukemia (ALL). Poor adherence to cytotoxic drug can cause relapse of the disease, increase therapeutic costs and may lead to death. *Aim.* Evaluation of adherence to medication in leukemic children in Egypt and identify possible steps to promote adherence in these children. *Methods.* study included children with ALL in complete remission at the maintenance phase of chemotherapy and taken 6-Mercaptopurine daily oral dose. Evaluation was done through specific questionnaire to the patients or his care giver which included details about the child, his family, his illness and details about the medication given and its circumstances. Also, determination of 6- mercaptopurine (6MP) metabolites was done for all children using thin layer chromatography in red cells. *Results.* forty three children with ALL were included in this study, 22 males and 21 females with age ranged from 19 months to 12 years. Non adherence was detected by 6-MP level in 32.5% of cases, -ve results obtained in 9 patients (20.9%) and very low levels (< 4.5 ng) in 5 patients (11.6%), and 58.1% detected by the questionnaire. Non adherence was significantly associated with low educational level (76%), 30.7 % of the non adherent children belong to a lower socio economic class, forgetfulness is the main cause of non adherence (46.2%), followed by refusal of the child to take the medicine (23%) other causes include negligence 11.5% and drug unavailability (11.5%). Mothers were the caregivers in 93 % of our patients, all well knows about the illness of their children and the consequence of stopping 6 MP, all were satisfied with the instructions for treatment, and for follow-up, although 51.1% found the cost to come to the hospital high and 48.8% find the time spent in each visit long. Age, sex, number of family members was not significant association. *Conclusion.* Results suggest that non adherence is mainly in-

fluenced by the educational level of the family and the low socio-economic condition More efforts should focus on methods of assessment and prediction of adherence to medicine especially in children with cancer and studying steps to promote such adherence, further study of this problem is needed urgently especially with the more usage of oral antineoplastic drugs.

0571

THROMBOTIC COMPLICATIONS IN ADULT PATIENTS WITH ACUTE LEUKEMIA: A SINGLE CENTER EXPERIENCE IN MÉXICO. INCIDENCE, RISK FACTORS AND SURVIVAL

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Background. Acute leukemias are hematopoietic malignancies that may be accompanied by abnormalities in hemostasis. Thrombotic complications are the second leading cause of death in patients with cancer, and the pathogenesis is multifactorial. The use of catheters, surgery, prolonged immobilization, the start of chemotherapy (including L-asparaginase), among others, are all risk factors involved in the incidence of thrombosis. *Objectives.* To describe clinical characteristics, frequency of thrombotic events, risk factors and survival of adults with acute leukemia treated at the Instituto Nacional de Ciencias Médicas y Nutrición, "Salvador Zubirán", Mexico City. *Methods.* A retrospective cohort of adult patients, diagnosed with acute leukemia in a specialized center, from October 2003 to December 2009. Analysis of thrombotic events, frequencies and proportions, survival curves by Kaplan-Meier, univariate and multivariate analysis were performed to determine the risk of thrombosis. Informed consent was not required. *Results.* We analyzed 181 patients with a median age of 33 years, 44.2% were women and 55.8% were male. The most common subtype was acute lymphoid leukemia (ALL) with 45.8%, being phenotype B the most frequent with 87.95%. Fifteen cases with thrombosis (8.3%) were documented, of which 53.3% were related to the use of a catheter, followed by DVT in 26.7%, acute myocardial infarction (AMI)/ischemic stroke in 13.3% and PE in 6.7%. Doppler ultrasound was the preferred diagnostic tool in 80% of cases, and the median time to develop thrombosis was 92 days, with 33.3% of events occurring during the first 30 days of diagnosis. L-asparaginase was administered in 11% of the patients, and only in 3 cases of this group (1.6%) an episode of thrombosis was also recorded. With regard to mortality, of the 15 patients with thrombosis, 27% were alive without evidence of disease at last follow-up, and 73% had died, being disease progression (48% of cases) the most common cause of death. We did not find recurrent episodes of thrombosis, and none of the events had an impact on mortality. There were no risk factors related to thrombosis in our study. The median overall survival was 349 days (range 257 to 440 days), with a follow-up of 3.5 years. *Conclusions.* The present study confirms that the incidence of thrombosis in this mexican adult population is comparable to that reported around the globe. However, only a third of these cases were diagnosed during the first month, contrary to what has been published in recent series. And, although catheter-related thrombosis was the most frequent event in this group, our study did not confirm that this or any other factor (age, subtype of leukemia, type of chemotherapy, platelet count or WBC at diagnosis) were associated with an increased risk of thrombosis or that the thrombosis per se could be considered a negative prognostic factor regarding overall survival.

0572

PULMONARY COMPLICATIONS IN SURVIVORS OF CHILDHOOD HEMATOLOGICAL MALIGNANCIES:SINGLE CENTER EXPERIENCE

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Background. Children treated for cancer face the risk of complications later in life, including pulmonary dysfunction. *Aim of study.* To evaluate the frequency and severity of pulmonary complications in survivors of childhood leukemia and lymphoma treated with chemotherapy alone or combined with radiotherapy. *Methods.* 70 childhood cancer survivors (44 males and 26 females) were enrolled in this study after parental consent.They included survivors of acute lymphoblastic leukemia (n=25), acute myeloid leukemia (n=5), Hodgkin disease (n=20) and non Hodgkin lymphoma (n=20).Their age at diagnosis varied between 1-14 years (median 4 years), and their age at evaluation varied between 3-25 years (median 10 years). Exclusion criteria included the presence of primary or secondary disease in the thorax, recent major surgery, previous thoracic surgery, known major non neoplastic lung disease, bone marrow or stem cell transplantation. Patients having evidence of chest infection at time of evaluation were temporarily excluded till at least four weeks after resolution of infection.Pulmonary complications were assessed through history taking, chest examination, high resolution computed tomography (HRCT) chest, and pulmonary function testing (PFTs). *Results.*Although most survivors had no clinical pulmonary compromise,40% had abnormal PFTs including: (14.3%) obstructive pattern, (5.7%) restrictive pattern and (20%) mixed pattern. There was no difference in PFTs between the groups in relation to malignancy diagnosis(P=0.37). Significant pulmonary dysfunction was seen in children older than 10 years of age at evaluation (P=0.003). Duration since completion of therapy was not signicantly related to PFTs in multivariate analysis. Patients treated with combined chemotherapy and radiotherapy showed higher percentage of complications (72.7%) compared to those treated with chemotherapy alone (25%) (P=0.001). Cumulative dose of Bleomycin caused significant abnormal PFTs compared to other chemotherapeutic agents (P=0.04),whereas administration of methotrexate was a significant factor related to pulmonary dysfunction(p=0.002). Only male patients who received combined therapy showed higher frequency of both restrictive and obstructive lung disease, abnormal respiratory reactance and peripheral airway disease when compared to chemotherapy only group (P=0.007, P=0.04, P=0.002, P=0.003, P=0.05 respectively), there was no significant difference in female patients. Survivors with abnormal CT chest findings (n=14) had lower FVC%, FEV1% and PEF% when compared to individuals with normal CT (P=0.001, P <0.001, P=0.001 respectively). *Conclusion.* Subclinical pulmonary function abnormalities are found in survivors of childhood hematological malignancies previously treated and off therapy .Pulmonary dysfunction is more evident with combined chemotherapy and radiotherapy. Bleomycin and methotrexate are the most incriminated chemotherapeutic agents,and males are at higher risk than females. Specific and extended follow up is warranted especially in the presence of risk factors or previously detected pulmonary problems.

Acute myeloid leukemia - Biology 2

0573

DO NATURAL KILLER CELL KILLER IMMUNOGLOBULIN-LIKE RECEPTOR GROUP GENETICS AFFECT RISK OF ACUTE MYELOID LEUKAEMIA DEVELOPMENT AND TREATMENT OUTCOME?

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Characterising natural killer cell (NK) reactivity using Killer Immunoglobulin-like Receptor (KIR) genetics has proven useful in predicting outcome of haematological malignancy after stem cell transplantation (SCT). We recently showed that stem cell donors with the activatory NK receptors KIR 2DS1, 3DS1 and the co-inherited 2DL5a, were associated with less relapse following T-deplete SCT for de novo Acute Myeloid Leukaemia (AML) but not other haematological malignancy. Interestingly, this effect was not seen in secondary AML. To investigate whether KIR genetics also affect AML biology outside the context of SCT, we performed KIR genotyping on DNA obtained at diagnosis from patients enrolled on the Medical Research Council (MRC) AML 10 and 15 trials. Patients received four courses of chemotherapy according to MRC protocols. KIR genes were identified using Qiagen© SSP PCR KIR genotyping kits. The frequency of KIR genes in 469 de novo and 38 secondary AML patients was compared to the gene distribution in a normal control population of 246 donors. HLA typing for KIR ligands (Cw, Bw, A3, A11) in AML patients was performed and combined with KIR genotype data to identify individuals missing an HLA ligand for their inhibitory KIR. In de novo AML, the KIR gene frequency did not differ significantly from the normal control population. However, in secondary AML (related to prior therapy, MDS or MPD), the frequency of the activating KIR 2DS2 was significantly lower (26%) than in normal controls (44%) or de novo AML (51%) (p=0.004). The activatory KIR haplotype B was also less common in individuals with therapy-related AML than in those without therapy-related AML (27% v 67%, p=0.005). In contrast to our previous findings in AML SCT, the activatory KIR 2DS1, 3DS1 and the coinherited 2DL5a, were not associated with improved outcome after chemotherapy for either de novo or secondary AML. HLA typing data for KIR ligands and KIR genotype data was combined in a model to predict those with the most activatory NK populations. The results of this analysis of KIR frequencies and HLA groups together in AML patients will be presented.

0574

LEGIUS SYNDROM'S GENE, SPRED1, IS FREQUENTLY INACTIVATED IN CHILDREN ACUTE MYELOBLASTIC LEUKEMIAS (AML)

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Background. Germ line loss of function SPRED1mutations are associated to Legius syndrom (LS), an autosomal dominant condition characterized by café au lait macules, freckling and sometimes Noonan-like appereance or learning difficulties. SPRED 1 is a member of Sprouty/Spred family of membrane associated negative regulators of ERK activation. SPRED1specifically inhibits MAPKs signaling by suppressing RAF phosphorylation and activation. We preaviously reported a case of AML in a 11-month old boy with SPRED1 germline mutation. *Aims.* We sought further explore the possible involvement of SPRED1 alterations in paediatric leukemia. *Methods.* We performed whole gene SPRED1 mutation screening at genomic and RNA levels as described (Passmant 2009) in 210 leukemia diagnostic samples: 67 AML, 140 Lymphoblastic Acute Leukemias (ALL), and ten Juvenile MyeloMonocytic Leukemia, JMML. AML samples were screened for NRAS, Ki-RAS, PTPN11, B-RAF1, FLT3-ITD, gene mutations. SPRED1 expression level was quantified by RQ-RT-PCR in diagnostic leukemic (32 AML, 54 ALL, 5 JMML) and in paired normal bone marrow samples obtained at the complete remission (CR), (32: 28 AML and 2 ALL). Physiological level of SPRED1 expression was determined in normal hematopoietic cells (10 healthy donors Bone Marrow, BM, one CD34+ sample, one thymus and 5 blood mononuclear cells). SPRED1, ERK1/2 and MEK protein expression and phosphorylation were evaluated by Western Blot and IHC on normal (BM, AML samples at CR) and pathological samples (AML at the diagnosis). *Results.* Three novel SPRED1 heterozygous nucleotide variations were identified in two B-ALL and one AML: one isosemantic transition c.674C>T, and two missense mutations: c.124G>A (p.Val42Ile) and c.1089A >G (pIle363Met). These nucleotide variations were confirmed in leukemia RNA at the time of diagnosis and in the remission BM. Patients did not have phenotypic features of Legius syndrome or other NF1 like diseases. SPRED1 expression was significantly decreased in AML compared to ALL (p<10-3) and normal hematopoietic samples (p <10-5). SPRED 1 expression levels varied among AML samples from 10-1 to 10-3 fold the amount measured in the normal BM. Lowest levels were correlated to FLT3 ITD mutations (p = 0.008) and highest with N-RAS mutations (p= 0.013). In AML -paired complete remission samples SPRED1 expression rose to a level similar to those of normal BM. This normalization of SPRED1 expression was correlated to the decrease of MEK/ERK protein expression and phosphorylation. *Conclusions.* SPRED1 mutations are rare in paediatric leukemia. The three novel variations described are germline mutations not associated to any characteristic feature of Legius syndrome. SPRED1 expression is strongly inhibited in the vast majority of AML both at transcript and protein levels. The loss of SPRED1 protein contributes to the loss of inhibition of MEK and ERK activation and to the increase of phospho-ERK1/2 et phosphor-MEK. This is the first observation of a role of SPRED1 gene in the signalling RAS-dependent cascade in children AML.

Reference

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0575

SMALL SUBPOPULATIONS OF NG2 EXPRESSING BLASTS IN THE CONTEXT OF A PANMYELOID IMMUNOPHENOTYPE PREDICT FOR INVERSION INV(16) IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

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Background. Individualized treatment is currently developed in the context of AML therapy. As a first step into this direction, treatment stratification based on cytogenetic or molecular aberrations is implicated in many current treatment protocols. Cytogenetic or molecular testing of reciprocal translocations is still the basis of this approach, but takes at least one or two days to be assessed. Flow cytometry is a standard procedure performed in the diagnostic workup of patients with AML. Recent work has shown that flow cytometric immunophenotyping allows for rapid prediction of several important cytogenetic or molecular aberrations, e.g. t(8;21), 11q23, or *mNPM1* gene. *Aim.* The aim of the present study was to identify an immunophenotypic pattern predicting the presence of inv(16) in AML patients. *Methods.* An 8-color approach (until 2006 3-color) was performed for diagnosing AML. Therefore, a FACS-Canto II (until 2006 FACS-Calibur; BD Biosciences) was used. 1,421 AML patients treated in different multicenter studies of the SAL study group were included. The NG2 monoclonal antibody (moAB 7.1, Beckman Coulter) has been used to predict the presence of aberrations at chromosome 11q23 (MLL gene) in AML and ALL, but no association was reported for inv(16) so far. The inv(16) and/or *CBFβ-MYH11* was detected in 85 patients using FISH as well as PCR. *Results.* As a first step, the panmyeloid antigen expression (CD13+CD33+CD117+cyMPO+CD34+), CD14, or aberrant CD2 expression was shown to reach only a moderate sensitivity or specificity: 100%/71%, 59%/92%, and 28%/98%. Second, we discovered that the consideration of a low NG2 expression (1%-19%) as a unique marker was already highly sensitive and specific

(80%/95%) in predicting inv(16). The combination of NG2 and pan-myeloid antigen expression only increased specificity to 99%. Finally, an integrated antigen pattern using NG2 expression plus all the above mentioned phenotypic characteristics lead to a sensitivity and specificity of 95% and 96%, respectively. Two of the 4 false negative patients showed a complex karyotype including trisomy 8. Three of those 4 patients did not achieve a CR, were MRD positive after induction therapy, or relapsed later. *Conclusions.* Using the immunophenotypic characteristics described above it was possible to predict the presence of inv(16) in AML with a high sensitivity and specificity of at least 95%. Since inv(16) is sometimes hard to assess in standard metaphase preparations and requires secondary confirmation using FISH or PCR assays, flow cytometry might provide a fast and reliable screening method for rapid risk-based treatment stratification.

0576

FREQUENCY AND PROGNOSTIC SIGNIFICANCE OF MONOSOMAL KARYOTYPE IN ACUTE MYELOID LEUKEMIA

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Monosomal karyotype (MK), defined as 2 or more monosomies, or a single monosomy in the presence of structural abnormalities, has recently been reported as identifying a distinct subset of acute myeloid leukemia (AML) with an extremely poor prognosis. We retrospectively evaluated all AML cases with MK diagnosed in our Department over a period of 13 years and explored potential associations with clinicobiological features and outcome. Overall, karyotypic data obtained from conventional cytogenetic analysis of unstimulated bone marrow cells were available in 549 AML cases. In our series, MK was found at a frequency of 11.3% (62/549 cases), similar to what has been reported recently in an independent patient cohort. Ninety-two percent (57/62) of MK cases were found to have a complex karyotype. Additionally, 98% (61/62) of MK cases were assigned to the unfavorable cytogenetic risk category. The median age of MK cases was 60.5 (range, 18-88) years. MK increased with age, being present in 5.5% of cases below the age of 30 but in 15% of those over age 60 (X2-test: P=0.022). Of 51 cases with available data, 24 (47%) concerned secondary AML, whereas the remaining 27 cases (53%) were de novo AML. At diagnosis, the median white blood cell count of MK cases was 3.25x109/l (range, 0.7-82) and the median LDH value 349 IU (range, 100-3360). For statistical purposes, MK cases were compared to a group of 51 AML cases with unfavorable karyotypic profile yet without MK (UWMK) who were treated with similar, "3+7"based regimens. Complete remission rates after induction treatment were 37% in UWMK patients versus 27% in those with MK (P=0.33). The median overall survival (OS) of UMWK patients was 15 months versus only 6.5 months in those with MK, with 3-year OS rates of 16% and 8%, respectively MK (P =0.003). Thus, MK defines a sizeable subset of patients with unfavorable cytogenetics, often with secondary AML and advanced age, who have a particularly poor prognosis even when compared to other cases with an unfavourable cytogenetic risk profile.

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COMPARISON OF PROGNOSTIC MARKES IN ACUTE MYELOID LEUKEMIA EXCLUDING PROMYELOCYTIC LEUKEMIA

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Background. Recently, several cytogenetic and molecular features, such as FLT3 alterations and NPM1 gene mutation, have been described in acute myeloid leukemia (AML). On the other hand, variables of nuclear chromatin texture have been described as independent risk factors in several malignancies (ALL, melanoma, multiple myeloma). *Aims.* Analyze the correlations of molecular features, morphometric characteristics and methylation and compare the influence on overall survival in adult patients with AML. *Methods.* Diagnosis was made by bone marrow (BM) cytology and karyotype, and cases were classified by WHO criteria. Cases of promyelocytic leukemia were excluded. We examined mutations of FLT3 and NPM1, besides methylation of p15, p16, p57, p73 and ER. Blasts from the diagnostic BM cytology were digitalized and interactively segmented. We studied nuclear texture variables derived from the co-occurrence matrix and fractal dimension. We compared the influence on overall survival of cytogenetic, molecular and nuclear texture features on overall survival of the patients. *Results.* We studied 86 cases of AML. Median age: 54 years. PB leukocytes: 30.0x10^9/l. In 61 cases the karyotype was available: 6 had a low risk karyotype, 34 had a normal, 4 had an otherwise intermediary risk and 17 a high risk karyotype. FLT3-TKD mutation was found in 8.2%, FLT3-ITD in 23.5% and 29.4% had NPM1 mutation (44.0% of these had also FLT3-ITD). p15 was methylated in 26.7%, p16 in 11.6%, p57 in 1.2%, p73 in 23.3% and ER in 3.5% of the patients. Blast nuclei of AML without maturation showed higher entropy (p=0.008), contrast (p=0.024), energy (p=0.049) and diagonal moment (p=0.016) and lower a second angular moment (p=0.008). In the univariate Cox analysis were significant: PB leukocytes (p=0.09), FLT3-ITD mutation (p=0.09), local homogeneity (p=0.1), cluster prominence (p=0.08) and "goodness of fit"($R^2$45) of the chromatin fractal dimension according to Minkowski (p=0.07). In multivariate analysis the FLT3-ITD mutation (p=0.001) and $R^2$45 (p=0.025) were independent factors of poor and good prognosis, respectively. Karyotype alone had no influence on survival. *Conclusions.* In our study, FLT3-ITD and chromatin fractal characteristics were more important risk factors for overall survival than karyotype and gene methylation status.

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0578

LAG3 (LYMPHOCYTE-ACTIVATION GENE 3) IS AFFECTED BY DNA HYPERMETHYLATION IN AML AND MDS PATIENTS

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LAG3 (lymphocyte-activation gene 3) is involved in T-cell regulation as an activator of antigen-presenting cells through MHC class II signaling, leading to increased antigen-specific T-cell responses *in vivo*. The aim of this study was to identify T-cell regulation genes, which are influenced by DNA methylation. *LAG3* is one of these genes, which inactivation through aberrant DNA methylation could contribute to the ability of cancer cells to escape the control of immune system (IS). Mononuclear cells (MNC) from peripheral blood or bone marrow of 10 de novo AML patients at diagnosis (age 42-65, median 58), 8 MDS patients (age 60-85, median 65) and 4 healthy donors (age 42-55, median 49) were subjected to methylation analysis. Informed consent from all AML and MDS patients were obtained. Besides *LAG3* other 23 genes involved in T-cell regulation were studied using methylation-restriction endonucleases followed by RQ-PCR. Bisulfite sequencing was used to validate these results and to extend number of examined samples. Levels of *LAG3* gene expression were measured by TaqMan gene expression assay. The restriction-based methylation analyses showed hypermethylation of 7 genes in AML and 6 genes in MDS patients. *LAG3* is one of the most promising. Frequency of hypermethylation of this gene was as follows: 5/10 AML and 3/8 MDS patients compared with 0/4 healthy donor samples. Further we made bisulfite sequencing that confirmed the same DNA methylation frequency of *LAG3* gene in other 8 AML patients. By both methods we examined DNA methylation status of *LAG3* gene in altogether 18 AML patients. We also performed gene expression profiling of *LAG3* on the subset of 19 AML patients at diagnosis and 11 healthy controls. We have found significant downregulation of its expression in AML patients at diagnosis. Our data suggest that hypermethylation of *LAG3* could play a role during process of leukemogenesis and contribute to molecular mechanisms of escape from immunological surveillance. It is a very promising gene to further evaluate its potential prognostic impact on disease outcome.

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IDENTIFICATION OF SECONDARY GENETIC CHANGES IN ACUTE PROMYELOCYTIC LEUKEMIA

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Acute promyelocytic leukemia is characterized by the chromosomal translocation t(15;17), resulting in the formation of PML-RARA gene.

Animal models have shown although this fusion protein is necessary it is not sufficient for leukemia development. We generated FISH probes by sequence independent amplification for chromosomal regions which have been described as altered in APL patients. The following loci were used to analyze a series of 23 APL patient samples after obtaining the inform concent:TP53, MYC, CDKN2A, CDKN1B, RB1, RASA3, NF1, hTERT, ERG, ABCB1 and the region between PML and telomere of chromosome 15. We detected regions which are well-known to be amplified like 8q24 or deleted like 17p13 in APL, along with others like 15q24.1-qter which may harbor novel tumor suppressor gene. Deletions were detected in CDKN2A locus (30% cases), CDKN1B (26% cases), RB1 (26% cases), P53 (17% cases), ABCB1 (43% cases), PML-TEL (22%cases) and hTERT (30%cases). Duplications were also detected in MYC (13% cases), ERG (4%), NF1 (4%), and PML-TEL(4%). Our FISH assay confirmed the accuracy by which DNA copy numbers were detected by BAC array CGH in these tumor suppressors and oncogenes loci in different studies.Taken together, these results suggest that the PML/RARA fusion gene needs several other cooperating genetic lesions to cause APL.

0580

DISCOVERY OF THERAPEUTIC TARGETS FOR COMBINATION THERAPY WITH VALPROIC ACID IN ACUTE MYELOID LEUKEMIA BY INTEGRATING SCREENS IN HUMAN AND RAT AML WITH CHEMICAL-GENETIC SCREEN IN CENORHABDITIS ELEGANS

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Valproic acid (VPA) has been introduced for relapsed advance Acute Myeloid Leukemia (AML) in clinical trials. Only a subset of patients respond to this treatment. Using the Brown Norwegian Myeloid Leukemia rat as model of progressive disease under VPA therapy, we screened the phosphoproteome for molecular targets of VPA. The preclinical rat model Brown Norwegian Myeloid Leukemia (BNML) was treated with 170 mg/kg VPA twice-daily, demonstrating significant increases in survival in comparison to controls ($p = 0.004$). To screen for molecular targets of VPA in this highly responsive model, phosphoproteome analysis was performed by difference gel electrophoresis (DIGE) separation and subsequent differential gel software analysis. Phosphoproteins were isolated from leukemic blasts in the spleen prior to harvesting by immobilized metal ion affinity chromatograpy (IMAC) and identification via Orbitrap mass-spectrometry. Seven of the phosphoproteins found to be significantly differentially expressed in VPA treated BNML rats compared to controls, were investigated for functionality. This was performed by RNAi in Bristol N2 strain of C. elegans at larval stage L1, 24 hours prior to exposure to 15 mM VPA for 72 hours. 4 of 7 gene knock-downs resulted in larval developmental arrest, defined as synthetic lethality. To investigate whether this lethality was a result from apoptosis the CED-1::GFP transgenic reporter assay was employed to quantify germline cell death following RNAi depletion and VPA exposure. Results showed increased numbers of apoptotic corpses by all genes examined. Knock-down of the 4 candidate genes in the transgenic cep-1::CED-1::GFP expressing C. elegans p53 ortholog, CEP-1, resulted in increased basal level of germline apoptosis independent of CEP-1. This suggests that a similar combinational treatment of AML patients might be benefitial, regardless of p53 status. Human AML cell line MOLM-13 was co-treated with VPA and a small molecule inhibitor against prospective target ACTB, cytochalasin B, to test this hypothesis. Inhibition of actin polymerization resulted in increased apoptosis and decreased proliferation when supplemented by VPA, as determined by DNA specific staining with Hoechst and WST-1 colorimetric assay respectively. Results indicate combination of such drugs may be beneficial in treatment of AML. Further, we used a chemical genetics synthetic lethal RNAi screen in Caenorhabditis elegans to a) explore the mechanisms of VPA regulated phosphoproteins, b) unravel why a subset of primary AML cells proliferate after treatment with VPA, c) find novel interactors of VPA by exploring chromatin associated genes, and d) find new targets for combination treatment with VPA. Indeed, we were able to discover novel genes, which increased the effect of VPA *in vitro*, in all screens. Interestingly, we identified 15 genes involved in suppression of VPA amongst the chromatin associated genes. Especially the enhancer of VPA SERBP1 (phosphoprotein screen), and suppressor UTX (chromatin gene screen) were of interest. UTX is shown to be somatically mutated in several cancers. We conclude that novel therapeutic targets can be targeted to increase the efficacy of VPA in AML.

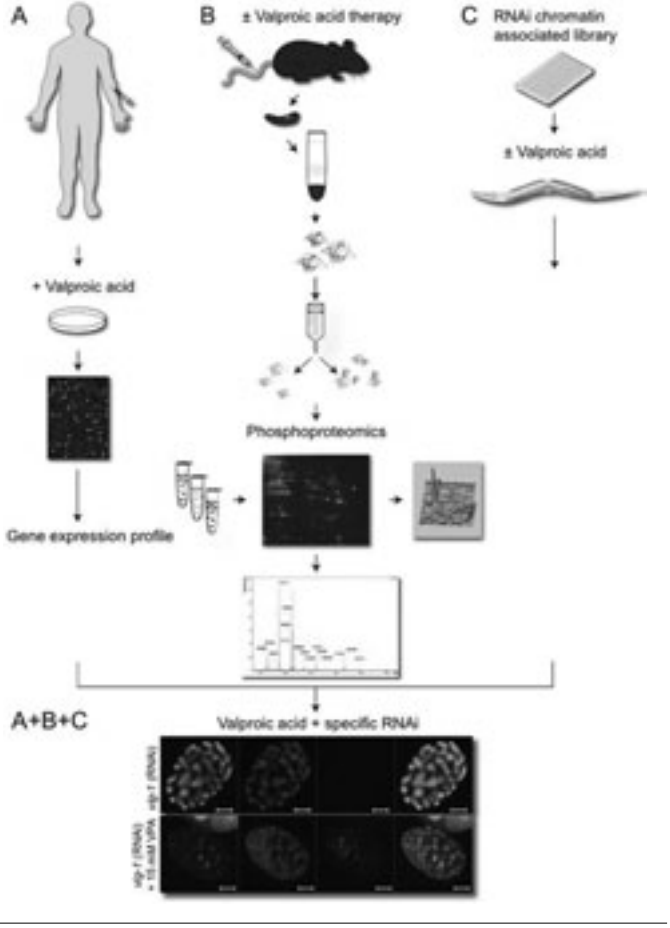

Figure 1.

0581

THE PRESENCE OF FLT3-ITD AND HIGH BAALC EXPRESSION ARE INDEPENDENT PROGNOSTIC MARKERS FOR POOR OUTCOME IN PEDIATRIC AML - A MOLECULAR CHARACTERIZATION OF PATIENTS TREATED WITHIN THE NOPHO PROTOCOLS

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Background. AML is a clinically and biologically heterogeneous disease entity that constitutes 15% to 20% of childhood leukemia. Historically, treatment of AML has been ineffective but identification of disease subgroups and risk stratified treatment based on genetic markers and treatment response has improved prognosis over the last decades. Apart from cytogenetic aberrations, additional genetic markers have been identified as possible prognostic markers, including the mutation status of the *FLT3*, *NPM1*, *CEBPA* and *WT1* genes as well as gene expression levels of *ERG*, *MN1* and *BAALC*. The prognostic significance of mutations in *FLT3*, *NPM1*, *CEBPA* and *WT1* has been thoroughly studied in adult AML but there are also many studies performed on childhood AML. However, gene expression of *ERG*, *MN1* and *BAALC* and their prognostic relevance in pediatric AML have to our knowledge not been studied. Furthermore, most studies have not investigated all

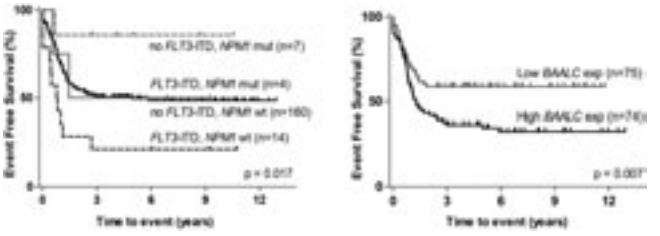

Figure 1. Kaplan-Meier curves.

these genetic markers in relation to each other and most studies have only focused on normal karyotype AML (CN-AML). *Aim.* To perform a thorough evaluation of *FLT3*, *NPM1*, *CEBPA* and *WT1* gene mutations as well as *ERG*, *MN1*, *BAALC*, *FLT3* and *WT1* gene expression as prognostic markers in pediatric AML, all treated within the same NOPHO protocols, and compare their prognostic strength in relation to each other. *Method.* The presence of *FLT3*, *NPM1*, *CEBPA* and *WT1* gene mutations and gene expression levels of *ERG*, *MN1*, *BAALC*, *FLT3* and *WT1* were investigated in 213 pediatric AML samples collected at diagnosis. All patients were enrolled in the NOPHO 1993 or NOPHO 2004 protocols. *Results.* The following results were obtained. 1) *FLT3*-ITD, *NPM1*, *CEBPA* and *WT1* mutations were most common in CN-AML pediatric AML and they commonly co-existed. 2) The presence of *FLT3*-ITD in the absence of *NPM1* mutation was associated with significantly shorter event free survival (EFS), see K-M curve. 3) The presence of an *NPM1* mutation in the absence of an *FLT3*-ITD correlated with improved EFS (see K-M curve) and the presence of an *NPM1* mutation was associated with a better overall survival (OS) in patients with normal karyotype (p=0.038). 4) No significant correlation with survival was found for *FLT3*-TKD, *CEBPA* or *WT1* gene mutations. 5) Gene expression levels of *ERG*, *MN1* and *BAALC* displayed a strong positive correlation with each other. 6) High levels of *ERG* and *BAALC* transcripts at diagnosis were associated with a significant shorter EFS (*ERG* p=0.002 and *BAALC* see graph). 7) No significant correlation with survival was found for *MN1*, *FLT3* or *WT1* gene expression. 8) In multivariate analysis, the presence of *FLT3*-ITD and high *BAALC* gene expression were independent markers for lower EFS. *Conclusions.* We can therefore conclude that analyzing the mutational status of *FLT3* and *NPM1* at diagnosis is important for correct prognostic stratification of pediatric AML patients and that determining the *ERG* and *BAALC* gene expression levels can add valuable information.

0582

ROLE OF SERINE339 IN CXCR4-MEDIATED MIGRATION, HOMING AND ENGRAFTMENT OF LEUKEMIC CELLS

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Background. The CXCR4 chemokine receptor is a major regulator of cell migration, and its overexpression has been associated with a poor prognosis notably in acute myeloid leukemia (AML). We have recently shown that the PIM1 serine/threonine kinase mediates phosphorylation of the serine residue 339 in the intracellular C-terminal tail of the receptor that interferes with CXCR4 receptor recycling and signaling. *Aim.* To study the role of Ser339 phosphorylation in CXCR4 mediated homing and migration, we stably expressed wildtype CXCR4 (WT) or CXCR4 mutants that abrogate phosphorylation (S339A) or imitate constitutive phosphorylation (S339E) in Kasumi-1 human AML cells lacking endogenous CXCR4 expression. *Methods.* The impact of these mutations on CXCR4 function was studied by measuring receptor internalization/recycling and downstream signals by cellular imaging, flow cytrometry and Transwell migration assay. *Results*. In the presence of the ligand (CXCL12), both the normal and mutated receptors were internalized. Recycling of the receptors was observed for all variants with a slight but significant increased recycling capacity of the S339E variant. Expression of both CXCR4 mutants resulted in enhanced ERK activation when compared to the WT receptor. Intracellular calcium efflux upon ligand binding was markedly affected by the mutations: expression of CXCR4-S339A resulted in an increased efflux whereas expression of CXCR4-S339E was associated with decreased efflux when compared to WT CXCR4. Functionally, expression of WT or mutant CXCR4 was able to restore migration capacity towards CXCL12. Expression of the mutant receptors resulted in increased chemotaxis associated with increased chemokinesis (random migration capacity). Transplantation experiments in NOD-SCID-IL2 -/- (NSG) mice allowed addressing the role of Ser339 in homing and engraftment. In contrast to mock-transduced cells, Kasumi-1 cells expressing WT CXCR4 showed efficient bone marrow homing and engraftment measured 24 hours and 7 days after transplantation. Interestingly, cells expressing the CXCR4-S339A or CXCR4-S339E mutant showed a significantly impaired homing and engraftment capacity. However, long-term expansion (measured 7 weeks post transplant) of grafted cells from the epiphyseal area to the diaphysis of the long bones was observed for all variants, suggesting that CXCR4-S339 may play a prominent role during the process of homing and engraftment in the bone marrow rather than during expansion of the leukemic cells. Homing and engraftment was mostly directed to the bone marrow with only sporadic presence of cells in other CXCL12 expressing organs such as the spleen, the liver, or the meningeal space. Interestingly, *in vitro* adhesion and detachment assays revealed that cells expressing the CXCR4 mutants showed an increased adhesion capacity that correlated with their chemotactic potential. In addition, they displayed significantly reduced detachment properties when compared to cells expressing CXCR4-WT supporting the reduced homing capacity observed *in vivo*. *Conclusions.* Our data suggest that Ser339 phosphorylation is likely to act as an important fine-tuning mechanism for CXCR4-mediated adhesion, homing and migration of leukemic cells. Targeted interference with phosphorylation of Ser339 may therefore constitute a novel strategy to therapeutically control CXCR4 functions in normal and malignant cells.

0583

SEQUENTIAL GENOMIC PROFILING IDENTIFIES POTENTIAL DRIVER ABERRATIONS IN ACUTE MYELOID LEUKEMIA FOLLOWING MYELODYSPLASTIC SYNDROME

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Background. Myelodysplastic syndromes (MDS) are clonal stem cell disorders characterized by ineffective hematopoiesis leading to peripheral cytopenia. MDS patients exhibit an increased risk of progression towards secondary acute myeloid leukemia (sAML) with poor prognosis. The mechanisms underlying transformation from MDS to sAML are largely unknown. *Aims.* To identify genetic lesions that are associated with leukemogenesis we performed sequential genome-wide human single-nucleotide polymorphism (SNP) array 6.0 profiling in 32 MDS [WHO categories: RA (n=3), RCMD (n=10), RAEB-I (n=5), RAEB-II (n=8), CMML-2 (n=1), not classified (n=5)] and the corresponding sAML samples. *Results.* In total, 22 (69%) patients acquired additional genomic aberrations (copy number alterations [CNA] and/or uniparental disomies [UPD]) at diagnosis of sAML as compared to the corresponding MDS sample. The most frequent acquired numerical aberration was trisomy 8 (n=6) followed by monosomy 7 (n=3); recurrent submicroscopic losses were identified at 17q11.2 (n=6) encompassing *NF1*, and at 21q22.12 (n=2) including *RUNX1*; of note, biallelic 17q11.2 and 21q22.12 losses were detected in one sAML each. Acquired UPD were detected in 6 (19%) cases affecting the following chromosomal regions: 1p (n=1), 11q (n=1), 13q (n=1), 20q (n=1), and 21q (n=2). Paired sequencing of candidate genes in 1p and 13q revealed UPD related homozygous mutation patterns for pre-existing heterozygous *NRAS* (p.G12S; chromosomal band 1p13.2) and *FLT3* (internal tandem duplication; 13q12.2) mutations in the corresponding sAML cases. Paired sequencing analysis of candidate genes known to be mutated in AML (*NPM1*, *TP53*) revealed that in both *NPM1* mutated sAML cases the mutation was already present at the time of MDS (RCMD each) diagnosis suggesting NPM1 mutations to be an early event in malignant myeloid transformation. In contrast, we also observed the acquisition of novel mutations during transformation. For example, a case initially diagnosed as RCMD with 5q- acquired a $TP53^{V173M}$ mutation during transformation to sAML with complex karyotype. Further analyses of candidate genes with potential leukemogenic relevance, in particular those mapping to 11q (*MLL*, *CBL*) and 21q (*RUNX1*) are currently underway. On the other hand, genomic profiling of one sAML case following 5'-azacitidine treatment of RAEB-II identified a normal genomic status in 11q and 13q, two regions found to be gained in the corresponding MDS sample. This suggests that these aberrations are not necessarily linked to transformation, and that distinct pre-existing clones might be selected by treatment for progression to AML. *Summary/Conclusions.* In our study more than two thirds of MDS cases ac-

quired additional genetic abnormalities during progression to sAML. Homozygous mutation patterns of known oncogenes, such as *NRAS* and *FLT3*, as well as complete loss of tumor suppressor genes or transcription factors, such as *NF1* and *RUNX1*, seem to play an important role in disease progression. Novel molecular analyses like next generation sequencing will facilitate to disclose additional genomic/genetic aberrations involved in this multistep process.

0584

GENOME-WIDE ANALYSIS OF REVERSIBLE EPIGENETIC ALTERATIONS MEDIATED BY RUNX1/ETO

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The reciprocal translocation of chromosomes 8 and 21, t(8;21), results in the formation of the RUNX1/ETO fusion gene that initiates acute myeloid leukaemia by recruiting co-repressor complexes to DNA. RUNX1/ETO interferes with the function of its wild-type counterpart, RUNX1, by directly targeting RUNX1 binding sites. However, the expression of RUNX1/ETO alone does not result in leukaemia and requires secondary mutations and currently it is unclear to which extent the precise epigenotype of t(8;21) cell depends on the presence of the original tumorigenic stimulus. The main purpuse of our study was to (i) identify RUNX1/ETO bound target regions, (ii) determine the chromatin structure of RUNX1/ETO target regions and (iii) most and foremost investigate to which extent these features can be reprogrammed by the selective removal of RUNX1/ETO. To this end, we used a combination of small interfering RNA-mediated RUNX1/ETO depletion, DNaseI accessibility studies, genome-wide chromatin immunoprecipitation (ChIP-seq) and expression profiling in t(8;21) carrying cell lines and cells from patients. RUNX1/ETO was found to co-localize with RUNX1, demonstrating that the fusion protein follows the binding pattern of the wild type protein but does not function primarily by displacing it. We also demonstrate that the RUNX1 binding profile and the sequence composition of RUNX1 binding cis-regulatory elements in t(8;21) and non-t(8;21) leukaemic cells is different. Integrated analysis of gene expression profiles and the RUNX1/ETO ChIP-seq readout showed that genes containing RUNX1/ETO binding sites are mostly up regulated by RUNX1/ETO knockdown. Depletion of RUNX1/ETO resulted in decreased expression of early myeloid progenitor antigens and increased expression of genes typical for differentiated myeloid cells, such as SPI.1, c-FMS, C/EBPalpha, BPI as well as the antiproliferative genes IGFBP7 and SLA. We also show that depletion of RUNX1/ETO leads to an increase of H3K9 Acetylation and RNA Polymerase II at RUNX1/ETO bound genes. Finally we demonstrate that the level of DNA-methylation is inversely correlated with the level of gene expression, DNAseI accessibility, H3K9Ac and H2AZ recruitment. However, even prolonged RUNX1/ETO depletion does not significantly influence DNA methylation, indicating that the relief of epigenetic silencing of tumour suppressor genes requires additional therapeutic strategies.

0585

CO-INHIBITION OF MDM2 AND HDAC SYNERGISTICALLY ACTIVATE P53 MEDIATED APOPTOSIS IN ACUTE MYELOID LEUKEMIA *IN VITRO* AND *IN VIVO*

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Although TP53 mutations are rare in acute myeloid leukemia (AML), wild-type p53 function is habitually annulled through over expression of MDM2 or through various mechanisms including epigenetic silencing via histone deacetylases (HDACs). We hypothesized that binary antagonism of MDM2 and HDACs, with nutlin-3 and valproic acid would additively inhibit growth in leukemic cells expressing wild-type TP53 and induce p53-mediated apoptosis. *In vitro* studies with the combination demonstrated synergistic induction of apoptosis in AML cell lines and patient cells. Mechanisms included massive induction of p53, acetylated p53 and p53 target genes in comparison to either agent alone. The inhibitory effect of the combinational therapy upon proliferation was correlated to the clinical parameters of the patients, where CD34

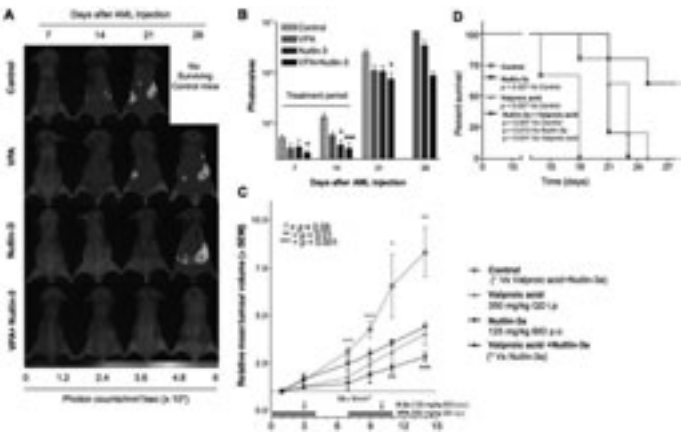

Figure 1.

negative patient samples demonstrated significantly better response in comparison to CD34 positive samples. To evaluate the combination *in vivo*, we developed an orthotopic, NOD/SCID IL2rγnull xenograft model of MOLM-13 (AML FAB M5a; wt TP53) expressing firefly luciferase. Survival analysis and bioluminescent imaging demonstrated the superior *in vivo* efficacy of the dual inhibition of MDM2 and HDAC in comparison to controls. Our results suggest the concomitant targeting of MDM2-p53 and HDAC inhibition, may be an effective therapeutic strategy for the treatment of AML.

0586

ACUTE MYELOID LEUKEMIA INDUCES BONE MARROW FAILURE BY INDUCING DORMANCY IN NORMAL HEMATOPOIETIC STEM CELLS

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Background. Bone marrow failure (reduced production of neutrophils, platelets and erythrocytes) is an important cause of morbidity and mortality in acute myeloid leukemia (AML). We have previously shown that normal hematopoietic stem cells (HSCs) are relatively preserved in AML while normal progenitors and differentiated blood cells are reduced indicating a block in differentiation of HSCs (Haematologica 2010; 95, suppl.2, Abstract 0031). *Aim.* Understand how AML induces a block in differentiation of normal HSCs. *Patients and Methods.*Primary samples were obtained from patients attending St Bartholomew's Hospital, London following informed consent. Controls were untreated patients with a normal blood count and marrow examination with a non-leukemia diagnosis. 1. Cell cycle analysis of HSCs. We have previously shown that the CD34+ compartment of some AMLs (termed CD34- negative AML) is normal and contains normal stem-progenitor cells. The cell cycle status of residual HSCs (CD34+ CD38- cells) in bone marrow from patients with CD34-negative AML and controls was examined using the Ki67 assay. In addition, the proliferation of HSCs in the bone marrow of NOD/SCID/Interleukin 2 receptor gamma chain null mice transplanted with human AML cells (or controls) was assessed using the BRDU assay. 2. *In vitro* co-culture assay. To investigate whether the effect of AML on HSCs is dependent on cell-cell contact or due to secretion factors from AML, we cultured AML cells and

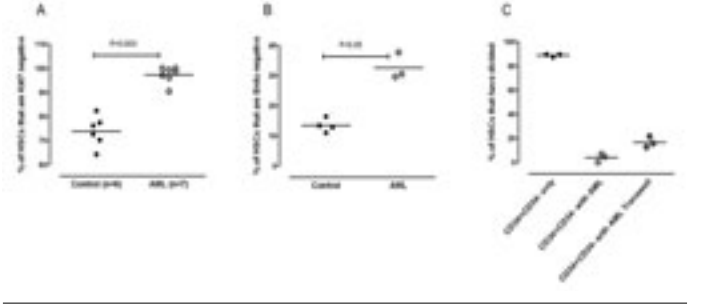

Figure 1.

normal HSCs (CD34+ CD38- cells) together or separated by a porous membrane (0.4 micron pore size to prevent cell contact). MS-5 stromal cells were used to support the AML and HSCs. Division of the HSCs was monitored using BRDU assay. *Results.* 1. HSCs are inappropriately quiescent in the context of AML. Normal HSCs in bone marrow from AML patients were more quiescent compared to HSCs in the control group (Figure 1A). Normal HSCs were shown to divide less in mice transplanted with AML than controls using BRDU assay (Figure 1B). 2. AML secretes a soluble factor that induces quiescence in normal HSCs. Normal CD34+CD38- cells divided less when cultured with AML than alone (Figure 1C). AML reduced division in normal CD34+ CD38- cells even when they are not in direct contact with HSCs (Figure 1C) suggesting a soluble factor is responsible. *Conclusion.* AML induces bone marrow failure by increasing quiescence in normal HSCs. This quiescence is inappropriate given the hematopoietic stress that would be expected to induce HSC cycling. This process appears to be mediated by a soluble factor. We are currently trying to identify this factor.

0587

MIR-34B HYPERMETHYLATION AND CREB OVEREXPRESSION MAY IDENTIFY MDS THAT EVOLVED TO AML

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Background. The cAMP response element binding protein (CREB) has been shown to be over-expressed in acute leukemia promoting abnormal proliferation, cell cycle progression, and clonogenic potential *in vitro* and *in vivo*. CREB has been previously reported to be a direct target of the microRNA, miR-34b. Low expression of miR-34b in myeloid leukemia cell lines was demonstrated to be due to hypermethylation of its promoter region. *Aim.* Our aim is to study *in vivo* the role of miR-34b in leukemogenesis. *Methods and Results.* HL-60 and K562 cell lines has been transfected with lentivirus for stable expression of MiR-34b and monitored for reduced expression of CREB. The transfected cell lines were transplanted in NOD-SCID mice and in flank tumor mice models. A decreased CREB expression was confirmed, whereas engraftment and disease progression *in vivo* were reduced by miR-34b expression. These results confirmed an important tumor-suppressor function for MiR-34b in AML. Mir-34b expression was monitored by RQ-PCR in a large cohort of 113 patients affected by de novo AML, and in 49 pediatric patients with diagnoses of myelodysplastic syndrome or myeloproliferative disorder (MDS/JMML). The distribution of MiR-34b expression in 113 AML patients at diagnosis was significantly downregulated (RQ = 0.176) with respect to healthy bone marrow CD19-CD3-sorted population (RQ = 1). MDS/JMML patients presented higher levels of MiR-34b (RQ = 5.5) compared to AML at diagnosis (RQ = 1). Methylation Specific-PCR revealed 65.5 % (74/113) of AML patients to be methylated at the miR-34b promoter region. Methylated AML patients had lower MiR-34b expression (RQ = 0.075), with respect to the unmethylated patients (RQ = 0.373). Moreover, CREB protein expression correlated to the methylation status of the MiR-34b promoter region in AML. By contrast, all healthy samples and the 49 MDS/JMML specimens never show methylation at the miR-34b promoter region. CREB protein expression was not detectable in MDS/JMML patients with MiR-34b promoter hypomethylation. These results indicated that methylation of the MiR-34b/c region is a tumor-specific phenomenon and that MiR-34b controls CREB protein expression. We examined the DNA of 3 MDS patients who evolved to AML. Results showed that the MiR-34b promoter was exclusively methylated in the onset of AML, together with a decrease of MiR-34b expression levels (RQmean-MDS = 0.41 vs RQmean-AML = 0.26). We used RNA to study changes in gene expression profiles from MDS to AML using GeneChip HG U133 Plus 2.0 on 4 paired patient samples. Supervised analysis of gene expression profiles identified 11 differentially overexpressed CREB-target genes (PRKACB, FDX1, NRXN2, PROSC, ADAM10, RAB7L1, NPR3, ITM2C, LATS2, CDK6 and HOXA7 ($p < 0.001$) between the MDS stage and the evolution to AML. Unsupervised hierarchical clustering analysis using these 11 genes divided the 4 pairs into two separated groups, revealing CREB over-expression and activation of the CREB pathway a feature of disease progression from MDS to AML. *Conclusion.* We consider MiR-34b promoter hypermethylation to be a critical early event for the onset of AML through the activation of the CREB pathway.

0588

T(6;11)(Q27;Q23)MLL/AF6 ENHANCES RAS PATHWAY WEAKENING AF6 FUNCTION IN MYELOID CELLS

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Background. Chromosomal rearrangements involving the human MLL gene at 11q23 are associated with the development of acute leukemias, and have been related to different outcome depending on the MLL translocation partner. The t(6;11)(q27;q23) translocation is characterized by MLL/AF6 expression, known to be a bad prognostic marker in pediatric acute myeloid leukaemia (AML). AF6 is a cytoplasmic protein that can bind RAS through its Ras Association (RA) domain, sequestering and therefore inactivating its GTP-bound active form. The exact function of the MLL/AF6 chimera and its role in the tumorigenic mechanism is still unclear.*Aim.* Here we focus on unravelling the process of leukemogenesis activated by MLL/AF6 fusion gene. *Methods.* Immunofluorescence experiment are performed to detect the localization of AF6 and RAS in healthy primary culture or in t(6;11) cell lines such as ML-2 and SHI-1. Transfection experiment are achieved to knock down AF6 or MLL/AF6 expression. Real time PCR and western blot analysis show the extent of the knockdown and the effect on downstream targets. Pathway inhibition assays confirm the important involvement of the RAS pathway in this MLL/AF6 translocated myeloid leukemia. *Results.* Results show the cytoplasmic co-localization and the interaction of AF6 and RAS in healthy bone marrow cells. This binding is demonstrated to reduced RAS-GTP levels. AF6 silencing in healthy bone marrow cells induces an increase in the expression of RAS pathway proteins by western blot, demonstrating that AF6 is crucial in maintaining the homeostasis of RAS active form and consequently of its targets. In MLL-leukemia, the chimera MLL-AF6 is found into nucleous promoting the sequestration of the cytoplasmic AF6 into the nucleus as well. RAS-GTP levels were high. The silencing of the chimera in ML-2 and SHI-1, by RNA interference, shows a change in AF6 sub-cellular localization. In fact, AF6 returns in the cytoplasm and co-localize with RAS, decreasing the availability of RAS-GTP active form. Effects on the downstream pathway, after MLL/AF6 silencing, such as increase in the phosphorilation of RAF, MEK, ERK is also evident. The implication of the RAS via in t(6;11)(q27;q23) AML cells is further confirmed by using specific RAS pathway inhibitors (PD98059 and U0126). After drugs administration ML2 and SHI1 cell lines, significatively increase cell mortality and decrease colony formation capability, at the same extent as seen after MLL/AF6 silencing. *Conclusions.* We assume that AF6 is a cytoplasmic protein that interacts with RAS-GTP in healthy bone marrow cells, preventing an over-activation of its downstream signalling pathway. In t(6;11)-MLL/AF6 translocated AML cells, the chimera promotes AF6 removal from the cytoplasm and its reclusion into the nucleus, thereby preventing its interaction with RAS and its normal function within the hematopoietic cells. These results suggest one possible mechanism by which MLL/AF6 acts in AML to be further investigated.

0589

HIGH-THROUGHPUT BISULFITE AMPLICON SEQUENCING OF THE 14Q32 IMPRINTED DOMAIN IDENTIFIES DNA METHYLATION SIGNATURES IN ACUTE MYELOID LEUKAEMIA

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Several studies have shown that aberrant epigenetic programming, including DNA methylation, plays a role in tumorigenesis. We have previously reported that in Acute Myeloid Leukaemia (AML) a subset of miRNAs clustered in 14q32 imprinted domain show a differential expression in cytogenetically distinct subtypes of AML. The domain harbours differentially methylated regions (DMRs) that regulate the expression of the encompassed genes and several binding sites for CTCF, an enhancer blocking protein, whose binding to DNA is inhibited by methylation. Hypermethylation of CTCF binding sites prevents the activity of the protein and correlates with overexpression of regulated genes.The aim of this study is to determine whether a change of DNA methylation pattern at 14q32 may occur in AML thus affecting the expression of genes and miRNAs regulated by imprinting.Bisulfite amplicon sequencing with Roche 454 GS FLX Titanium has been performed to analyse the DNA methylation pattern of 7 CTCF binding sites and surrounding sequences located at 14q32. The

region spanning 8 kb and overlapping the promoter of the gene MEG3, includes also the MEG3-DMR and 2 CpG islands. Twenty-four AML patients, including 14 cases with t(15;17) and 3 cases with t(8;21) translocations, 3 cases with inversion (inv16) and 4 normal karyotype (NK) have been selected for the study. Remission samples for patients with t(15;17) and NK, 4 normal bone marrow from healthy donors and 2 non-infiltrated bone marrow from Non-Hodgkin Lymphoma patients, were included in the analysis as controls. High-throughput sequencing of 8 amplicons ranging from 347 bp to 492 bp generated over 600.000 reads with an average sequence depth of 615 reads per amplicon. A total of 160 CpG-dinucleotides have been analysed with an average of 20 CpGs per amplicon.Unsupervised hierarchical cluster analysis showed distinctive DNA methylation signatures associated with cytogenetically different subclasses of AML. Notably, DNA methylation in 14q32 segregates leukaemia's diagnostic specimens from remission and normal bone marrow samples. The analysis segregates also CpGs belonging to the same amplicon, suggesting that CpG methylation is non-randomly distributed in the area. AMLs displayed hypermethylation as compared to controls, with a prominent signature in AML with t(15;17). No significant differences were detected between normal bone marrow and remission samples. Statistical analysis with ANOVA showed that 89 CpGs, distributed in 5 regions, were differentially methylated (p-value < 0.05) among all cases. In particular, 5 of 20 CpGs embedded in 3 CTCF binding sites, were highly methylated in patients with t(15;17) as compared to controls. This pattern is consistent with miRNAs overexpression in AML with t(15;17) previously described. A highly distinctive methylation profile was detected in a region with all 33 CpGs exceeding the significance threshold (p < 0.05).This study shows that changes in 14q32 DNA methylation profile occur in cytogenetically distinct subclasses of AML. It is likely that alteration of DNA methylation in this region could determine miRNAs deregulation.

0590

PRAME-INDUCED INHIBITION OF RETINOIC ACID RECEPTOR SIGNALING-MEDIATED DIFFERENTIATION IN AML CAN BE OVERCOME BY HIGH-DOSE ATRA

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Background. In acute myeloid leukemia (AML) without retinoic acid receptor (RAR) rearrangement the effect of all-trans retinoic acid (ATRA) is still poorly understood besides a previously discovered correlation with NPM1 mutation in cytogenetically normal AML. Recently, the leukemia-associated antigen PRAME has been shown to be a dominant repressor of retinoic acid receptor (RAR) signaling in the presence of ATRA. *Aims.* Within this study, we now wanted to focus our efforts on further investigating the impact of PRAME expression on the response to ATRA treatment in AML. *Methods.* We profiled gene expression in diagnostic AML samples (n=88) derived from our AML HD98B trial for AML patients older than 60 years, in which ATRA was administered in addition to intensive chemotherapy. Gene expression findings were then further investigated in leukemia cell line models by shRNA based PRAME knock-down in PRAME-expressing cells (K562, THP-1) and PRAME knock-in in PRAME-negative cell lines (KG-1, OCI-AML2). *Results.* Profiling gene expression revealed a PRAME-associated gene expression pattern consisting of 1051 genes (ClassComparison analysis, P<0.05), which was significantly enriched for genes involved in the retinoic acid metabolic process as determined by DAVID functional annotation analysis. In AML cell line models we could demonstrate that cell proliferation and differentiation in PRAME-negative versus -positive cell lines were different. While in PRAME-negative AML cells a reduction of the proliferation rate from 49% to 23% was observed at low ATRA concentrations (10e-8mol/l), increasing ATRA levels could not further decrease proliferation (23% at 10e-5mol/l). In PRAME-positive cells increasing ATRA concentrations were associated with a continuous decrease in the proliferation rate (26% at 10e-8mol/l and 6.5% at 10e-5mol/l, respectively) and increase in differentiated, CD66b expressing cells (from 1.5% at 10e-8mol/l to 5% at 10e-5mol/l). shRNA-mediated knock-down of PRAME increased the ATRA-induced differentiation >4fold and overexpression of PRAME in negative cell lines reduced the expression of CD66b ~2fold. In line, our primary AML patient data also suggests that the repressor activity of high PRAME levels can be overcome by ATRA treatment in addition to conventional chemotherapy as determined by a trend towards better outcome in ATRA-treated PRAME-positive cases (p=0.11, log rank test). *Summary/Conclusions:* In PRAME-positive cells PRAME may block differentiation and increase proliferation via blocking RAR-signaling, which might be overcome with ATRA. Thus, in addition to its immunological role, PRAME expression seems to be involved in leukemic cell growth and impaired differentiation, and might represent a marker, which can be targeted by both ATRA-treatment and immunotherapeutic approaches in AML.

0591

ACETYLATION OF MYELOID-SPECIFIC TRANSCRIPTION FACTORS DURING ATRA-DEPENDET MYELOID DIFFERENTION OF THE NB4 AND HL-60 CELL LINES

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Previously, we identified a new signaling pathway operating during G-CSF-triggered granulocytic differentiation. We found that G-CSF activates synthesis of the enzyme Nicotinamide Phosphorybosyltransferase (Nampt) which converted nicotinamide into NAD+ with followed activation of NAD+-dependent protein deacetylase SIRT1. We also found that SIRT1 binds to and activates myeloid-specific transcription factors C/EBPα and C/EBPβ. However, mechanisms and functional outcomes of acetylation/deacetylation of myeloid-specific transcription factors are not fully understood. In the present work we aimed to analyse whether myeloid transcription factors, C/EBPα, C/EBPβ and PU.1 could be acetylated and if SIRT1 is involved in this process. We treated two promyelocytic cell lines, NB4 and HL60, with ATRA for 2 days and studied acetylation status as well as intracellular localization of above mentioned transcription factors. We found, that all three proteins were acetylated in both cell lines. In NB4 cells, acetylated C/EBPα was localized predominantly in the nucleus and its localization was not changed after stimulation with ATRA. Acetylated PU.1 was found in both nucleus and cytoplasm and ATRA treatment led to a slight increase in cytoplasmic acetylated PU.1 protein. C/EBPβ was only weakly acetylated and localized predominantly in the cytoplasm in untreated and ATRA treated cells. In HL-60 cells, acetylated C/EBPα protein was localized in the cytoplasm and was translocated into the nucleus after treatment with ATRA. Acetylated PU.1 protein was found in both cytoplasm and nucleus and incubation with ATRA resulted in slight increase of acetylated PU.1. C/EBPß was weakly acetylated in control and ATRA-treated cells. We also found that all three transcription factors interacted with SIRT1. In both cell lines, C/EBPα:SIRT1 as well as C/EBPβ:SIRT1 protein complexes were detected in the cytoplasm and in the nucleus and ATRA treatment led to nuclear import of these complexes. Contrary, PU.1:SIRT1 complexes were localized exclusively in the cytoplasm independent of ATRA treatment. Differences in the intracellular localization of acetylated PU.1 and C/EBPα transcription factors could be explained by the reciprocal feedback inhibition of C/EBPα and PU.1 for terminal granulocytic vs monocytic differentiation. Since granulocytic differentiation requires C/EBPα and monocytic PU.1. However, exact effects of the acetylation/deacetylation of myeloid transcription factors have to be analyzed more in the details.

0592

EXPRESSION OF DIFFERENTIATION-DEFECTIVE ISOFORM IV OF G-CSFR IS NOT AFFECTED IN MYELOID CELLS OF CN PATIENTS

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Severe congenital neutropenia (CN) is a preleukemic syndrome with a cumulative incidence to develop acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) of ca. 20 %. More than 80 % of CN patients who developed leukemia have acquired mutations in the gene encoding granulocyte-colony stimulating factor receptor (G-CSFR). Moreover, CN patients, who required doses of G-CSF higher than 8 ug/kg/d for treatment, have a higher incidence of AML/MDS, in comparison to patients with lower G-CSF doses. Therefore, we assume that aberrant G-CSFR signaling pathway could be involved in the leukemogenic transformation in CN patients. Previously, it has been shown that elevated expression of the isoform IV of G-CSFR in standard risk AML patients who received G-CSF therapy was correlated

with increased 5-year cumulative relapse incidence, in comparison to AML patients who did not receive G-CSF. Isoform IV of the G-CSFR has the membrane proximal tail responsible for proliferative functions, but replaces the carboxy-terminal region critical for maturation with a novel sequence, ablating its ability to drive neutrophilic differentiation. In the present study we aimed to compare the expression levels of isoform IV and isoform I in myeloid cells of CN patients, CyN patients and healthy individuals. We analysed 22 CN patients (7 with ELA2 mutations, 7 with HAX1 mutations and 8 patients with unknown genetic defects), 4 CyN patients and 10 healthy individuals. We found that in healthy individuals expression levels of isoform I of G-CSFR were more than 200 (78-264) fold higher than of isoform IV. There were no significant differences in the expression ratio of isoform I to isoform IV between CN patients (median=348.7), CyN patients (median=429.7) and healthy individuals (median=199.0). There were also no differences between CN patients with different genotypes. Only one patient developed leukemia and revealed a ratio of 175. We therefore hypothesize that the expression of Isoform IV is not involved in the underlying disorder of congenital neutropenia.

Acute myeloid leukemia - Clinical 2

0593

A PHASE 2 STUDY OF LENALIDOMIDE AS MONOTHERAPY AND IN COMBINATION WITH CYTARABINE, DAUNORUBICIN AND ETOPOSIDE FOR HIGH-RISK MDS/AML WITH CHROMOSOME 5 ABNORMALITIES; THE NCRI LEN5 STUDY

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High risk myelodysplasia (MDS) and acute myeloid leukaemia (AML) with associated chromosomal changes involving chromosome 5, especially as part of a complex karyotype, rarely has a durable response to cytotoxic chemotherapy. Conversely MDS patients with an isolated deletion of the long arm of chromosome 5 (del5q) frequently have a dramatic response to immunomodulatory therapy with lenalidomide. We therefore conducted a phase 2 non randomised study between August 2009 and May 2010 to assess the safety, tolerability and efficacy of lenalidomide monotherapy, followed by lenalidomide with intensive chemotherapy in patients with primary/relapsed/refractory high risk MDS or AML with abnormalities of chromosome 5. The initial lenalidomide monotherapy consisted of 10mg daily days 1-21 of a 28 day cycle. If a complete response was achieved this was consolidated with combination ADE (3+3+5 -days)- cytarabine 100mg/m2 BD, daunorubicin 50mg/m2 OD, etoposide 100mg/m2 OD) and with same dosing of lenalidomide, if a partial response was achieved a further cycle of lenalidomide monotherapy was administered, if progressing then combination induction chemotherapy with ADE (10+3+5) and lenalidomide was administered. Informed consent was obtained from 14 patients, median age 66 (range 40-75). 4 had high risk MDS, 10 had AML. 9 had primary, 3 relapsed and 2 refractory disease. Karyotypic abnormalities consisted of del5q alone in 2 and del5q with additional abnormalities in12. All 14 patients received the initial lenalidomide monotherapy and 9 patients received combination therapy. 1 patient had only monotherapy. 4 patients discontinued the study. The primary endpoints were early death rate (EDR), the proportion of patients recovering their platelets and surviving 42 days post combination chemotherapy and response rate. Stopping rules were set for review after 10 patients had received combination chemotherapy. 3 patients died during the initial monotherapy, an early death rate of 27%, with no patients with blasts >5% achieving a remission. 1 patient with blasts <5% achieved CR after 2 cycles and continues on maintenance therapy. Of the 9 patients who received combination therapy 4 achieved CR/CRp (44%) 2 patients achieved PR (22%), for an ORR of 66%. 2 patients successfully underwent allogeneic transplantation following achievement of remission. 15 SAE's were reported, which consisted primarily of haematological toxicity, grade 3 ALT rise (22%) and venous thrombo-embolism (11%). In view of the unacceptable EDR and lack of response to monotherapy the trial was halted for consideration of amendment and simultaneous review of data for the 9 patients who had received combination chemotherapy. There were no early deaths with combination therapy, however 7 had failed to achieve platelet recovery and the stopping rule was activated. In conclusion Lenalidomide monotherapy at a dose of 10mg daily is ineffective as induction therapy in MDS/AML patients with increased marrow blasts. Lenalidomide combined with ADE chemotherapy has predictable toxicity and has efficacy even in this particularly adverse patient cohort which warrants further investigation.

0594

OUTCOME OF RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA (AML)

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Background. 20% to 30% of AML patients relapse after allo-SCT. For these patients there is no standard therapy. They may receive intensive salvage, donor lymphocyte infusion (DLI) or palliative chemotherapy. Recently, new agents such as lenalidomide showed promising activity. We retrospectively analyse the outcome of 51 patients from our Institution who relapsed from AML following allo SCT. *Methods.* We selected patients with a diagnosis of AML according to WHO criteria who received allo-SCT and relapsed between Jan 2000 and Oct 2010 from our database. Primary endpoint was response rate and duration after salvage treatment. Secondary end points were the overall survival (OS) and relapse free survival (RFS) for responsive patients. At relapse, patients received either curative (anthracycline and/or high dose cytarabine and/or gemtuzumab) or non curative salvage (low dose cytarabine and/or and/or Azacytidine oral chemotherapy or best supportive care (BSC)). OS was calculated from the date of relapse. *Results.* Initial patients characteristics included: median age=48 years [range: 16-69], favourable-risk (n=1; 2%) intermediate-risk (n=29; 57%) or poor-risk cytogenetics (n=18; 35%). Thirty four patients (67%) had been transplanted in complete remission (31 [61%] in CR1 and 3 [6%] in CR2), and 17 (33%) with refractory disease. The conditioning regimen was myeloablative (MAC) and reduced intensity (RIC) for 9 (18%) and 42 (82%) patients respectively. Thirty patients (59%) received an allograft from matched related donor (MRD), 8 (16%) from matched unrelated donor (MUD), 12 (23%) from cord bloods and 1 (2%) from haplomismatch donor. Median time from SCT to relapse was 3.9 months [0.8-90], 29 patients (57%) relapsed <6 months after SCT. Twenty two patients (43%) received curative salvage treatment, 22 (43%) non intensive chemotherapy and 7 (14%) BSC. Among the 22 patients who received intensive salvage therapy, 16 achieved CR (72%). There were 2 (9%) toxic deaths related to high dose chemotherapy and 1 patient died from extensive chronic graft-versus-host disease after a DLI. With a median follow up at 13 months [3-33], the median RFS was 5.4 months. The overall median survival (OS) was 3.4 months. Factors influencing survival were: treatment (curative vs non curative: median OS= 6.7 vs 1.8 months, p=0.015), time between allo SCT and relapse (>6 months versus <6 months, median OS= 7.7 months versus 1.9 respectively; p=0.009). Disease characteristics (cytogenetics, WHO classification) and status before graft did not significantly impact the OS. *Conclusions.* Our results confirm that the prognosis of the patients who relapse after allo SCT is very poor with a median 3.4 months survival underlining the need for new therapies. Initial CR and salvage treatment significantly affect survival after relapse. In a selected group of 43% of patients who relapsed after allo SCT and received intensive chemotherapy, 72% achieved CR and survived significantly longer.

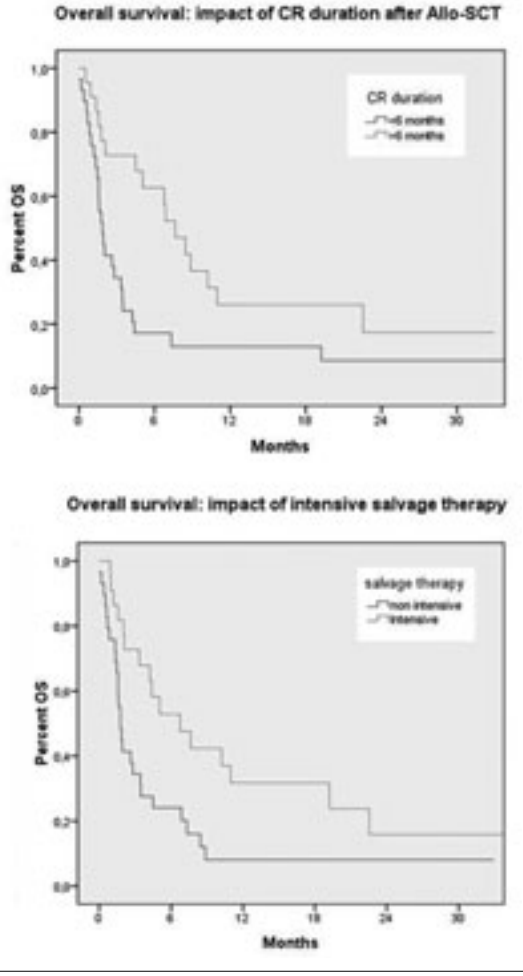

Figure 1.

0595

CONTINUOUS SEQUENTIAL INFUSION OF FLUDARABINE AND CYTARABINE FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) AND LOW BONE MARROW (BM) BLAST COUNT (20-30%)

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Background. According to WHO classification, the presence in the BM of 20 % or more blast cell is required for diagnosis of AML. Notwithstanding, the same WHO panel suggested that the 20% blast threshold is not a mandate to treat the patient as having AML, in that therapeutic decisions must always be based on the clinical situation after all information is considered. Accordingly, the treatment of these patients, namely in the elderly, is controversial. *Aims.* To investigate in a phase II trial the efficacy and toxicity of a regimen including fludarabine (F) and ARA-C (CI-FLA) in a series of 40 untreated patients aged more than 60 years with 20-30% BM blast count. A comparison with 100 patients with higher BM blast percentage uniformly treated in the same period is also presented. *Methods.* F at loading dose of 10 mg/sqm over 15 min at day 0, and after three hours and half ARA-C at a loading dose of 390 mg/sqm over 3 hours were given; then, F (20 mg/sqm/ci/24 hours for a total of 72 hours) and ARA-C (1440 mg/sqm/ci/24 hours for a total of 96 hours) were started. G-CSF was added at day +15 at a dose of 5 microg/kg. Patients achieving CR were programmed to receive a reduced course of CI-FLA, followed by G-CSF from day 15 to mobilize CD34+ cells and perform autologous stem cell transplantation (ASCT). Between June 2001 and October 2010, 40 out of 140 patients (28%) were found with 20-30% BM blast count. Median age was 67 years (61-81). Cytogenetic analysis was successful in 38 patients (95%) and showed normal karyotype (intermediate) in 24 patients (63%), while 14 patients (37%) had different chromosomal abnormalities and were classified as unfavourable. *Results.* Overall, 27 patients (67%) achieved CR. There were 5 induction deaths (12%), while 8 patients (20%) were refractory to induction treatment. The median number of days to neutrophil >0.5x10E9/l and platelet >20x10E9/l was 19 (7-34) and 20 (9-38), respectively. Documented infections occurred in 5 cases (12%). Twenty-two patients (81% of remitters) were eligible for consolidation and monitorized for mobilization of CD34+ cells, collection being successful in 15 of them (68%). Median number of CD34+ cells/kg collected was 6.8x10E6 (2.5-40.3), median number of apheresis being 2 (1-2). Thirteen patients (32% of the whole population) received ASCT. Median disease free survival (DFS) and overall survival were 9 and 10 months, respectively. Survival at 5 years is projected to 23%. The only parameter significantly related to DFS duration was the presence of unfavorable cytogenetics. In particular, DFS was 29 months for patients with diploid karyotype as opposed to 7 months for those with adverse one (p:0.001). Finally no difference was found with patients with > 30 % BM blast count as to CR achievement and duration, toxicity and overall survival. *Conclusions.* CI-FLA is effective and well-tolerated in elderly patients with low blast count AML. Therapeutic results are encouraging as to CR achievement and ASCT feasibility; however best results are achievable in the subgroup of patients with diploid karyotype.

0596

A HYPERTENSIVE PEAK SIGNIFICANTLY PRECEDES THE OCCURRENCE OF DIFFERENTIATION SYNDROME (DS) IN APL PATIENTS TREATED WITH AIDA BASED REGIMEN

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Background. Cardiovascular manifestations observed during DS, a life threatening side effect of ATRA, include hypotension, weight gain and pericardial effusion. The observation that several APL patients develop a hypertensive peak before the occurrence of DS led us to investigate its predictive value for such complication. *Patients.* 51 pts (31 female, 20 male) with genetically confirmed APL were treated with the Spanish PETHEMA LPA99 trial between 2004 and 2010. Median age was 40 years (range, 7-71). 2 pts (4%), 31 pts (61%) and 18 pts (35%), respectively, were Sanz's low, intermediate and high risk score. Additional cytogenetic abnormalities were observed in 39% of the pts. Median body mass index (BMI) was 24 kg/m^2 (range, 14-40). 43 pts achieved CR (86%). DS prophylaxis included prednisone 0.5 mg/kg from d1 to d15 in high risk pts (WBC >10 G/l). *Results.* According to Frankel's criteria 16 pts (32%) developed DS. Median time to onset of DS was 15 days (range, 2-29).Weight gain, pericardial effusion and hypotension were noted respectively in 50%, 37.5% and 18.7% of pts who developed DS. A hypertensive peak was observed 24 to 96 hours (median 36) before DS in 7 (43.7%) of the pts who developed this complication, of whom only one had a history of hypertension. Systolic and diastolic blood pressure values varied from 160 to 260 mmHg and 95 to 110 mmHg, respectively. 4 pts died from DS. By univariate analysis, age ≥ 40 (P=0.034), BMI ≥ 30 (P=0.009), baseline WBC ≥ 10G/l (P=0.034), serum creatinine >1.4mg (P<0.001) and absence of additional cytogenetic abnormalities (P=0.009) were associated with DS. A hypertensive peak was seen in 43.7% of the patients who developed DS compared to 11.7% of those without DS (P=0.011). Occurrence of a hypertensive peak was independent from the use of steroid prophylaxis (P=0.45), but was significantly associated with high BMI (P=0.003). *Conclusion.* Hypertensive peaks during induction treatment of APL may have a predictive value on the occurrence of DS.

0597

VALUE OF NPM GENE MUTATIONS IN RESPONSE ASSESSMENT AND MINIMAL RESIDUAL DISEASE EVALUATION IN DENOVO CYTOGENETICALLY NORMAL NPM+ AML

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Background. In normal karyotype AML patients the presence of Nucleophosmin (NPM) gene mutations at diagnosis is considered a good prognostic marker and FLT3 ITD negative NPM+ patients are not anymore considered for allogeneic tranplant in first complete haeamatological remission (CR). For minimal residual disease (MRD) evaluation the study of NPM gene mutations is not routinely performed and WT1 gene expression is considered the standard method for all patients. *Aims.* We evaluated NPM gene mutations as a possible indicator of quality of response and as marker of minimal residual disease (MRD), in comparison with WT1. *Patients and Methods.* One-hundred-fifty-eight bone marrow samples of twenty-four consecutive normal karyotype NPM+ denovo AML patients (median age 54 years) treated between May 2004 and June 2010 and achieving CR after two courses of conventional chemotherapy were studied. Follow-up analyses were censorized at the time of allogeneic transplantation or at the last bone marrow evaluation. A four-color flow cytometer was used to perform immunophenotype (IF). FLT3 ITD were performed according to the international standards of quality. NPM A and B DNA mutations and WT1 expression were studied by a quantitative Real Time PCR. *Results.* In 19 out of 22 patients in whom IF was done at response evaluation the flow cytometric analyses did not detect the clonal population observed at diagnosis (immunophenotypic CR, 86%). In 12/24 patients samples were negative for NPM mutations (molecular CR, 50%). Relapses in molecular CR patients and in those with persistence of NPM mutations were 5/12 (42%) and 11/12 (92%), respectively ($p < 0.05$). Patients with the first two consecutive NPM negative samples had a lower relapse rate compared to the other patients [3/11 (27%) vs 12/13 (92%); $p < 0.01$]. FLT3 ITD was detected in 8 patients. Five of them relapsed (median DFS 6 months, range 1-8), 3 are still in molecular CR (median DFS 21 months, range 8-69). In the follow up study of 15 patients who achieved a molecular CR the reappearance of NPM mutations was followed by haematological relapse in 8/9 patients (89%) with a median interval of 3,5 months (range 1-6 months). In 4 of these 8 NPM+ patients WT1 expression and IF were normal at the time of NPM gene evaluation. The median interval between WT1 increase and haematological relapse was 1 month (range 0-4). The median DFS in the 9 patients who achieved two consecutive negative NPM determinations and those who did not (15 patients) were 17 months (range 6-69 months) and 7.5 months (range 0-12 months), respectively. *Conclusions.* our preliminary analysis shows that the achievement of at least two consecutive negative NPM determinations is associated with prolonged haematological remissions. In the follow up of molecular CR patients the reappearance of NPM gene mutations is almost always followed by clinical relapse and may be considered an earlier relapse-marker than WT1 increase. Monitoring NPM gene mutations at response evaluation and in the follow up might help in defining subgroups of patients with high relapse risk and therefore likely to benefit of an early allogeneic transplant.

0598

ACUTE MYELOID LEUKEMIA PATIENTS WITH HIGHER BONE MARROW DENDRITIC CELL LEVELS IN COMPLETE REMISSION HAVE SUPERIOR DISEASE FREE SURVIVAL

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Background. Dendritic cells (DC) are unique lineage-negative antigen-presenting leukocytes which play a critical role in the regulation of the adaptive immune response. There is limited information on a potential association between bone marrow (BM) DC levels and minimal residual disease (MRD) status and outcome in patients with acute myeloid leukemia (AML). *Aims.* To establish levels of DC at AML diagnosis and during follow-up and relate the findings to the presence of MRD and disease free survival (DFS). *Methods.* Fifty-six out of 62 AML patients (≤ 65 years; 90%) achieved complete remission (CR) following intensive chemotherapy treatment at Karolinska University Hospital 2002-2006. Immunophenotyping by four-color flow cytometry (FC) on BM was performed at diagnosis to determine DC levels and aberrant blast features for MRD follow-up. Plasmacytoid DC (pDC) and myeloid DC (mDC) were identified by lin-/HLA-DR+/CD123+ and lin-/HLA-DR+/CD11c+ phenotypes, respectively and expressed as percentage of the total nucleated BM cell count. DC and MRD levels were assessed at CR (n=51) and after completion chemotherapy (n=26). A hospital control group (n=10) was used to determine reference DC levels in BM. Median follow-up time for patients alive was 78 months. *Results.* In the control group the median levels of pDC and mDC were 0.10% (range 0.02-0.27%) and 0.07% (range 0.02-0.19%), respectively. At AML diagnosis (n=62), pDC (median 0.00; range 0.00-1.2%) and mDC (median 0.00; range 0.00-0.8%) could be detected in only 15 and 17 patients, respectively. In all but two patients, DC levels increased at CR with median levels of pDC 0.16% (range 0-1.16%) and mDC 0.09% (range 0-1.36%). At the end of treatment the levels had in-

Table 1.

Levels of dendritic cells expressed as percentage of the total bone marrow cell count in AML patients at diagnosis, complete remission, follow-up, and relapse and in hospital controls

		p(DC) median	p(DC) range	m(DC) median	m(DC) range
AML patients	Diagnosis (n=62)	0.00	0.00-1.20	0.00	0.00-0.80
	CR (n=51)	0.16	0.00-1.16	0.09	0.00-1.36
	Post cons (n=26)	0.28	0.02-0.84	0.14	0.02-0.27
	Relapse (n=20)	0.05	0.00-0.65	0.03	0.00-0.37
Hospital controls	(n=10)	0.10	0.02-0.27	0.07	0.02-0.19

creased further, median pDC 0.28% (range 0.02-0.84%) and median mDC 0.14% (range 0.02-0.27). Patients with higher levels (defined as above the median value) of pDC at CR and after consolidation chemotherapy had longer DFS than patients with lower values, 22.5 vs. 11.5 months. No difference in DFS was observed in relation to mDC at CR (12.5 vs. 14 months) but patients with higher mDC after completion of consolidation chemotherapy had superior DFS (40 vs. 22.5 months). The number of patients with allogeneic SCT in first CR did not differ between the groups. MRD levels above 0.01-0.1% (depending on the sensitivity of the analysis) were detected in 80% of patients at CR and 35% at the end of post consolidation treatment. There was no significant association between DC levels and presence of MRD. Among 30 patients with relapse, DC levels were measured in 20 patients and were found to be almost as low as at diagnosis; median pDC 0.05% (range 0-0.65%), median mDC 0.03% (range 0-0.37%). *Summary/Conclusions.* No DC were seen in BM at AML diagnosis but both pDC and mDC regenerated after CR, sometimes to much higher levels by comparison to those in control patients. DC diminished again at relapse. Patients with higher levels of DC at CR and after completion of chemotherapy seem to have superior DFS. There was no correlation between MRD and DC levels. asa.derolf@karolinska.se

0599

FLUDARABINE, CYTARABINE, IDARUBICIN AND ETOPOSIDE (FLAIE) SCHEDULE IS SAFE AND ACTIVE FOR YOUNG PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA: RESULTS OF A MULTICENTRIC PHASE III ITALIAN STUDY

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Background. Fludarabine plus Cytarabine and Idarubicine (FLAI) was proved to be an effective and well tollerated induction regimen for treatment of acute myeloid leukaemia (AML). The trial objective was to assess the efficacy of Etoposide when used in combination with FLAI schedule. *Design and Methods.* We retrospectively report clinical outcome results of 101 newly diagnosed and younger than 60 years AML patients (median age 46 years, range 18-60 years) treated in a Phase III clinical trial, with FLAIE induction chemotherapy, including Etoposide to the FLAI schedule. Induction consisted of Fludarabine 25mg/m2/day on days 1-5, Idarubicin 6 mg/m2/day on days 1, 3 and 5, Cytarabine 2 g/m2 infused in 4 h, daily on days1-5 and Etoposide 100 mg/m2/day on day 1-5. After induction, all the patients underwent consolidation with Cytarabine (2 g /sqm i.v. infusion on days 1-5) and Idarubicin (12 mg/ sqm i.v. infusion on days 1, 3 and 5). All the patients shared the same strategy for intensification, that was allogenic or autologous stem cell transplantation. After consolidation, maintenance treatment with Cytarabine was given to patients who obtained a complete remission but who could not undergo allogenic or autologous stem cell transplantation. More than half of the patients had abnormal karyotypes. Molecular analysis at diagnosis for the more frequent abnormalities was performed. Duration of CR and overall survival was estimated according to the Kaplan-Meier method. The CR duration was dated from start of CR to first evidence of recurrence. *Results.* After informed consent was obtained, the patient received a single induction course of FLAIE; 73 pts obtained a CR (72.2%) and 8 pts a CRp (7.9%) for an overall response rate of 80.2%. Fifteen patients (14.9%) had resistant disease, and 5 (4.9%) died during induction. After a median follow-up of 33 months, 75 patients (76%) are in continuous CR. The median CR duration and OS were 45 and 55 months, respectively. 11 pts underwent ABMT and 44 a BMT. Relapses were more frequent in patients who were not submitted to allogenic stem cell transplantation. Of the 55 transplanted patients, 28 (51.9%; 1 with chromosome 7 abnormality), were alive in CR after a median follow up of 10 months (range, 2 to 45 months) after transplantation, 14 (25.9%) relapsed (median DFS 4 months), and 13 (24%) died in CR or CRp of transplant related complications. The most common grade 3 adverse events included gastro-intestinal toxicities(i.e. nausea, vomiting, mucositis and diarrhoea), liver dysfunction, and skin rash. *Conclusion.* The combination of etoposide to FLAI is safe and active. Further studies exploring different dosing and scheduling are warranted, particularly in patients with poor-risk AML.

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0600

PRETREATMENT RISK FACTORS AND IMPORTANCE OF COMORBIDITY FOR OVERALL SURVIVAL, COMPLETE REMISSION AND EARLY DEATH IN PATIENTS WITH ACUTE MYELOD LEUKEMIA

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Background. The prognosis and clinical course of acute myeloid leukemia (AML) differs among patients depending on specific the features of the disease, such as cytogenetics, white blood cell (WBC) count and serum lactate-dehydrogenase (LDH) level. Moreover, patient-related factors including age, performance status and pre- or coexisting diseases (comorbidity) are of prognostic importance, too. *Aims.* The aims of this study were to determine the pretreatment risk factors for overall survival (OS), rate of complete remission (CR) and early death in patients with AML, as well as to estimate the influence of comorbidity on patients' outcome. *Methods.* This single-center study involved 145 patients with nonpromyelocytic AML with 58-month follow-up. The following parameters were estimated as the risk factors for OS, CR and early death: age, WBC (<30x109/L vs ≥30x109/L), level of serum lactate dehydrogenase (LDH) more than 1.5 x upper limit of normal and expression of CD34 antigen on leukemic blasts (<10% vs ≥10%). Performance status evaluated by Eastern Cooperative Oncology Group, ranged 0-4 (<1 vs ≥2). Cytogenetic risk group was assessed by recommendation of European LeukemiaNet: favorable, intermediate-I, intermediate-II and adverse group. Comorbidities were evaluated by using the hematopoietic cell transplantation-specific comorbidity index (HCT-CI). Patients treated by Medical Research Council 12. The risk factors were identified using the univariate and multivariate analysis. *Results.* The median patients' age was 55.6 years, range 18-79 years. The most significant risk factor for poor OS was the adverse cytogenetics: p=0.008, relative risk (RR)=1.406 (95% CI 1.092-1.810). The age ≥55 years indicated to be the most significant risk factor for poor rate of CR: p=0.001, RR=0.214 (95% CI 0.111-0.413). The most significant factor for early death was the HCT-CI ≥3: p=0.001, RR=0.357 (95% CI 0.202-0.630). The significant cut-off age for poor OS and CR was ≥55. The incidence of HCT-CI≥3 was significantly increased in patients with age of ≥55 years than in patients under age 55 (p<0.001). Half of the patients aged ≥55 years had HCT-CI≥3, other half of the patients aged <55 years had HCT-CI=0. Infection as a comorbidity had an impact on HCT-CI score (according HCT-CI definition) in most of patients (91.7%) with the age of <55 years. We estimated the risk factors for both of these groups. The most significant risk factor for OS in the group under age 55 was adverse cytogenetics: p<0.001, RR=1.731 (95% CI 1.239-2.342), while in the group with age ≥.55 years it was HCT-CI≥3: p=0.008, RR =1.195 (95% CI 1,011-1,412). In the group aged <55 years, the most significant factor for poor CR rate and for early death: p=0.007, RR=0.063 (95% CI 0.008-0.471) was infection as a comorbidity: p=0.005, RR=3.125 (95% CI 1.410-6.928), while in patients with age ≥55 years it was elevated serum LDH level for CR: p=0.049, RR=0.27, (95% CI 0.0784-0.9968) and leukocytosis: p=0.007, RR=1.0098 (95% CI 1.0027-1.0170) for early death. *Conclusions.* This study identified the pretreatment risk factors for OS, CR and early death in patients with AML. Comorbidity, evaluated by HCT-CI has important influence on OS and early death in these patients.

0601

AZACYTIDINE FOR ACUTE MYELOID LEUKEMIA IN ELDERLY OR FRAIL PATIENTS: A PHASE II STUDY (SAKK 30/07)

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Introduction. Acute Myeloid Leukemia (AML) in the elderly is difficult to treat. Azacytidine has been shown to improve survival in patients (pts) with myelodysplastic syndromes. We present results of a phase II trial treating elderly or frail AML pts with azacytidine. *Methods*. AML pts unfit for intensive chemotherapy, with a WHO performance status ≤ 3 were eligible. Trial therapy consisted of azacytidine 100 mg/m2 injected subcutaneously on 5 consecutive days every 28 days for up to 6 cycles stopping at 6 cycles if no hematological improvement (HI) was observed, stopping early in case of progression or complications, and continuing beyond 6 months for responding pts. The primary endpoint was complete (CR) or partial remission(PR) within 6 months. *Results*. Between September 2008 and January 2010, 45 evaluable pts across 10 Swiss centers were accrued with a median follow-up of 11 months (95% confidence interval (C.I.) [9.3, 13.5]). 27 (60%) were male, median age was 74 (55-86) years and 35 (79%) had performance status 0-1. Pts received a median of 3 (1-14) cycles. Treatment was terminated because of lack of response after 6 cycles in 3 pts and earlier in 37 pts (disease progression in 12, toxicity in 4, patient refusal in 2, death with leukemia in 11 and poor performance status in 3, other in 5). Five pts remain on therapy. The median hospital stay in 27 pts admitted during the first treatment cycle was 13 days (1-30). Adverse events of grade III or higher most frequently reported were constitutional or hematologic, i.e. fatigue in 6, febrile neutropenia in 18, infections in 16, dyspnea in 7, thromboembolic complications in 2, hemorrhage in 5 and retinal detachment in 5. Among all 45 pts, 8 (18%,95% C.I.: 8-32%.) achieved CR/Cri, 6 (13%) HI and 15 (33%) stable disease within 6 months. Up to now 35 (78%) died. Median overall survival was 6 months. Age, gender and cytogenetic risk group had no significant impact on response, but there was a borderline association when pts were grouped by blast count (> or ≤ 30%, p=0.08). *Conclusions*. The current results show that the modified azacytidine schedule is a feasible option for elderly or frail AML pts in an outpatient setting with moderate, mainly hematologic, toxicity and response in a substantial proportion of pts, although the predetermined level of expected efficacy was not reached.

0602

LOW-DOSE LENALIDOMIDE COUPLED WITH LOW-DOSE CYTARABINE INDUCES COMPLETE REMISSION OF ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS WITH UNFAVORABLE CITOGENETICS: PRELIMINARY RESULTS OF A PHASE II STUDY

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Background. Elderly acute myeloid leukemia (AML) patients frequently fail chemotherapy. Median survival does not usually exceed few months. Recently, two groups demonstrated the efficacy of high-dose lenalidomide (50 mg/day, days 1-28). However, no reports demonstrated the efficacy of low-dose lenalidomide in combination with other drugs. *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 AML aged >70 years. The primary endpoint of the study was to determine the complete response (CR) rate according to SWOG criteria. *Methods*. Sixteen patients (median age 76 years, range: 70-80) were consecutively enrolled in the study. Median white blood cell count at diagnosis was 7.5 $x10^9$/l (range: 0.59-44$x10^9$/l), whereas median haemoglobin was 9.4 g/dl and median platelet count was 44$x10^9$/l. Two out of sixteen patients had a normal karyotype, whereas 14/16 presented with an intermediate or unfavourable karyotype. Twelve patients had a de novo AML, whereas 4 patients had a secondary AML (2 after MDS, 1 after CMPD, 1 after myelofibrosis). All 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 The study was registered at EMEA with the EUDRACT no 2008-006790-33, and is still recruiting patients. All patients gave written informed consent. *Results*. Two out of 16 patients are still receiving induction therapy, and are not evaluable for response. Five out of 16 patients died in aplasia while receiving the first induction cycle of therapy, and are not evaluable for response. Nine patients received at least one cycle of therapy and are evaluable for response. Among these patients, 4/9 (44%) cleared peripheral blood blasts at the end of the second week of the first cycle, with recovery of normal WBC, hemoglobin and platelets values after a median of 36 days (range: 31-42) from the start of chemotherapy. Three out of 4 responding patients are still in morphologic, cytogenetic and FISH CR after 11, 10 and 8 months from the start of therapy, respectively. The remaining patient died after receiving the third cycle of therapy while in CR due to a multi organ failure after an infectious complication. The other 5 patients who completed at least one cycle of therapy did not respond at all and rapidly died due to progressive disease. At present, 3/16 patients are alive in continuous CR, 2/16 are alive with active disease, 2/16 are too early and 8/16 died either in aplasia (5) or in progressive disease (3). Notably, all responding patients presented at diagnosis patients with low blast count and unfavorable cytogenetics. *Conclusions*. low-dose lenalidomide has clinical activity, when coupled with low-dose cytarabine, in an extremely poor-prognosis subset of AML patients. *Acknowledgements*. Celgene is gratefully acknowledged for providing Lenalidomide for the patients. The study was supported in part by AIL Pesaro Onlus.

0603

FENOTIPING ANALYSIS OF NK CELL AT DIAGNOSIS OF ACUTE MYELOID LEUKEMIA (AML): CLINICAL CORRELATION WITH OUTCOME

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Purpose. To study the expression of NKp30, NKp46, DNAm ad NCR fenotype in NK cells of patients with Acute Myeloid Leukaemia (AML) at diagnosis and their correlation with survival (OS) and relapse free survival (RFS). We decided not perform analysis of APL patients because all them are alive and in remission (PETHEMA LPA 2005 protocol). *Patients*. 48 patients diagnosed of AML from 2006 to 2010 were included. The median age is 65 year (21-87); a 60% of the patients are male. The FAB categories were: M0:8; M1: 4, M2:7;M3: 7; M4: 6; M5: 15, M6:1. Karyotype were: Good risk: 10 (22%); Intermediate: 19(43%); High risk: 15 (34%). White blood counts were 16X109/L (1,2-339x109/L); median bone marrow blast was 55% (21%-98%). 30 patients were treated according to LMA-2007 PETHEMA protocol (induction protocol Cytarabine 200 mg/m2 x7 days and Idarrubicine 12mg/m2x3 days; 10 patients were treated previously to LMA2007 with the same induction schedule, consolidation and 2 cycles of Ara C intensification (only normal Karyotypes and high-risk patients were submitted to allogenic bone marrow transplantation). Only one patient was treated with Azacitidine. *Methods*. NK cells from patients with AML were sorted in FACS. Expression of NK markers such as DNAM-1, NKP 46, NKp30, NCR phenotype were perfomerd against control of similar age. We also tested the effect of age in expression of DNAM-1 on NK cells comparing with normal controls (n=21). We tested also the expression of DNAM-ligands on AML blasts CD112 and CD155 and the correlation with DNAM-1 in NK cells. *Results*. Expression of DNAM-1, NCRs, NKp46 and NKp30 on NK cells in patients <65 was decreased compared with age-matched controls (p<0.04). Older patients differ only in NKp46 expression (p<0.03). It was a significant inverse correlation between DNAM-1 expression on NK cells and CD112 in AML blasts. DNAM-1 on NK cells is down regulated after co-culture with AML blasts expression DNAM-ligands. Nowadays, 14 of the 48 patients are alive. The median OS is 13,467 months. The median RFS was 50.966 months. In univariate analysis, OS was correlated

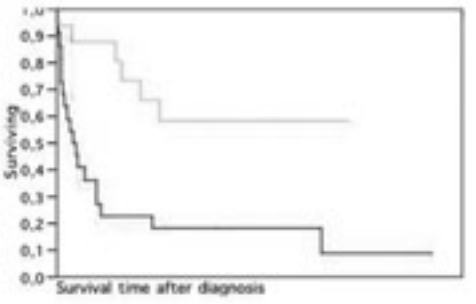

Figure 1.

with age>65 (Long-rank test: p<.0002); expression of NKp-46 (p<0.00160), NCR phenotype (Long-ran p<0,0112); expression of DNAM-1 (p<0,0297). Multivariate analysis was performed and only age (p=0,0001) and NKp-46 (p=0.05) was correlated with OS. This results point out NKp-46 in NK populations of AML patients at diagnosis as an independent factor of OS.

0604

DAY 14 BONE MARROW RESULT HAS LOW POSITIVE PREDICTIVE VALUE FOR REMISSION FAILURE IN ACUTE MYELOID LEUKEMIA

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Background. The initial objective of induction therapy for patients with acute myeloid leukemia (AML) is to achieve a complete remission (CR). The conventional measure of response following induction chemotherapy is the percentage of myeloblasts on a bone marrow biopsy (BMB) performed 28 days after chemotherapy. Previous studies have suggested persistent leukemia on a BMB at day 14 (D14-BMB) is significant, and some protocols recommend immediate further treatment when persistent myeloblasts are present on D14-BMB. *Aims*. At our centre, we routinely perform D14-BMB, with the intent to immediately administer further chemotherapy if persistent myeloblasts are found. The aims of this study are to assess our adherence to this strategy, and to assess how the results of D14-BMB impact on treatment outcome. *Methods*. This was a retrospective review of consecutive patients with a new diagnosis of AML treated at our centre between 1993 and 2007. Patients were included if they had induction chemotherapy and had D14-BMB. Through chart review, their subsequent course was determined, including any therapy prior to Day 28, and subsequent bone marrow biopsy results. *Results*. There were 129 patients who had induction therapy for AML and had a D14-BMB. Forty two (32.5%) had residual leukemia on day 14, defined by myeloblasts >4%; of these, 17 had immediate reinduction chemotherapy, 5 patients were changed to supportive care, and 20 had no further action until a repeat BMB was performed at time of peripheral blood count recovery. The CR rate on the recovery or day 28 marrow for the whole group was 67.4%. The CR rate for patients with residual leukemia on day 14 was 45.2%. For patients who had additional chemotherapy after day 14 it was 47% versus 55% for patient with persistent leukemia on day 14 who had no additional chemotherapy. The CR rate for patients who had no residual leukemia on day 14 was 82.4%. For the entire group, the results of the D14-BMB resulted in any intervention in 17% of patients. The finding of a positive D14-BMB resulted in any intervention in 52% of patients. The positive predictive value of the presence of myeloblasts >4% on the day 14 BMB, in patients receiving no further action until repeat BMB at peripheral blood count recovery, was only 45%. The negative predictive value for the finding of no residual leukemia on day 14 BMB was 78%. *Conclusions.* These data confirm that the day 14 BMB result is an important prognostic factor for remission status at day 28. However, nearly half of the patients with persistent myeloblasts on day 14 BMB had no further treatment until a repeat BMB was done around day 28, and over half of these patients were in remission on day 28. In our hands, the day 14 marrow has prognostic significance but low utility in altering treatment during the induction phase of patients with AML.

0605

CLOFARABINE THERAPY IN ELDERLY AML - AN IRISH REVIEW

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Background. Development of acute myeloid leukemia (AML) in elderly patients is considered a condition with a very poor prognosis. Standard treatment with low-dose cytarabine has few responses in high-risk patients and only an 18% overall response rate (ORR). Clofarabine is a second-generation purine nucleoside analogue which has activity versus AML and may produce improved responses especially in high-risk groups. *Aims*. We aim to examine the outcome of elderly patients, not fit for intensive therapy, treated with clofarabine as an initial, single agent therapy. *Methods*. This is a retrospective study of patients diagnosed with AML and treated first line with single agent clofarabine across five centers in Ireland. These patients were considered unsuitable for higher intensity treatment due to age or performance status. *Results*. A total of 24 patients were treated in 5 Irish centers between July 2007 and June 2010. 83% were males. The median age at the commencement of treatment was 74 years (range 64-80) and 79% of patients (n=19) were >70 years. Of the patients for whom analysis at diagnosis was available, 60% (9/15) had a poor risk genetic profile according to WHO classification. Patients received a median of 2 cycles of clofarabine treatment (range 1-3). 83% of patients (n=20) were treated with 20mg/m^2 for 5 days per cycle, the remainder received 30mg/m^2 for their first cycle. The overall response rate was 54% (n=13). Complete remission rate was 29% (n=7), with 8% of patients (n=2) having a complete response without count recovery. The principle adverse event was neutropenic sepsis and the 30-day mortality rate was 25%. The 6-month mortality rate was 62% and the 12-month mortality rate was 89%. The overall median time of survival was 16.5 weeks (range 1-384).In those who responded it was 39 weeks (range 6.5-384 weeks) and of non-responders was 4 weeks (range 1-20). Median survival in patients > 70 years was 17 weeks (range 1-130 weeks, mean 25.5 weeks). *Conclusion*. Clofarabine as a single agent was associated with promising activity in a poor risk group of elderly AML patients with predominantly poor risk cytogenetics with a CR/CRi of 37%. Unfortunately these responses were not durable with only 11% of patients alive at 12 months. Despite a possible improvement in response rates with new agents such as Clofarabine, the optimal post-remission strategy for these patients remains a major challenge and requires further studies.

0606

THE PREDICTIVE VALUE OF HEMATOPOIETIC CELL TRANSPLANTATION COMORBIDITY INDEX FOR EARLY DEATH AND SURVIVAL IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. The Hematopoietic cell transplantation comorbidity index (HCTCI) predicts non-relapse mortality and overall survival (OS) after stem cell transplantation. HCTCI score is predictive of early death and survival in elderly patients with acute myeloid leukemia (AML). *Aims*. The aim of this study was to determine the prognostic role of HCTCI for early death and OS in adult AML patients of all ages. *Methods*. In the single-center, retrospective study, we analyzed the outcome of 233 AML patients aged >=18 years (excluding promyelocytic AML) treated between 1993 and 2010. Eighty three percent of patients received chemotherapy. The rest of patients (17%) died before treatment (median survival 5 days, range 3-17 days) and they make a part of early death group (38%). Comorbidities were evaluated by using the Hematopoietic cell transplantation comorbidity index (HCTCI). *Results*. The median patients age was 57,3 years, ranged 18-85. Elderly population (above 60 years) makes 46% (107 Pts.) of entire group. The patient population was divided into those with HCTCI scores of 0 (low score), 1 or 2 (intermediate), or 3 and more (high score) according to original

description of the HCTCI scoring system. Forty eight per cent of patients had low HCTCI scores of 0, 24% had intermediate scores of 1-2 and 28% high scores of 3 or more. Early death rates in the three groups defined as death before specific treatment or within 30 days from time of commencing induction treatment were 30,6%, 42% and 69% respectively (Chi-Square P <0.00001). The significant difference in early death rates was present in subgroup analysis in elderly patients (>60 years, p<0.00001) as well as in younger patients (<60 years, p<0.05). When we compared all patients in early death with other patients the significantly higher WBC number (p<0.001), and HCTCI score (p<0.001) were found. OS was a median of 7,5 months in patients with a low HCTCI score of 0, 4,5 months in those with an intermediate score of 1-2, and 0,6 months in those with a high score 3 and more (p<0.001). In subgroup analysis, HCTCI score influence survival in elderly patients (p<0.01). In patients younger than 60 years there was significantly shorter survival in high HCTCI score group (0,77 months) comparing with low HCTCI score group (9,4 months) (p = 0.03327) but not in other comparisons. *Summary/Conclusions*. The results from this study indicated that HCTCI score is predictive of early death in adult patients with AML and overall survival in elderly patients with AML. High HCTCI score also predicts shorter survival in younger patients with AML.

0607

IMPACT OF FLUDARABINE-BASED INDUCTION THERAPY ON CLINICAL OUTCOME OF CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA (CN-AML) WITH FLT3-ITD AND/OR NPM1 MUTATION

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Background. Cytogenetically normal acute myeloid leukemia (CN-AML) defines a clinical entity with an heterogeneous outcome, as 5-year survival rates range from 24 to 42%. In this patients, accounting for about 45% of total AML cases, many important prognostic factors have been identified in the last years. Among these, mutations of FLT3 and NPM1 genes are the most widely studied. Fludarabine-based induction therapy displayed interesting results in AML, with high rates of complete remission (CR). However, no specific data are available about fludarabine effect on the outcome of CN-AML patients according to specific molecular "signature". *Aims*. To assess the impact of molecular abnormalities in CN-AML patients treated with fludarabine, we analyzed 137 patients treated with a fludarabine-based induction therapy, evaluated response according to internal tandem duplication (ITD) in FLT3 gene and/or nucleophosmin (NPM1) mutation. *Methods.* One hundred thirty seven patients with CN-AML and assessed for FLT3-ITD and NPM1 mutations were included in the study. All patients received fludarabine as a part of induction course, according to the institutional protocols. Patients considered at high risk of relapse for disease characteristics at diagnosis or poor response to induction therapy were considered candidate to allogeneic stem cell transplantation (SCT); in transplanted patients, survival was censored at the time of SCT. *Results.* Median age of patients (73 males and 64 females) was 54 years (range: 20-79), with 61 (44.5%) aged more than 55. Hyperleukocytosis (WBC > 30 x 10^9/L) and CD34 positivity were present in 50 (36.5%) and 54 (39.5%) cases, respectively. FLT3-ITD was detected in 44/137 (32%) patients. No association was found between FLT3 status and age, FAB subtype or CD34 expression, but FLT3+ patients had significantly higher WBC count (50 ± 75 x 10^9/L vs 42 ± 74 x 10^9/L, p=0.03). NPM1 mutation was studied in 100/137 (73%) cases and detected in 41/100 (41%) patients. NPM1 mutation was found in 17/32 (53%) FLT3+ patients and in 24/68 (35%) FLT3- ones (χ^2=2.17, p=0.14). All patients were evaluable for response to therapy. CR was achieved in 100/137 (73%) cases, and was affected only by age and CD34 positivity, while no correlation was found with WBC count, FLT3 and NPM1 mutations. Leukemia free survival (LFS) was significantly shorter in FLT3-ITD+ patients compared to un-mutated ones (11 vs 28 months, p=0.05), and in CD34 positive cases (10 vs 22 months, p=0.02). No differences were found considering WBC, age and NPM1 status. Overall survival (OS) was worse in elderly patients (p=0.001) and CD34+ cases (p=0.002), while FLT3 and NPM1 mutations did not have a significant impact. Considering survival by the combination of FLT3 and NPM1, LFS was similar in the four (FLT3+/NPM+, FLT3+/NPM-, FLT3-/NPM+, FLT3-/NPM-) groups, while a trend for longer OS was observed in FLT3-/NPM1+ patients compared to all other groups (5-yrs OS 60% vs 30%), even if difference didn't reach statistical significance. *Summary/conclusions.* Our data suggest that fludarabine was able to improve LFS and OS in FLT3-ITD+ patients, but did not completely abrogate the advantage of NPM1 mutation.

0608

EARLY APOPTOTIC RESPONSE AND CLINICAL OUTCOME OF INDUCTION CHEMOTHERAPY IN PATIENTS WITH DE NOVO ACUTE MYELOID LEUKEMIA. PILOT TRIAL

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Background. Many studies revealed that apoptosis is one of the major mechanisms of action of cytotoxic drugs used in treatment of leukemia. In *in vitro* conditions, leukemic cells undergo apoptosis after exposure to cytotoxic drugs within several days in culture but less is known about those effects *in vivo*. *Objective.* In pilot trial we evaluated morphologically recognized apoptosis measured as apoptotic index (AI) during induction antileukemic treatment and its relationship with clinical features and initial treatment outcome (CR/NR). *Materials and Methods.* We analyzed bone marrow samples (particles) from a cohort of 35 patients suffering from *de novo* AML, aged 46 yrs (19-64 yrs), treated with similar antileukemic induction treatment (ADE/MAE 26 pts, and DA/MC schedule 8 pts). After obtaining informed consent, samples were taken at diagnosis and also at 3rd day (D3) from start of therapy (48h of exposure *in vivo*). Analysis was performed on morphological level by counting cells with morphologically recognized apoptosis on at least 1000 cells and expressed as AI (%). We evaluated bcl-2 positivity in leukemic cells by immunohistochemistry with use of commercial Bcl-2 antibody and imaging kits (LSAB2 and CSA, Dako Danmark) at treshold of 20% bcl-2+ cells. Statistical analysis included parametric and nonparametric tests. *Results.* According to morphology of blasts, 20 patients had myeloid leukemia (3 with M1 and 17 with M2). Monocytic morphology was present in 15 patients (12 had M4 and 3 had M5 type). No dysplastic features were found. Twenty three patients achieved CR (65%) and 12 were non responders. Initial mean AI was 3.4±2.2%. There was no difference in initial AI between patients according to leukemia type (myeloid/monocyte) or prognostic karyotype groups (ANOVA & Kruskal Wallis & Mann Whitney U tests p>0.05). AI was increased to 8.1±4.7% at D3 (t-test p<0.01). We have not found differences in rise of AI concerning leukemia type or cytogenetic groups. When we analyzed increase of AI and outcome of induction therapy we have found that patients achieved CR have significantly higher therapy induced AI at D3 than nonresponders (ΔAI 6.4±5% vs. 2.5±1.9%, t-test and Mann Whitney p<0.05). Bcl-2 nega-

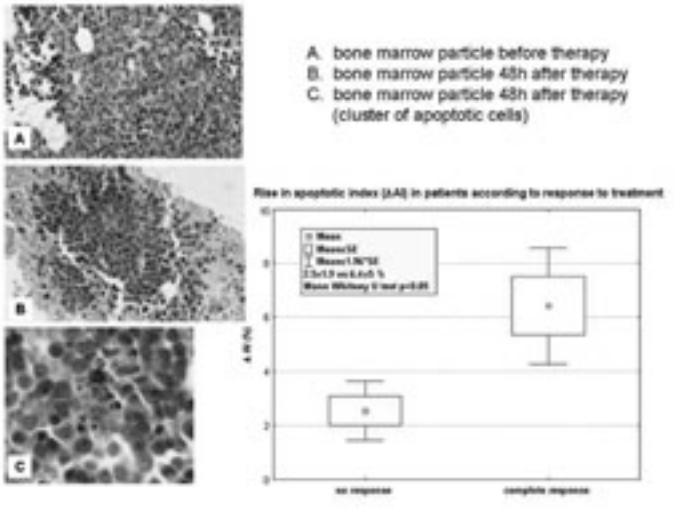

Figure 1. Apoptosis in bone marrow.

tive patients in CR had higher increase of ΔAI (6.1±1.9%) than patients with NR (1.8±1.6%). That difference was much lower in group of bcl-2 positive patients. *Conclusion.* Our results in a small cohort of patients revealed that we can also measure treatment induce apoptosis *in vivo*, during induction treatment of AML like in *in vitro* assays. That increase in apoptosis have impact on treatment outcome and possibly have prognostic significance like blast clearance, but further studies are needed.

0609

SECONDARY HEMATOLOGICAL MALIGNANCIES AFTER TREATMENT OF NON-METASTATIC BREAST CANCER

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Background. Survival of early breast cancer patients improved due to modern chemotherapies in last two decades. However long term toxicities as secondary hematological malignancies due to improved survival have been a major concern. *Aim.* We aimed to determine the frequency of secondary hematological malignancies in non-metastatic breast cancer patients (BCP) who received adjuvant chemotherapy and radiotherapy. *Method.* Data of BCPs followed at Hacettepe University Insititute of Oncology, Department of Medical Oncology between years 2004 to 2010 were analyzed retrospectively. *Results.* There were 1319 non-metastatic BCPs out of 1475 followed between years 2004 to 2010. 1183 (89,7%) patients among non-metastatic BCPs received adjuvant radiotherapy and/or neoadjuvant/adjuvant chemotherapy. 1066 (80,8%) patients received adjuvant or neo-adjuvant cytotoxic chemotherapy. Adjuvant radiotherapy was applied to 960 of non-metastatic BCPs (72,8%). 228 (17,3%) patients received only adjuvant/neoadjuvant chemotherapy and 117 (8,9%) patients received only adjuvant radiotherapy. 11 (1%) out of 1066 adjuvant/neoadjuvant chemotherapy received BCPs were also treated with granulocyte colony stimulating factors (GCSF). The frequency of secondary hematological malignancies among adjuvant or neo-adjuvant chemotherapy received BCPs was 0,56% (6/1066) and 0,59% (7/1183) among radiotherapy and/or chemotherapy treated non-metastatic BCPs. There were five AML patients; three of them were AML - FAB M3 and two of them could not be subclassified. One patient had multiple myeloma and the other had diffuse large B cell lymphoma. However the latter did not receive cytotoxic chemotherapy for breast cancer. *Summary/conclusions.* Treatment-associated secondary hematological malignancies, especially myeloid leukemias is a growing problem due to high prevelance of breast cancer and dismal outcome of secondary leukemias. Further studies are neded to determine the risk for other hematological malignancies with contributing factors; radiotherapy and novel chemotherapy agents.

0610

COMPARATIVE STUDY OF ACUTE MYELOID LEUKEMIA WITH INV(3)/T(3;3) AND OTHER ACUTE MYELOID LEUKEMIAS ASSOCIATED WITH ABNORMALITIES OF 3Q: A GROUP OF ENTITIES WITH THE SAME CLINICAL BEHAVIOR?

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Background. Acquired abnormalities of the long arm of chromosome 3 have been reported in approximately 3-3.5% of patients with acute myeloid leukemia (AML). However, the revised 2008 WHO classification recognizes only AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) as an independent clinicopathological entity with a poor outcome. Controversy exists concerning if all AML with any 3q abnormality could be reunited in the same group. *Aims.* Our purpose was to compare, from a biological and clinical perspective, a group of patients with AML inv(3) or t(3;3) with another group carrying different abnormalities of 3q at q21 or q26. *Methods.* We collected 55 patients [35 with AML inv(3)/t(3;3), 50 ± 20 years, range 14-84, men 54%, and 20 with AML associated with other 3q aberrations, 53 ± 19 years, range 2-79, women 55%]. The group of AML abn(3q) included four cases of t(2;3), three of t(1;3), three of del(3), two of t(3;10), two of der(3)t(1;3), and one of t(3;21), t(3;11), t(3;6), dup(3), del(3)t(3;12) and del(3)t(3;16). Among others, we recorded at diagnosis the existence of a previous history of MDS, chemotherapy or radiotherapy; blood cell counts, presence and degree of cellular dysplasia, serum lactate dehydrogenase, data from bone marrow aspirate and/or biopsy, immunophenotype of blasts, and other associated cytogenetic findings. The therapeutic approach (including hematopoietic stem cell transplantation, HSCT), response to treatment, overall survival (OS) and disease-free survival (DFS) were also recorded. In the statistical analysis, relations between variables were studied using the Fischer exact test (categorical) and the Mann Whitney U test (continuous). *Results*. There were no differences in sex, age, history of previous MDS or chemo-/radiotherapy, clinical presentation, WBC and platelet counts, haemoglobin level, serum LDH, degree of cellular dysplasia (significant in both groups) and bone marrow findings. The immunophenotype of blasts was similar (immature myeloid pattern, CD33+CD13+CD117+HLA-DR+, with frequent aberrant CD7 expression), but the frequency of CD34 positive cases was higher in the inv(3)/t(3;3) group (96% vs. 73%, p=0.04). Of note, the association with monosomy 7 was significantly more prevalent in the inv(3)/t(3;3) group (43% vs. 10%, p=0.01), while the presence of del(5q) or a complex caryotype were found in similar proportions (9% vs. 10%, and 26% vs. 30%, respectively). When intensive chemotherapy was used (80% and 78% of the cases), bone marrow remission after first cycle of induction was achieved only in 6% of patients with inv(3)/t(3;3) and 20% in the other group. Allogeneic HSCT was performed in 29% of patients with inv(3)/t(3;3) compared to none in the group of patients with other 3q aberration (p=0.05). Mortality rate and median OS and DFS were similar in both groups, extremely unfavorable. *Conclusions.* In our experience, clinical behaviour of AML with inv(3)/t(3;3) and other AML abn(3q) is similar. Although the association with monosomy 7 is significantly more frequent in the first group, this does not seem to influence outcome. Our results are in concordance with a recent refinement of cytogenetic classification in AML (Grinwade *et al.*, 2010), which includes both subset of AML patients together in the group of adverse prognosis.

0611

OUTCOME OF THERAPY RELATED ACUTE MYELOID LEUKEMIA - A SINGLE CENTRE EXPERIENCE

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Background. Therapy related acute myeloid leukemia (t-AML) is the most serious late complication of prior chemo (CT) and /or radiotherapy (RT) for malignant or nonmalignant diseases. This uncommon distinct entity is considered to have poor prognosis. *Aim.* To evaluate the prognostic parameters affecting outcome in t-AML managed in a single center. *Methods.* A total of 42 patients (pts) with t-AML (median age 56.07 years, range 23-84; 30 females) were diagnosed between 2000 - 2010. Prior malignancy had 37/42 (88.15%) pts - 9 hematological [CLL -2(4.8%), NHL- 4(9.5%), HL -2(4.8%), B-ALL 1(2.4%)] and 28 solid cancers, predominantly of breast - 14(33.3%) and cervix - 6(14.3%). Five (11.9%) pts. had no malignancies (2 multiple sclerosis, 3 connective tissue diseases). History of previous CT alone had 18/42 (42.9%), RT alone 11/42 (26.19%) and combined CT+RT 13/42 (30.1%) pts. G-CSF during therapy for prior disease had been administered in 3/42 (7.1%) pts. Therapy related myelodysplastic syndrome (t-MDS) preceded t-AML in16/42 (38.1%) pts. On t-AML diagnosis median ECOG performance status was 2.5 (ECOG≤1- 7/42; 16.7% pts.) and median HCTI-comorbidity index was 3.69 (HCTI-CI<3- 10/42;

23.8%). Cytogenetic risk groups were assessed according to refined MRC criteria. *Results.* For all 42 pts median WBC count was 27.23x109/L (range 1.1-177), platelet count 62.29x109/L (6-207), hemoglobin level 87.83g/L (47-133), peripheral blood blast percentage 30.7% (0-98), bone marrow blasts 66.7% (22-98), LDH 1216 U/L (257-6204). DIC was registered in 30/42 (71.4%) pts. Median latency period from prior CT/RT was 54.62 months (6-243); primary disease was active in 11/42(26.2%) pts. FAB distribution was as follows: M0-1(2.4%), M1-3(7.1%), M2- 15(35.7%), M3- 3(7.1%), M4- 6(14.3%), M5- 6(14.3%), M6- 1(2.4%). In 5 pts FAB could not be determined and 2 (4.8%) pts had T/My AML. Coexpression of B and T lymphoid markers was registered in 7/39 (17.9%) and 6/39 (15.4%) pts, respectively. Cytogenetic analysis was eligible in 33/42 (78.6%) pts: favourable karyotype 5/33 (15.2%), intermediate 14/33 (42.4%) and unfavorable 14/33 (42.4%). Normal karyotype was registered in 9/33 (21.4%) pts, while complex and monosomal karyotypes were registered in 8/33 (24.2%) and 9/33 (27.3%) pts, respectively. Abnormalities of chromosomes 5 and/or 7 were present in 10/33 (30.3%) pts, either alone or within complex/monosomal karyotypes. Total of 24 (57.1%) pts received standard induction CT (3+7 and variants). Haematologic complete remission (CR) was achieved in 10 (23.8%). Early death within two weeks occurred in 8 (19%) pts. For all 42 pts, the median overall survival (OS) was 5.94 months (range 0.5-34); in 10pts achieving CR median disease free survival (DFS) was 11.8 months (range 4-32 months). Among all parameters assessed only pretreatment karyotype, ECOG PS, HCT-CI and activity of primary disease had impact on outcome. ECOG PS, HCT-CI and active primary disease had impact on OS ($p<0.05$). Favorable karyotype proved a good prognostic parameter concerning outcome, CR rate, OS and DFS ($p<0.05$). *Conclusion.* Survival in t-AML is very poor. Among all evaluated patient-related and disease-related parameters only pretreatment karyotype proved significant concerning CR rate, OS and DFS, while ECOG PS, HCT-CI and active primary disease influenced only OS.

0612

CLOFARABINE IN THE TREATMENT OF ACUTE MYELOID LEUKEMIA - SINGLE CENTER EXPERIENCE

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Background. Clofarabine (CLO) is a purine nucleoside antimetabolite. It demonstrated its efficacy and safety not only in acute lymfoblastic leukemia patients (pts.), but also in pts. with acute myeloid leukemia (AML). *Aims.* To evaluate efficacy and safety of CLO in salvage treatment of AML. *Methods.* A retrospective analysis of pts. with AML treated with clofarabine-based regiments. For refractory or hematological relapsed AML we used combination with antracycline (CLO 22.5 mg/m2 once daily intravenously for 5 days plus idarubicine 9 mg/m2 intravenously for 3 days, consolidation cycle with 25% dose reduction) and for pts. with molecular relapse CLO in monotherapy (CLO 40 mg/m2 intravenously for 5 days, consolidation cycle in the same dose). *Results.* In the study period 4/2009 - 9/2010 we have treated 21 pts. with CLO-based regiments (9 pts. with molecular relapse and 12 pts. with hematological relapse or refractory AML). The mean age was 49 years (range 18-67 years). 7 pts. had favorable, 10 pts. intermediate and 4 pts. adverse cytogenetic risk. In relapsed pts., observed time to molecular relapse was 5.3 months (n=9) and to hematological relapse 10.2 months (n=4). From 9 pts. with molecular relapse 6 pts. (67%) achieved complete molecular remission, 1 pts. (11%) partial molecular remission and 2 (22%) had molecular or hematological progression. From 12 pts. with refractory and relapsed AML 5 (42%) achieved complete remission (CR), 3 (25%) partial remission (PR), 1 (8%) stable disease (SD), 2 (16%) progression and 1 died before assessment. 6 pts. received 1-3 consolidation cycles and 10 pts. underwent allogeneic hematopoietic stem cell transplantation (allo HSCT) (6 pts. in CR, 4 pts. with active disease). We observed neutropenia and thrombocytopenia gr. III-IV (according CTCAE 4.0) in 100% of CLO treated pts. Infections were the most frequent complications associated with CLO therapy - 4 pts. (44%) treated for molecular relapse and 8 pts. (66%) with relapsed or refractory AML, 5 (42%) of them had severe infection (pneumonia). The frequency of infectious complication decreased after introduction of posaconazole prophylaxis (66% vs.33% respectively). Mortality in molecular relapse group and relapsed/refractory disease group was 22% and 67%, respectively. 6 months disease free survival (DFS) in molecular relapse group was 85% and overall survival (OS) 75% and in relapsed/refractory disease group 75% and 50% respectively. *Summary/Conclusions.* The use of clofarabine shown 78% response rate in pts. with molecular relapse AML and 67% in pts. with hematological relapse or refractory AML. The most serious complications are severe infections, which could be reduced using posaconazole prophylaxis.

0613

VALIDATION OF BONE MARROW ASPIRATES IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA AT DAY 14 OF INDUCTION THERAPY

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Background. Current treatment of non-M3 acute myeloid leukemia (AML) usually includes 7+3 chemotherapy followed by a bone marrow assessment between day 14 (D14) and 17 after treatment initiation. This evaluation may change decisions concerning therapy and have prognostic implication. *Aims.* The objectives of this study were to evaluate different modalities of D14 bone marrow aspirate (BMA) evaluation (quantitatively and qualitatively), determine the inter-observer agreement and compare the results with D14 bone marrow biopsy (BMB). We included 119 patients with AML who received 7+3 and had D14 bone marrow evaluation. The analysis was performed by two independent observers, blind to patient and remission status. The evaluation included confirmation of the diagnosis of AML and identification of residual leukemia in a quantitative (percentage of blasts) and qualitative (Likert scale) manner. The qualitative assessment of blasts was determined by stratification in 5 categories: definitely infiltrated, probably infiltrated, doubtful, probably free and definitely free. *Results.* The evaluation of the BMA (n=107) by both observers using a Likert scale yielded a significant agreement between observers (Kappa w = 0.737, 95% CI 0.642 to 0.832, $p<0.001$). The correlation of the quantitative evaluation was also significant ($rs=0.798$, $p<0.001$). ROC curves were obtained correlating the BMA quantification of blasts and Likert scale by both observers with bone marrow biopsy results (n=82). The areas under the curve (AUC) were 0.924 and 0.946 for observer 1 and 0.867 and 0.870 for observer 2 for assessments of the number of blasts and Likert scale, respectively. Comparing the ROC curves between the two methods of BMA evaluation (quantitative and qualitative) by the same observe we found the differences between AUC of 0.025 observer 1 ($p = 0.220$) and 0.002 for observer 2 ($p = 0.967$). The evaluation of different cutoff points for blasts percentages in BMA showed a better sensitivity and specificity at the rate below 6% and 7% blasts for observers 1 and 2, respectively. A similar analysis for the Likert scale showed the best cutoff point as the 4th item of the scale (probably infiltrated) for both observers. *Conclusion.* Evaluation of bone marrow at D14 using quantitative or qualitative scale had a significant agreement between observers. Both tests were predictive of bone marrow involvement comparing with biopsy. The evaluation using a qualitative scale is easier to perform.

0614

THE COST-EFFECTIVENESS OF A NEW DIAGNOSTIC TEST THAT IDENTIFIES NEW PROGNOSTIC SUBTYPES IN ACUTE MYELOID LEUKEMIA

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Background. Acute myeloid leukemia (AML) is a heterogeneous disease consisting of cytogenetic and molecular subtypes with a prognostic impact. Currently, patients are classified into risk groups mainly based on the presence of cytogenetic abnormalities. However, large differences in survival outcome still exist within the intermediate risk group. Therefore, many studies now focus on identifying new prognostic subtypes within the intermediate risk group. Based on this research, a new diagnostic test, the AMLprofiler, has been developed that identifies some of these new prognostic subtypes. *Aim.* The aim of this study is to evaluate the cost-effectiveness of the new diagnostic test for patients with AML aged 18-60 years. *Methods.* An individual patient simulation decision-analytic model has been developed, because the cost-effectiveness cannot be evaluated alongside a clinical trial due to the small incidence of the new subtypes. The structure of the model and the identification of relevant parameters were based on the literature and interviews with clinical experts. All input parameters were estimated from clinical trial data from patients aged 18-60 years. The model was validated by comparing the model-based survival results with the results from the clinical trials and the literature. The model was used to estimate survival outcome and costs of two alter-

natives: current care and care if the AMLprofiler is used at time of diagnosis. Resource use was derived from all patients diagnosed in 2008 and 2009 in two hospitals. The resource use was combined with Dutch tariffs to calculate the costs for the Netherlands. *Results.* The AMLprofiler identifies patients who could be treated with chemotherapy instead of a (allogeneic) stem cell transplantation (SCT). These patients would not have to be exposed to the high risks of the transplantation. Other patients were identified who would need the most intensive treatment to have some chance to be cured. These patients coull be given other types of allogeneic SCT (from a matched unrelated donor or cord blood transplant) instead of autologous SCT or chemotherapy. Combining this information in the decision-analytic model showed that the use of the AMLprofiler could lead to higher 5-year overall survival and lower costs. *Conclusion.* The AMLprofiler has the potential to be a dominant choice over current tests as the use of the AMLprofiler could lead to higher overall survival and lower costs. The health outcome could be further improved if targeted treatments were to become available for the specific subtypes of AML.

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Biology of thrombosis

0615

LARGE PNH CLONES ARE UNCOMMON IN PATIENTS WITH INTRAABDOMINAL THROMBOSIS

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Background. Paroxysmal Nocturnal Hemoglobinuria (PNH) usually presents with hemolysis or cytopenias. Thrombosis, often at unusual sites-intraabdominal, cerebral or dermal may develop during the course of the disease. However there is a paucity of data on how many cases of PNH present primarily as thrombosis. Current guidelines recommend screening for PNH in patients with the above mentioned sites of thrombosis .We screened patients with intraabdominal thrombosis to determine the frequency of the PNH clone by flow cytometry in this population *Aim.* To assess the presence of PNH clones in RBCs and granulocytes, by flow cytometry in patients with intrabdominal thrombosis. *Methods.* Patients with intra abdominal thrombosis -Budd Chiari Syndrome (BCS), Extra Hepatic Portal Vein obstruction (EHPVO), mesenteric, iliac or renal vasculature as confirmed by imaging studies and referred for thrombophilia workup to the department of Hematology, PGIMER, Chandigarh were included in the study. Patients with recent transfusions and blood samples more than 48 hours old were excluded from analysis. EDTA peripheral blood samples were analysed by flow cytometry using CD55, CD59 on both RBCs and granulocytes and additionally CD16 on granulocytes. 10,000 events were acquired and analysed on the FACS Calibur (BD Biosciences).Patients with > 5% deficient RBCs and granulocytes were labelled positive for PNH clone .Normal controls were included in each run. *Results.* In the 3 year period, 86 adult cases of intra abdominal thrombosis were tested. There were 50 (58%), 27 (32%), 3 cases of EHPVO, BCS and Superior mesenteric vein thrombosis respectively. Iliac vein thrombosis, isolated arterial and combined arterial and venous thrombosis was seen in a single case each. Recurrent thrombosis with involvement of the abdominal vessels at least once was seen in 3 cases. Patients with EHPVO had significant cytopenias as compared to those with BCS. Small populations of CD55 deficient RBCs were seen in 8 cases (9.3%). None of the cases showed significant CD 59 negativity on the RBCs. Whereas 3(3%) cases had granulocytes with CD 59 deficiency, CD 55 deficiency was seen in a single case. Results for CD 16 showed presence of a clone in at least 11 out of the 52 cases tested. This was not significant when compared with normals. *Summary/Conclusion.* Flow cytometry with CD55 and CD 59 yielded isolated small clones with a PNH phenotype in the RBCs or granulocuytes of 12 (13.9%) cases patients with intraabdominal thrombosis. No case had deficiencies on both red cells and granulocytes. CD 16 was not a useful marker for detecting PNH cells in this population. Large clones of PNH type cells, reported to occur in thrombosis in known patients of PNH on follow up, were conspicuously absent in the study population. PNH with a primary thrombotic presentation is therefore rare in cases with intraabdominal thrombosis. In a resource constraint setting, effectivity of screening all patients with intraabdominal thrombosis for PNH therefore needs reconsideration.

0616

This abstract has been withdrawn by the authors.

0617

NITRIC OXIDE STIMULATES AND REGULATES ERYTHROPOIETIN RECEPTOR IN ENDOTHELIAL CELLS

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Erythropoietin (EPO) is a cytokine that stimulates erythropoiesis and also induces its own receptor (EPOR) during differentiation of erythroid progenitor cells. However, as we showed previously, EPOR is expressed in cells beyond the erythroid lineage including endothelial cells. EPOR in endothelial cells is further increased at low oxygen tension. With EPO stimulation of endothelial cells during hypoxia, there was also a corresponding increase in nitric oxide (NO) production and endothelial NO synthase (eNOS) expression. We now present evidence for NO regula-

tion of endothelial EPOR expression. We use primary human umbilical vein endothelial cells (HUVECs) and the human bone marrow microvascular endothelial cell line (TrHBMEC) to study effect of NO donor diethylenetriamine NONOate (DETANO) on EPOR expression. HUVEC treated with 10-50 μM of DETANO at different oxygen tension for 24 hours showed statistically significant induction of EPOR gene expression at 5% and 2% of oxygen with 25 μM of DETANO and 50 μM of DETANO at 2% oxygen. Also TrHBMEC cultured at 21 and 2% oxygen for 3, 24, 48 hours with 50 μM DETANO demonstrated a time and oxygen dependent induction of EPOR mRNA expression after 24 hours particularly at low oxygen tension. In TrHBMEC and HUVEC EPOR protein was significantly induced by DETANO at 2% oxygen. We used EPOR promoter/luciferase reporter gene assay to examine if NO could regulate EPOR expression at transcriptional level. HeLa cells were transfected with different constructs containing the EPOR 5' UTR and extending upstream to about 2 kb and 200 bp from the transcription start site. DETANO treated HeLa cells, with the 2 kb promoter construct, increased luciferase activity at 2% oxygen about 50 %, and 70 % with the 200 bp construct. There was no induction of EPOR promoter activity after DETANO stimulation at 21% of oxygen, suggesting that NO regulated EPOR expression at the transcriptional level in promoter region only at low oxygen tension. Different signaling pathways in TrHBMEC were examined after DETANO stimulation and we found that DETANO activated MAPK kinase in TrHBMEC after 30 min both at 21% and more at 2% oxygen. The effect of EPO on MAPK activation was not statistically significant. However, both EPO and DETANO induction of EPOR were blocked with MAPK inhibitor PD98059, suggesting direct effect of DETANO on MAPK kinase activation. These data provide a new effect of NO on EPOR expression and regulation in endothelial cells under normal and low oxygen tensions.

0618

NEW METHOD OF PROTHROMBOTIC TENDENCIES PREDICTION IN SEPSIS: SPATIAL CLOT GROWTH

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Background/Aims. Inflammation in sepsis activates blood coagulation that may lead to thromboses of the vessels of the microcirculation and consequently organ failure. So estimation of haemostasis system state is extremely important. Conventional diagnostic methods are insensitive to procoagulant changes in sepsis. Our aim was to study changes in haemostasis system state for patients with sepsis and septic shock by a method of spatial clot growth created in our laboratory and to compare the sensitivity of this new method to conventional ones. *Methods.* 16 patients (age 21-60) with hematological malignancies and sepsis were enrolled in the study. All patients have been surveyed from 1st to 5th day and on 7th, 14th, 21st and 28th days from the infection beginning. The spatial clot growth is the original method of investigation of haemostasis. Its principle consists in registration of clot formation after local activation of coagulation by immobilized tissue factor by light scattering in non-stirred thin layer of platelet-free plasma containing inhibitor of contact activation. Contemporarily clotting time tests, thromboelastography, plasma D-dimer level assay were performed. *Results.* Spatial clot growth showed growing hypercoagulation in 6 patients. Plasma D-dimer levels rose after that in 5 of them. Contrariwise, plasma D-dimer levels did not raise and were statistically significant lower if spatial clot growth did not show hypercoagulation. Mean values were 457μg/l and 234 μg/l, respectively (P<0.05). Other tests did not show hypercoagulation during all time of the study. Other 10 patients had elevated plasma D-dimer level on 1st day. It gradually reduced or stayed the same. Spatial clot growth showed normalization of coagulation in survivors or growing hypocoagulation in non-survivors. Routine tests showed normal and hypocoagulation or only hypocoagulation respectively. *Conclusions.* Spatial clot growth is more sensitive to the procoagulant changes of haemostasis system state than routine tests and can predict prothrombotic tendencies in sepsis.

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0619

DIVERGENT ROLE IN THROMBOTIC AND INFLAMMATORY MECHANISMS REGULATED BY THE G455A POLYMORPHISM OF THE BETA CHAIN OF FIBRINOGEN IN PATIENTS WITH STABLE ANGINA

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Purpose. Patients with coronary artery disease (CAD) exhibit increased levels of thrombotic and inflammatory biomarkers including fibrinogen, interleukin 6 (IL-6) and sCD40L. However, it remains obscure, whether the genetic variability on fibrinogen beta chain modifies thrombosis and inflammation in those patients. In the present study we investigated the impact of the G455A genetic polymorphism on the aforementioned biomarkers. *Methods.* We genotyped 393 patients with documented CAD and 236 controls. The G455A polymorphism was determined by polymerase chain reaction (PCR) and the specific HaeIII restriction enzyme. Serum levels of fibrinogen were measured by the von Clauss method. Moreover, IL-6 and sCD40L levels were assessed by enzyme-linked immunosorbent assay (ELISA). *Results.* Genotype distribution was GG: 54.6%, GA: 36.8%, AA: 8.6% for controls and GG: 50.1%, GA: 42.0%, AA: 7.9% for CAD. Interleukin 6 levels (pg/ml) were enhanced in patients with CAD compared to controls (4.40±3.13 vs 3.41±2.76, p<0.01). However, the G455A polymorphism failed to affect IL-6 levels between GG+GA vs AA both in CAD and controls (p=NS). Similarly, sCD40L levels (ng/ml) were significantly higher in CAD compared to controls (2.07±1.47 vs 1.82±1.68, p<0.001). Although no difference was observed in sCD40L across the study genotypes both in controls and in CAD (p=NS), the G455A polymorphism defined sCD40L levels (GG+GA vs AA) in the total population (1.91±1.41 vs 2.77±2.23, p<0.05). Finally, fibrinogen levels (mg/dl) were significantly higher in CAD compared to controls (436.0±126.8 vs 373.2±91.7, p<0.001). Importantly, the present polymorphism affected significantly fibrinogen levels (GG+GA vs AA) not only in controls (366.6±85.8 vs439.1±122.3, p<0.05), but also in CAD (426.0±122.7 vs 521.8±113.1, p<0.001). *Conclusions.* The G455A genetic polymorphism has a striking effect on fibrinogen levels both in controls and in patients with coronary artery disease. In addition, it affects partly sCD40L in the total population. Our findings suggest that the G455A polymorphism affects the thrombotic process and consequently promotes atherosclerosis, especially via its significant impact on fibrinogen.

0620

IDENTIFICATION OF MOLECULAR BASIS OF ANTITHROMBIN DEFICIENCY: CLINICAL FEATURES OF 30 INVESTIGATED PROBANDS AND REPORT OF 12 NOVEL MUTATIONS

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Background. More than 200 mutations of antithrombin (AT) gene cause type I (quantitative) or type II (qualitative) deficiency. Type II is subclassified according to dysfunction of the reactive site (RS) or heparin binding site (HBS) or pleiotropic effects. Type II HBS is associated with a low thrombotic risk. *Aims.* To investigate the gene mutations causing AT deficiency. *Patients and Methods.* DNA of 23 patients with AT deficiency and venous thromboembolism (VTE) was sequenced according to Picard *et al.* (Thromb Haemost 2005;93:57). Seven asymptomatic women with AT deficiency identified by screening before pregnancy or oral contraceptive intake were also analyzed. *Results.* Twenty-one different mutations were identified in patients with VTE. Three novel (E34X, W307X, Y260_P352del -two cases) and five known (C-4X, A94V, R129X -two cases, R132X, R425QfsX8) mutations were found in ten heterozygous patients with type I deficiency. In two of them heterozygous factor V Leiden (+R129X) and prothrombin G20210A (+C-4X) were present. Four novel (E205K, E265K, D342G, E377D) and one known (M251I) mutations were found in five heterozygous patients with type II RS deficiency, in one of them associated with heterozygous factor V Leiden (+E377D); three known mutations (L270P, A404T, L409P -two cases) were found in four heterozygous patients with pleiotropic (quantitative-qualitative) AT deficiency. Two known mutations (P41L, R47C) were found in two heterozygous patients with type II HBS deficiency, in both cases with additional abnormalities (LAC and anti-beta-2-glicoprotein I in the former case, homozygous factor V Leiden in the latter). One novel heterozygous mutation (E180K) was found in one patient with type II HBS deficiency who had first VTE at 77 years of age. Finally, one patient with type I deficiency was double heterozygous for two novel mutations, L210PfsX43

and G2R; the father heterozygous for L210PfsX43 had type I deficiency, whereas heterozygous G2R mutation had null effect in the mother. Among the asymptomatic women, three with type I deficiency had two novel (S250IfsX16 and V303CfsX13) and one known (C-4X) heterozygous mutations. Four with type II HBS deficiency had three known heterozygous mutations (R47H, R47C, L99F -two cases), in one of them associated with heterozygous prothrombin G20210A (+L99F). In four pregnant women (one type I and three type II HBS) antithrombotic prohylaxis was tailored according to the phenotype. *Conclusions.* The molecular bases of AT deficiency are heterogeneous; their identification can provide data to understand AT structure-function and to give advice for antithrombotic prophylaxis tailored according to different AT deficiency subtypes.

0621

THE RELEASE OF PLATELET FACTOR 4 (PF4) IS IMPAIRED IN PATIENTS WITH ET, ESPECIALLY THOSE PRESENTING BLEEDING COMPLICATIONS

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Background. Essential thrombocythemia (ET) is characterised by bleeding tendency, thrombotic complications and qualitative platelet defects. PF4 is synthesized in megakaryocytes and stored in platelet α-granules. After platelet activation PF4 is released in high concentration in the vicinity of vessel wall injury. That is why PF4 is the most important marker of platelet activation. According to the literature, clot formation and stability are optimal at phisiological levels of PF4. Both increased and decreased PF4 levels in platelets disrupt normal clot development. Our previous results indicated that platelets of ET patients release less PF4 during *in vitro* clot formation, but the examined group of patients was too small. *Aim.* In this study we assessed serum PF4 level and plasma activity of main coagulation factors in a group of 106 ET patients and looked for correlation between these parameters and bleeding or thrombotic complications. *Methods.* We have examined 106 patients with ET (80 females and 28 males, mean age 54 (23-82). The control group (CG) consisted of 20 healthy persons: 6 males and 14 females (mean age 41 (31-54)). We evaluated serum level of PF4, cholesterol and triglycerides and plasma activity of factors: I, VIII, XII, AT, protein C and S. We assessed also von Willebrand factor antigen and activity and urokinase plasma level. *Results.* In 21 patients (19.8%) from ET group 37 thrombotic complications occurred an in 22 (20,75%) patients bleeding episodes were noticed. Concentration of PF4 was higher in ET patients serum as compared to the CG (median 86.25;P25-75%,66.69-98.18 versus 70.00;49.00-90.50IU/ml, p<0.05). To eliminate the influence of elevated PLT amount on PF4 concentration a ratio of PF4 per million platelets was calculated. The ratio PF4/ 1mln PLT was significantly lower in ET group as compared to CG (median 108.94,P25-75%,82.40-125.06 versus 251.71,180.52-387.36, p <0.001). Additionally, PF4/ 1mln PLT ratio was statistically significantly lower in patients presenting bleeding complications compared to patients with thrombotic complications (median 95.42,P25-75%:74.05-120.66, versus 121.29,P25-75%,93.39-154.88, p<0.05). When evaluating plasma activity of main coagulation factors we found an interesting tendency. Von Willebrand factor activity (vWF:RCo) was reduced below normal limit in 25% of ET patients with bleeding episodes, in 10,5 % of ET patients with thrombotic complications and in 21,8% of ET patients without any complications. Because of this, the median von Willebrand factor activity in patients with bleeding episodes was lower than in patients with thrombotic ones (median 66.50%, P25-75%, 48.90-90.70 versus 87.40%, 67.00-134.00, p<0.05). The urokinase serum level was higher in ET patients compared to CG (median 0.581, P25-75%, 0.456-0.823 versus 0.439; 0.354-0.558 ng/ml, p<0.05) but we didn't notice any difference between patients with bleeding and thrombotic complications. *Conclusions.* Platelets of ET patients presenting bleeding complications release significantly less PF4 during *in vitro* clot formation than platelets of ET patients with thrombotic complications, which may indicate impaired platelet function and impaired clot formation *in vivo*. Bleeding tendency observed in ET patients may be a result of the coexistence of impaired platelet function and some other causes of diathesis, such as low von Willebrand factor activity.

0622

SENSITIVE APTT FOR LUPUS ANTICOAGULANT (LA) DETECTION. IS IT USEFUL AS A FIRST STEP IN A BASED-TEST ALGORITHM IN CLINICALLY SUSPECTED ANTIPHOSPHOLIPID SYNDROME (APS) DIAGNOSIS?

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Background. According to the guidelines for LA detection updated in 2009 by the ISTH (V. Pengo *et al.*, JTH 2009; 7: 1737-40), testing for LA should be limited to patients with a significant probability of having APS, or unexplained prolonged aPTT. Single test is not sensitive to detect LA, being necessary to perfom two different tests: Diluted Russell Viper Venom Time (dRVVT) as first choice, followed by a sensitive aPTT (low phospholipid and silica as activator). Neverthless in some centers aPTT is the first choice for LA detection. *Aim.* To define a laboratory based algorithm depending on the test performed for the first step evaluation in LA detection in patients with APS. *Patients and Methods.* Along a period of two months, 220 patients underwent evaluation for suspected hypercoagulability status. In cases with clinical criteria of APS according to the Sapporo International Consensus Statement, the clinical files (demographic, clinical and inherited and acquired thrombophilic risk factors) of patients, were reviewed. Sensitive aPTT and dRVVT were performed using an ACL TOP-3G automatized coagulometer (Instrumentation Laboratory). *Results.* 220 patients were evaluated for hyperocoagulable status (including LA). Positive result for LA was detected in 43 patients (19.5%) male (M) 18 (41.8%) and female (F) 25 (58.1%) with a median age of 50 years (range: 5-82). Prolonged sensitive aPTT was detected in 19 of these 43 patients (44.2%), M 9 (47.4%) and F 10 (52.6%) with a median age of 52 years (range: 5-79). Clinical events were deep vein thrombosis/pulmonary embolism 14 (32.5%); cerebrovascular disease 9 (20.9%); arterial ischaemia 5 (11.6%); venous and arterial thrombosis 2 (4.6%); obstetric complications 1 (2.3%); connective tissue disease 8 patients (18.6%) and 4 (9.3%) prolonged aPTT. 17 patients were diagnosed of APS on the basis of clinical and biological data. 7 (41.2%) of these APS patients were negative for prolonged sensitive aPTT (aPTT ≤ 38.0 sec), 5 M and 2 F, median age of 52 years (range: 46-71), 4 patients presented venous thrombosis and 3 patients arterial thrombosis. No prolonged sensitive aPTT was detected in absence of LA positive detection. *Coclusions.* 1) According to the latest updated recommendations, dVRRT must be considered as the first choice as first step in LA detection due to the possibility of misdiagnosis when sensitive aPTT is performed as first choice. 2) Combination of both tests seems to be the better choice in performing a test-based algorithm for LA detection. 3) Guidelines for LA detection and their updating in facets of test performance including preanalytical, analytical, and postanalytical issues are necessary to improve knowledge and experience.

0623

ASSOCIATION BETWEEN INTERLEUKIN-6 AND CORONARY ARTERY DISEASE SEVERITY AMONG NON-ELDERY PATIENTS

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Background. Systemic inflammation and carotid atherosclerosis markers have been used to detect patients at high risk of cardiovascular disease. We investigated the association of hs-CRP and a cytokine panel (IL-6, IL-1b, IL-8, IL-10 and TNF-a) to coronary artery disease (CAD) extent, accounting for the effect of subclinical carotid atherosclerosis. *Methods.* Eighty-three patients (92.8 % males, 54.5±7 year-old) had their first coronary angiogram after an acute coronary syndrome (81.9%) or for recently diagnosed or suspected stable CAD (18.1%). Coronary arteries with stenosis ≥50% (CS), Gensini score (GS) and Hamsten score (HS) were used as indices of CAD severity. Carotid B-mode ultrasound was performed bilaterally to measure maximal carotid intima-media thickness (CIMT) and maximal carotid plaque thickness (CPT). Serum levels of lipid parameters, hs-CRP and a cytokine panel (IL-6, IL 1b, IL-8, IL-10 and TNF-a) were also measured. Spearman rank correlations, logistic regression models and ROC curves were used for statistical analysis. *Results.* Hs-CRP significantly correlated with IL-6 (0.422, p<0.001), but not with any other cytokine. IL-6 correlated strongly with all CAD severity scores (CS: r=0.362, p=0.001, GS: r=0.380, p < 0.001, and HS: r=0.370, p=0.001), but not with CIMT or CPT. In contrast, hs-CRP did not correlate with any of the above coronary or carotid ather-

osclerosis indices. Patients in the highest IL-6 concentration tertile had increased CAD scores and more often multi-vessel CAD (85.2% of cases) compared to patients in the middle (51.9%, p=0.018) or the lowest tertile (44.8%, p=0.002). Patients in the highest versus lowest IL-6 concentration tertile were more likely to have multi-vessel CAD, after adjustment for risk factors, age, gender and CIMT (adjusted OR: 2.464, 95% CI: 1.16-5.233, p=0.019) or CPT (adjusted OR: 2.523, p=0.015). At ROC curve analysis IL-6 was a significant albeit modest predictor of multi-vessel CAD (AUC=0.693, 95% CI: 0.577-0.809, p=0.003). Circulating IL-6 levels >4.8 pg/ml predicted multi-vessel disease with 64% sensitivity and 67% specificity. *Conclusions.* Among non-elderly patients with initially diagnosed or suspected CAD, circulating IL-6 levels were associated with CAD severity, suggesting an important link between IL-6 and coronary atherosclerosis.

0624

FIBRINOGEN MODIFICATION BY SHEAR STRESS ACTIVATED PLATELETS

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Background. Laminar shear stress in bulk circulation activates platelets to different levels and may initiate arterial thrombosis at sites of pathological blood flow. The exposition of platelet surface receptors is changed, and storage proteins and low molecular species either stored or formed de novo are released. The low molecular substance malondialdehyde (MDA) is formed as a consequence of lipid peroxidation and/or of prostaglandin's metabolism in platelets. MDA is reactive toward amino groups of proteins and nucleic acids, it has been inferred to have mutagenic and cytotoxic roles, possibly to be a participant in the onset of atherosclerosis. In a previous work we studied the influence of oxidative modification of fibrinogen on platelet dynamic adhesion and found significantly lower platelet adhesion on fibrinogen modified with MDA. *Aims.* The aims of the present study were to determine wheather MDA is formed in shear stressed platelets suspended in fibrinogen and if it modifies the fibrinogen molecules. *Methods.* Blood was drawn from healthy volunteers, who had not ingested any drug for at least two weeks, in accordance with the Ethical Committee regulations of our Institute. Washed blood platelets were isolated by differential centrifugation of blood and resuspended in Tyrode buffer at pH 7.4 in the presence of human fibrinogen (final concentration 1 mg/ml). High shear was applied with a cone and plate analyzer, the Impact-R (DiaMed) in acordance with manufacturer's manual. Samples (washed platelets - fibrinogen) were placed onto a polystyrene plate onto which a Teflon cone was perfectly fitted. After incubation (10 s) shear was applied (shear rate 1800 s^{-1}) for 1, 5, 15, 30 minutes. The protein carbonyls formed in platelet suspension were estimated using dinitrophenylhydrazine, the released serotonin and formed MDA derivatized with thiobarbituric acid were estimated using chromatographic methods. Dinitrophenylhydrazine derivatized proteins (DNPH-proteins) were analysed using SDS-PAGE and immunoblotting. Adhered platelets stained with May-Grunwald were analyzed by an image analyzing system. The platelet adhesion was recorded by an examination of the percentage of the total area covered with platelets, expressed as a surface coverage (SC, %). *Results.*We found the release of serotonine, time-dependend icreasing formation of MDA and DNPH-proteins and increasing platelet adhesion throughout the experiment. Both serotonine release and MDA formation reflected the platelet activation due to shear stress. The most pronounced bands of DNPH-proteins on SDS-PAGE immunoblots were the fibrinogen alpha, beta and gamma chains. *Summary/conclusions.* Fibrinogen is the protein most vulnerable to oxidative stress products and indeed the fibrinogen molecules were preferable modified with MDA. We found that MDA formed in platelets activated by shear stress and modified platelet proteins and fibrinogen in buffer. According to our previous results malondialdehyde modification of fibrinogen decreases platelet adhesion, therefore the MDA production by platelets activated by shear stress may possibly protect platelets from further activation.

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0625

SMOKING AND ADAMTS-13 LEVELS IN HEALTHY MALES

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Background. The procoagulant protein von Willebrand factor (vWF), which has prothrombotic activity, was reported to be elevated in smokers leading to increase incidence of thrombosis. vWF is down-regulated by ADAMTS-13 protease (members of a disintegrin and metalloprotease with thrombospondins 1 repeats). No reports on the effects of smoking on ADAMTS-13 are currently present, particularly in Arab ethnicity. *Aim.* To determine the effects of smoking on ADAMTS-13 antigen and activity levels in healthy Arab smokers in Kuwait. This will clarify the role of ADAMTS-13 in hemostasis and thrombosis, and adds on to the mechanism of thrombosis in smokers. *Methods.* 200 Arab males were recruited. After obtaining consent, venous blood samples from 80 smoker and 80 nonsmoker healthy subjects were collected after asking subjects to fast and refrain from smoking for 8 hours (smokers here were termed "smokers at rest"). Similar sampling was done for 40 smokers, who were asked to smoke one cigarette immediately before taking blood (termed "acute smokers"). For all blood samples, plasma was separated and used to measure ADAMTS-13 antigen and activity levels, as well as vWF collagen binding activity levels using commercial ELISA kits. *Results.* No difference in ADAMTS-13 antigen level was found between smokers at rest and nonsmokers, but ADAMTS-13 and vWF activities were significantly lower in smokers ($p<0.018$). Compared to smokers at rest, acute smokers had significantly higher levels of vWF activity and ADAMTS-13 antigen and activity levels ($p<0.01$). *Conclusions.* The increase in vWF activity in smokers is an acute mechanism that occurs in respond to endothelial injury caused by cigarette consumption. High vWF activity is accompanied by an increase in ADAMTS-13 activity as a natural physiological mechanism to degrade the elevated vWF molecules. If not followed by a subsequent smoke, the activities of both proteins subside. The repeated increase in vWF and constant degradation by ADAMTS-13 result in lower overall levels of both proteins in smokers (at rest) compared to nonsmokers who do not experience a similar (repeated) injury to the endothelium.

0626

ASSESSING THE IMPACT OF THE G58A POLYMORPHISM ON FIBRINOGEN A-CHAIN GENE IN PATIENTS WITH STABLE ANGINA: EFFECTS ON SPECIFIC MARKERS OF COAGULATION

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Purpose. Evidence suggests that the G58A polymorphism on fibrinogen a-chain gene is associated with increased fibrinogen levels in healthy individuals. However, it is still unclear, whether this polymorphism is associated with coagulation/thrombosis in patients with coronary artery disease (CAD). In the present study we examined the impact of this polymorphism on fibrinogen levels, D-dimers levels and plasminogen levels. *Methods.* The study population consisted of 395 subjects, 246 of which angiographically documented for CAD. The G58A polymorphism was detected by polymerase chain reaction (PCR) and appropriate restriction enzymes. Fibrinogen levels were measured by immunonephelometry, while plasminogen and D-dimers levels were measured by standard coagulometry techniques. *Results.* The genotype distribution was GG: 37.8%, GA: 39.4% and AA: 22.8% for patients with CAD, while GG: 33.5%, GA: 44.3% and AA: 22.2% for controls. Patients with CAD had significantly higher fibrinogen levels (mg/dl) than controls (434.7±132.7 vs 384.7±103.7, p=0.0002). However, in patients with CAD fibrinogen levels were not significant higher for 58AA homozygotes vs 58G carriers (453.6±131.4 vs 441.1±140.6, p=NS), while similar difference occurred in controls (AA: 385.2±129.4 vs GG+GA: 392.6±103.0, p=NS). Moreover, D-dimers levels (mg/L) were significantly higher in CAD patients than controls (409.7±188.2 vs 332.8±199.4, $p<0.0001$). In addition, there was a significant difference for 58G carriers vs 58AA homozygotes for CAD patients (506.4±418.8 vs 662.2±627.1, $p<0.05$), but not for controls (AA: 415.6±289.6 vs GG+GA: 355.9±276.5, p=NS). Finally, CAD patients and controls had no significant difference in plasminogen levels (u/ml) (119.8±79.1 vs 113.9±22.9, p=NS). Patients with CAD had no difference in plasminogen for 58AA homozygotes vs 58G carriers (110.2±20.6 vs

112.2±17.2, p=NS), while no significant difference was observed for controls (AA: 112.3±16.7 vs GG+GA: 114.3±23.5, p=NS). *Conclusions.* Our findings indicate that the G58A polymorphism on fibrinogen a-chain gene affects D-dimers levels in patients with coronary artery disease. These findings provide a possible mechanism by which this polymorphism may affect thrombotic process/coagulation independently of fibrinogen levels and may have important clinical implications.

0627

ALTERED COAGULATION PROCESS IN PATIENTS WITH HYPERTENSION: THE ROLE OF THE G455A POLYMORPHISM

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Purpose. Genetic polymorphism G455A on fibrinogen b-chain gene has been associated with the risk of coronary artery disease. However, it is unknown whether it affects the prothrombotic profile of patients with hypertension (HT). In the present study we examined the impact of this polymorphism on fibrinogen, D-dimers, factor V (fV) and factor X (fX) levels in the aforementioned population. *Methods.* The study population consisted of 266 HT and 165 non HT. The G455A polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, while circulating levels of fibrinogen were measured by the von Clauss method. D-dimers levels, fV and fX levels were measured by standard coagulometry techniques. *Results.* The genotype distribution in non HT and HT was GG: 50.9%, GA: 41.8%, AA: 9.7% and GG: 51.5%, GA: 37.6%, AA: 10.9% respectively. There was no significant difference in fibrinogen levels (μg/dl) between 455AA homozygotes and 455G allele carriers in non HT patients (448.3±34.6 vs 395.4±9.9, p=NS). Importantly, 455AA genotype presented with much more elevated levels of fibrinogen compared to the GG+GA in HT patients (535.4±25.3 vs 414.2±8.0, p<0.001). Moreover, HT 455AA homozygotes had significantly increased D-dimers levels (μg/l) compared to 455G allele carriers (640.3±83.6 vs 485.5±27.2, p<0.05). No difference was observed for non HT regarding D-dimers between the 455AA genotype and GG+GA (477.6±74. vs 450.8±40.7, p=NS). Interestingly, 455AA genotype presented with higher fV(%) and fX(%) levels compared to GG+GA in HT patients (133.6±5.8 vs 117.8±3.3, p<0.05, for fV) and (101.9±4.6 vs 92.2±2.4, p<0.05, for fX). However, no difference was observed in fV and fX levels between 455AA and GG+GA in non HT (105.8±11.6 vs 118.7±4.4, p=NS for fV) and (95.8±8.0 vs 119.4±29.1, p=NS for fX). *Conclusions.* The genetic polymorphism G455A on fibrinogen b-chain gene has a remarkable impact on prothrombotic profile of patients with hypertension, given its effect on fibrinogen, D-dimers, factor V and factor X levels. These findings provide evidence that this polymorphism affects significantly the mechanisms of the coagulation process in hypertensives.

0628

ALTERED ENDOTHELIUM-DEPENDENT RELAXATION INDUCED BY ERYTHROCYTE MEMBRANE FROM SUBJECTS WITH DIFFERENT HAEMOGLOBIN GENOTYPES IN ISOLATED RABBIT CAROTID ARTERIES

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Background. The literature contains conflicting reports concerning changes in vascular reactivity following interaction between erythrocytes and vascular endothelial cells. *Aim.* The goal of this study was to characterize the effect of constituents of erythrocytes from subjects with different Hb genotypes on acetylcholine-induced endothelium-dependent vasorelaxation. *Methods.* Isometric contractions of ring preparations (5mm long) of rabbit carotid artery (n=6) suspended in 20ml organ baths and bubbled with 95% O2, 5% CO2 were studied pharmacologically using standard *in vitro* techniques under an initial load of 1g, at 37oC and pH 7.4. Concentration-dependent contractile responses induced by phenylephrine (PE) as well as relaxation responses induced by acetylcholine (Ach) were examined and their respective EC70 and IC70 values obtained. Acetylcholine (IC70M)-induced relaxations of pre-contractions induced by EC70 M phenylephrine were examined in control rings as well as in rings exposed for 30 minutes to (a) intact washed erythrocytes (b) erythrocyte ghosts and (c) haemoglobin - all obtained from subjects of different haemoglobin genotypes (AA, AS and SS). Arterial rings were exposed to 50μl of each of the erythrocyte constituents at an adjusted haematocrit of 0.6. *Results.* Ach-induced relaxation was significantly (p<0.05) enhanced by AA erythrocytes (46.2±3.25 and 69.5±5.4% for control and test, respectively). In contrast, AS and SS erythrocytes as well as exposure to Hb did not significantly alter Ach relaxation whereas AS and SS ghosts significantly (p<0.01) attenuated Ach relaxation. Compared with AA, erythrocytes but not Hb from AS and SS subjects elicited significantly greater inhibition of Ach relaxation; futhermore, inhibition of Ach relaxation by erythrocyte ghosts was significantly greater with AS than SS. *Conclusions.* Our results show that a membrane-bound factor may account for the genotype-dependent attenuated Ach-induced relaxation following interaction between erythrocytes and vascular endothelial cells.

0629

A SINGLE NUCLEOTIDE POLYMORPHISM ON FIBRINOGEN BETA CHAIN GENE MODIFIES ATHEROGENESIS INDEPENDENTLY OF FIBRINOGEN LEVELS

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Purpose. Evidence suggests that impaired coagulation process contributes to atherogenesis and its clinical manifestations. In addition, the role of the genetic variability on fibrinogen beta chain gene on fibrinogen levels is widely speculated. In the present study we sought to examine whether a single nucleotide polymorphism could affect the coagulation cascade beyond its effect on fibrinogen. *Methods.* The study population consisted of 318 patients with documented coronary artery disease (CAD) and 221 controls. The G455A polymorphism was estimated by polymerase chain reaction (PCR) and the specific HaeIII restriction enzyme. Serum levels of D-dimers, factor (f) V, factor (f) X were measured by standard coagulometry techniques. *Results.* The genotype distribution was GG: 49.3%, GA: 41.2%, AA: 9.5% for CAD and GG: 55.2%, GA: 35.7%, AA: 9.1% for controls. Through the study (overall) population the G455A polymorphism affected significantly D-dimers levels (μg/l) between the G allele carriers and the AA homozygotes (median: 2.55[2.37-2.71] vs 2.68[2.38-2.83], p<0.05), as well as fV (%) (114.6±24.7 vs 126.8±26.6). No effect was observed on fX levels (p=NS). Moreover, there was no significant difference in the studied parameters across the genotypes in the control group (p=NS for all). Controversially to the control group, the G455A polymorphism defined D-dimers and fX and fV levels in CAD patients. More specifically, the AA homozygosity was associated with significant increased levels of D-dimers (median: 2.75[2.50-2.90] vs 2.60[2.40-2.70]), fV (135.0±20.5 vs 119.5±24.8) and fX (102.4±19.6 vs 90.1±22.8) compared to the G allele carriers (p<0.05 for all). *Conclusions.* A single nucleotide polymorphism on beta chain fibrinogen gene, regulates D-dimers, factor V and factor X levels in patients with advanced atherosclerosis. Our findings indicate that the G455A polymorphism may affect the coagulation, a responsible underlying mechanism for atherogenesis and this is beyond its effect on fibrinogen.

0630

ADMISSION MEAN PLATELET VOLUME PREDICTS LEFT VENTRICULAR SYSTOLIC DYSFUNCTION IN PATIENTS WITH ACUTE ST-ELEVATION MYOCARDIAL INFARCTION TREATED WITH PRIMARY ANGIOPLASTY

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Background. Left ventricular systolic dysfunction (LVSD) after acute myocardial infarction (AMI) worsens short- and long-term prognosis. Admission mean platelet volume (MPV), an indicator of platelet reactivity, is an independent predictor of impaired angiographic reperfusion and of both short- and long-term mortality after AMI. *Aim.* The aim of the study was to investigate the role of admission MPV on LVSD post AMI. *Methods.* The study included 360 patients (pts) with first acute ST-elevation myocardial infarction (STEMI), admitted to the University hospital from January 2003 to December 2007. In all the pts angioplasty of the culprit lesion was performed (only pts with < 12 h after the onset of symptoms were included and pts with malignant or inflammatory disease and cardiogenic shock were excluded from the study). Whole group characteristics: 70% man, mean age 63 years, diabetes in 27%, hypertension in 64%, current smoking in 35%, hyperlipidemia in 34%, infarct related artery: left anterior descending artery in 43%, left circumflex artery in 14%, right coronary artery in 43%, Killip class >1 in 23%, multivessel disease in 54%, TIMI flow: >1 pre PCI in 22%, >1 post PCI in 96%, time to treatment 4.8±3.6 h. MPV, platelet count (PLT) and C-reactive protein (CRP) were obtained at the time of admission

and samples were processed within 1 h of venepuncture. LVSD (defined as left ventricular ejection fraction <0.50) was assessed using transthoracic echocardiography (Simpson's method) within 6 days post AMI. *Results.* 126 pts (35%) had LVSD. Pts with LVSD were older, more frequently had anterior myocardial infarction, multivessel disease, heart failure on admission and longer time to reperfusion. Peak creatine kinase and admission troponin were significantly higher in pts with LVSD while CRP, admission creatinine, baseline and final TIMI flow, gender and cardiovascular risk factors were comparable in both groups. MPV was higher in pts with LVSD (8.82 vs. 8.46 fL, p<0.001), and increased with the degree of admission heart failure (8.46, 8.77, 9.32 fL for Killip class I-III respectively, p<0.001) while PLT did not differ between groups. MPV negatively correlated with PLT (r=-0.33, p<0.001) and positively with peak creatine kinase (r=0.16, p=0.03). ROC curve analysis showed that values of MPV>8.6 fL had 71.4% sensitivity and 57.3% specificity for predicting LVSD. After multivariable adjustment the independent predictors of LVSD were: MPV (OR 1.533, 95% CI 1.079-2.179), age (OR 1.095, 95% CI 1.029-1.165), anterior myocardial infarction (OR 6.094, 95% CI 1.808-20.538), multivessel disease (OR 3.538, 95% CI 1.105-11.333) and peak creatine kinase (OR 1.0006, 95% CI 1.0003-1.0009). *Conclusion.* Admission MPV is independent predictor of LVSD in pts with STEMI treated with primary angioplasty.

0631

ANALYSIS OF BLOOD COAGULATION PROCESS WITHIN MURAL THROMBI GENERATED UNDER WHOLE BLOOD FLOW CONDITIONS

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Background. Mural thrombus formation at sites of damaged vessel walls is fundamentals for both physiologic haemostasis and pathological intravascular thrombosis. Blood coagulation process playing a role in stabilizing thrombi has been studied so far basically in classic experimental approaches, such as APTT assays, that evaluate fibrin clot formation in a plasma solution devoid of blood cell components including platelets. However, such classic coagulation assays cannot necessarily reflect the *in vivo* mural thrombus formation, in which platelet adhesion/aggregation and blood coagulation can upregulate each other under whole blood flow conditions. *Aims.* We therefore established the *in vitro* assay system that can precisely evaluate the blood coagulation process during mural thrombus formation under whole blood flow conditions. *Methods.* Using an *in vitro* perfusion chamber system, blood coagulation during mural thrombogenesis under whole blood flow was visually evaluated by confocal laser scanning microscopy (CLMS) as the extent of intra-thrombus fibrin network formation, which was calculated as the ratio of fibrin relative to fibrinogen within mural thrombi generated on a collagen- or von Willebrand factor-coated surface in an immune-fluorescent method. The blood coagulation process within thrombi under flow was also evaluated in detail by the time-course changes of P-selectin expression, tissue factor (TF) accumulation, or thrombin binding on platelets. *Results.* Analysis by CLMS during perfusion of whole blood anticoagulated to various extent revealed that the size and shape of thrombi was dependent on the amount of intra-thrombus fibrin deposition under high shear rate conditions. The generation of platelet procoagulant activities was confirmed to occur with the sequence of (1) P-selectin expression, (2) TF accumulation, (3) thrombin binding, and eventually (4) fibrin deposition within platelet thrombi. *Summary/Conclusions.* Our system enables real-time observation of fibrin network formation in mural platelet thrombi, as well as analysis of the functional link between blood coagulation and mural thrombogenesis under whole blood flow conditions. With this approach, we visually evaluated successfully mural thrombogenicity of hemophilia patients with or without addition of activated coagulation factor VII, as well as the antithrombotic effects of 3-hydroxyl-3-methylglutaryl coenzyme A reductase inhibitors ("statins") under whole blood flow, an experimental situation most relevant for the *in vivo* haemostasis and thrombosis.

0632

HEMOSTATIC MARKERS EVALUATION IN A TRIAL OF THROMBOPROPHYLAXIS FOR NEWLY DIAGNOSED MYELOMA PATIENTS TREATED WITH LENALIDOMIDE AND DEXAMETHASONE

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Background. Multiple myeloma (MM) patients receiving lenalidomide and dexamethasone combined therapy have an increased risk of thrombosis. Low-molecular weight heparin (LMWH) and low-dose aspirin (ASA) are used as thromboprophylaxis in these patients. In a prospective, multicenter phase III trial (RV-MM-PI-209) of newly diagnosed MM patients treated with lenalidomide and low-dose dexamethasone during induction therapy, the safety and the efficacy of low-molecular weight heparin (LMWH) or low-dose aspirin (ASA) as anticoagulant prophylaxis was assessed. *Aims.* In a group of MM patients enrolled in the RV-MM-PI-209 trial, selected markers of hemostatic system activation were measured in order to evaluate: 1. the biomarkers' predictive value for thrombosis, and 2. the modulation of the biomarkers by thromboprophylaxis during lenalidomide administration. *Methods.* Induction treatment consisted of four 28-day cycles of lenalidomide (25 mg d 1-21) and low-dose dexamethasone (40 mg d 1,8,15,22). All eligible patients were randomly assigned to receive LMWH (Enoxaparin 40 mg/d, N=166) or ASA (100 mg/d, N=176) for the duration of the induction therapy. Plasma samples from 36 patients were available for analyses at baseline (T0) before starting treatments (22 LMWH/14 ASA). In addition, plasma samples from 15 study subjects (10 LMWH/5 ASA) were obtained at the end of induction therapy (T1). On all plasma samples we measured prothrombin fragment F1+2 and Tissue Factor (TF), as markers of coagulation activation, and thrombomodulin (TM) as marker of endothelial cell activation. Forty healthy subjects acted as the control group. *Results.* At baseline, the levels of F1+2 (138±10.7 vs 100±13 nmol/L; p=ns), TF (115±20 vs 69±10 ng/ml; p=0.03) and TM (34±5.7 vs 11±0.9 ng/ml; p<0.001) were increased in the 36 MM patients compared to healthy controls. To evaluate the effect of lenalidomide/dexamethasone therapy, in association with LMWH or ASA, data from patients having samples available at both T0 and T1 were compared. The results showed that a reduction occurred from T0 to T1 for F1+2 (124±13 vs 109±16 nmol/L; p=ns), TF (164±21 vs 64±6.9 ng/ml; p=0.02), and TM (32±4.3 vs 25±2.8 ng/ml; p=ns) plasma levels. In addition, the plasma levels of both F1+2 and TF at the end of the induction therapy were similar to that of control subjects. Differently, at the same time point, the levels of the endothelial activation marker TM, were still significantly higher compared to controls. The decrease of F1+2 observed at T1, the marker of thrombin generation, was statistically significant for the subgroup of patients under LMWH (p=0.01), but not in those on ASA. *Conclusions.* Our data confirm the occurrence of blood coagulation and endothelium activation in MM patients. After induction therapy, the levels of the two blood coagulation makers tended to normalize towards controls' values. Differently, TM was still significantly higher, suggesting an insult of the antitumor therapy on endothelium. The analysis of data according to the type of thromboprophylaxis, showed that F1+2 significantly decreased in those patients receiving LMWH, but not in those receiving ASA. No thrombotic events occurred in the analyzed subgroup of patients to allow evaluation the predictive value for thrombosis of these markers.

Cellular immunotherapy and vaccination

0633

POTENTIAL ROLE OF GLUTAMINE METABOLISM IN IMUNOMODULATION OF T-LYMPHOCYTES BY MESENCHYMAL STROMAL CELLS

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Background. Mesenchymal stromal cells (MSCs) exert an immune regulatory function and suppress T-cell proliferation, but the mechanisms underlying this property are not completely known. Glutamine, the most abundant free amino acid of the human body, is metabolized by the enzyme glutaminase generating glutamate. However, in situations of glutamine deficiency, the enzyme glutamine synthetase produces glutamine from glutamate. Glutamine regulates cell proliferation by activating protein kinase A and mTOR signaling. Interestingly, inhibition of the mTOR pathway leads to an increased generation of regulatory T-cell. *Aims.* In this work, we evaluated the potential role of MSCs in the modulation of glutamine levels, as an immunomodulatory mechanism acting upon T lymphocytes. *Methods*. Peripheral blood CD3+ T-cell from 3 individuals were activated by anti-CD3/CD28 beads and cultured or not with MSC. Following a 5 day period, CD4+ T-cells and CD8+ T-cells were purified and profiled by whole genome microarrays and selected genes validated by RT-PCR. T-lymphocytes from 3 independent individuals were similarly activated and cultured. In addition, we cultivated MSCs alone or in presence of activated T-cell supernatant. Following a 5 day period, glutamine and glutamate levels were analyzed in culture medium by hplc. In another experiment T-lymphocytes were co-cultivated, as described, and T-cell proliferation was analyzed upon addition of glutamine at different concentrations (0.5, 0.7 and 1.0mM). *Results*. As expected, proliferation of lymphocytes co-cultured with MSCs was significantly inhibited (cfse). Using the Ingenuity Pathway Analysis, our microarray data revealed that mTOR pathway was down regulated in lymphocytes immunomodulated by MSC. Concordantly, these immunomodulated lymphocytes expressed higher levels of genes associated with regulatory T-cells, such as IL10, FOXP3, CTLA-4 and GITR (validated by RT-PCR). Levels of glutamine were lower in the culture media of T-cells cultivated with MSCs (as compared to those cultured alone), while the levels of glutamate were higher. In line, the expression of glutamine synthetase was increased in immunomodulated lymphocytes. Interestingly, while high levels of glutamine were found in the media of MSCs or activated lymphocytes cultured alone; when the culture media from MSCs cultured alone was substituted by that of activated lymphocytes cultured alone (in the 3rd day of culture), glutamine levels at the last 5th culture day were strikingly reduced, indicating that the inflammatory stimuli provided by the media of activated T-cells lead MSCs to consume the glutamine present in the media. This mechanism can be responsible by the low levels of glutamine found in the co-culture of MSCs and lymphocytes. Finally, while the addition of glutamine to cocultures partially restored the proliferation of immunomodulated T-lymphocytes in a dose dependent manner, lymphocytes cultured alone showed no change in cell proliferation by addition of glutamine. We are currently addressing the potencial role of MSCs glutaminase, in the consumption of glutamine, by using a glutaminase inhibitor. *Conclusions*. Our results point to a new immunoregulatory mechanism, by witch MSCs would restrict lymphocyte proliferation, trough the consumption and deprivation of glutamine from the surroundings. Finally, reduced glutamine levels would implicate reduced mTOR signaling, contributing to an increase in the generation of regulatory T-cell.

0634

DELICATE BALANCE BETWEEN THE ABSOLUTE NUMBERS OF ANTIGEN-PRESENTING CELLS AND ANTIGEN-SPECIFIC T CELLS DETERMINES THE LIKELIHOOD OF SUCCESSFUL *IN-VITRO* PRIMING OF IMMUNE RESPONSES

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Background. In-vitro generation of primary T cell responses from a naive repertoire against selected antigens (Ag) is essential for the development of adoptive immunotherapeutic strategies. Although feasible, the limited robustness of the procedure hampers broad scale clinical application so far. *Aims.* In this study, we investigated in detail the role of the type of the antigen-presenting stimulator cells and antigen dosing in priming, survival, and expansion of antigen-specific precursor T cells (Tprec). *Methods.* We developed an *in vitro* model that allows kinetic functional monitoring of activation and proliferation of antigen-specific T cells using fluorescent dye (PKH or CFSE) labeling, CD137 counterstaining and quantitative flow cytometry. T cell clones (or purified naive T cell populations in a selected number of experiments) were stimulated with antigen-presenting cells (APC) derived from various sources, including monocytes, monocyte-derived dendritic cells (DCs), EBV-LCL and primary leukemic APC at CTL/stimulator (CTL/S) ratios ranging from 10/1 to 1/625 in the presence of variable numbers of innocent bystander T cells, mimicking different frequencies of antigen-specific Tprec within a total responder (R) cell population. *Results.* Using professional APC like monocyte-derived DCs as stimulator cells, optimal T cell activation was observed at CTL/S ratios critically ranging between 1/1 and 1/10. This phenomenon occurred irrespective of the presence of different numbers of innocent bystander T cells, illustrating that optimal T cell activation is determined by the specific Tprec/S ratio rather than the total cellular R/S ratio. Exposure of individual CTL to higher numbers of stimulators resulted in overstimulation and activation-induced cell death (AICD) of the specific T cells. Within the naive repertoire, specific Tprec frequencies for a single antigen are estimated to be as low as 1 in 1,000.000 to 1 in 10,000. Taken the above data into account, application of the widely chosen R/S ratio of 10/1 for priming of specific Tprec with a Tprec frequency of 1 in 10,000 would correspond to an effective Tprec/S ratio of 1/1000, which may often result in the induction of AICD of the specific Tprec. Our data show that a maximum of 10 APCs per Tprec results in optimal stimulation, but this may seem to be in conflict with the likelihood of specific Tprec to encounter an APC among the bulk of bystander cells. Simultaneous triggering of multiple Tprec by targeting multiple antigens may therefore lower the likelihood of induction of AICD in the specific Tprec. This was confirmed by the generation of primary immune responses against (completely) HLA-mismatched APC. Using stimulator cells with an inferior APC phenotype (e.g. monocytes or primary leukemic cells), similar dose-response relationships were observed, but the range of optimal Tprec/S ratios was broader and was shifted towards a higher APC amounts per Tprec. Increasing the antigen dose on the APC surface shifted the Tprec/S ratio towards lower amounts of stimulators per Tprec. *Conclusions.* In conclusion, our data showed that a delicate balance between the absolute numbers of antigen-specific Tprec and antigen-presenting stimulator cells determines the likelihood of successful priming and survival of antigen-specific Tprec.

0635

INTERLEUKIN-15 SKEWS HUMAN MONOCYTE DIFFERENTIATION INTO CD56+ DENDRITIC CELLS WITH CYTOLYTIC ACTIVITY AGAINST MYELOID LEUKEMIC CELLS

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Background. Recent research has underscored the potential clinical utility of dendritic cells (DCs) for antileukemia immunotherapy. Nonetheless, current DC-based immunotherapy strategies leave considerable room for improvement, particularly when it comes to the generation of DCs with maximal immunostimulatory potency. We and others have recently reported on a novel, improved protocol for DC generation using interleukin (IL)-15 (IL-15 DCs). *Aims.* A subpopulation of these IL-15 DCs was incidentally found to express the natural killer (NK) cell-related molecule CD56. The aim of the present study was to determine whether IL-15 DCs also possess NK cell-like effector functions that would allow them to exert direct cytolytic activity against myeloid leukemic cells. *Methods.* Human peripheral blood monocytes were allowed to differentiate into immature DCs for 24-36hr in the presence of granulocyte macrophage colony-stimulating factor and IL-15. CD56+ and CD56- IL-15 DC fractions were separated by positive immunomagnetic selection, and further matured for 18hr in the pres-

ence of the Toll-like receptor 7/8-agonist resiquimod. DC-mediated cytotoxicity towards the myeloid leukemic cell line K562 was measured by flow cytometry after overnight co-culture at different effector:target (E:T) cell ratios. Cytotoxicity blocking assays were performed using concanamycin A, an inhibitor of perforin/granzyme B-induced apoptosis, and neutralizing antibodies against tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). *Results.* Flow cytometric immunophenotyping confirmed the expression of CD56 on a subpopulation of IL-15 DCs (34.3±2.1%). Contamination of NK cells was excluded on the basis of phenotypic analysis. Interestingly, CD56+ IL-15 DCs induced apoptotic cell death in up to 22.6±1.0% of K562 cells at an E:T ratio of 50:1. Compared to their CD56- counterparts, CD56+ IL-15 DCs displayed significantly stronger tumoricidal activity towards K562 cells at E:T ratios 50:1 (P=0.0002) and 25:1 (P=0.0063). The perforin inhibitor concanamycin A partially abrogated the cytotoxic effect of CD56+ IL-15 DCs (-51.8±11.7%). A further significant decrease in cytotoxicity was obtained by treatment with anti-human TRAIL antibodies (-22.5±2.8%). *Summary/Conclusions.* Here, we describe the unique ability of IL-15 to promote rapid differentiation of monocytes into CD56+ DCs endowed with cytotoxic activity against the myeloid leukemic cell line K562. The perforin/granzyme B-pathway, and to a lesser extent TRAIL-induced apoptosis, were implicated in IL-15 DC-mediated cytotoxicity. The observation that IL-15 DCs exert a direct antileukemic action provides strong support for their use in cellular immunotherapy of myeloid leukemia.

0636

EX-VIVO ALLOGENEIC STIMULATION SIGNIFICANTLY IMPROVES EXPANSION OF CYTOKINE-INDUCED KILLER CELLS WITHOUT INCREASING THEIR ALLOREACTIVITY ACROSS HLA-BARRIERS

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Background. Cytokine-induced killer (CIK) cells are a heterogeneous subset of *ex-vivo* expanded T lymphocytes with a mixed T-NK phenotype and endowed with a HLA-unrestricted antitumor activity. CIK cells can be used in the autologous setting of cancer patients or as alternative to standard donor lymphocyte infusion following allogeneic hematopoietic cell transplant (HCT). CIK cells present a reduced risk to induce graft versus host disease (GVHD) in HLA identical siblings but retain a certain degree of alloreactivity when challenged across major HLA-barriers. Even if CIK cells can be efficiently *ex-vivo* expanded, their expansion rate has been reported to be greatly variable, ranging from only few to more than 1000 folds. This variability may be a potential limitation for their effective clinical applications in patients with poor expansion rates. *Aims.* We evaluated whether alloreactivity of CIK cells might be exploited as a new method to favorably increase their *ex-vivo* expansion preserving the antitumor ability and safety profile. Our hypothesis is that a timed allogeneic stimulation might provide CIK cells with a proliferation boost and potentially decrease the overall alloreactivity versus hypothetical third party recipients. *Methods.* Starting from healthy donors (n=7), we compared in parallel the standard expansion protocol of CIK cells, based on IFN-γ, Ab anti-CD3 and IL2, with a new one including the addition of irradiated allogeneic PBMC (ratio 1:1) as stimulators on day +7 and +14. After 4 weeks we compared expansion rates, antitumor activity and residual alloreactivity across major HLA-barriers (assessed by MLR versus third party HLA-mismatched PBMC, different from those used as stimulators during the experimental expansion). *Results.* Allo-stimulated CIKs (AS-CIK) presented significantly higher expansion rates (median 131 fold, range 24-720) compared to standard controls (median 32 fold, range 9-121, p <0.03). The expansion of the CD3+CD56+ fraction, main responsible for the antitumor activity, was significantly higher (2243 fold, range 443-4319) within AS-CIKs compared to controls (403 fold, range 67-1722, p< 0.03) (Fig. 1). AS-CIKs retained effective tumor killing ability (64%, 57%, 52% and 43% of specific killing, assessed versus osteosarcoma cell lines, at 40:1, 20:1, 10:1 and 5:1 effector/target ratio respectively) that resulted comparable with controls. The alloreactivity of AS-CIK cells against third party HLA-mismatched PBMC was reduced, without reaching statistical significance, compared to controls; the median percentage of alloreactive-proliferating cells within AS-CIKs was 29% (range 27,7-31,8) compared to 45,5% (range 30,2-54) of controls. The membrane expression of CD8+, CD4+ and NKG2D molecules was comparable between AS-CIKs and standard counterparts (p>0.05). Interestingly, AS-CIKs and standard controls presented a similar lifespan with a comparable telomere length (median 8.5 vs 8.7 Kb). *Conclusions.* Our data suggest that *ex-vivo* alloreactivity of CIK cells may be exploited to obtain significantly higher expansion rates without affecting the resulting antitumor ability, safety profile, phenotype or lifespan. If validated into clinical settings, these findings may improve the efficacy of adoptive immunotherapy with CIK cells; the reduced alloreactivity versus third party might have important implications into HCT settings to generate CIK cells with a further reduced risk of GVHD across major HLA-barriers.

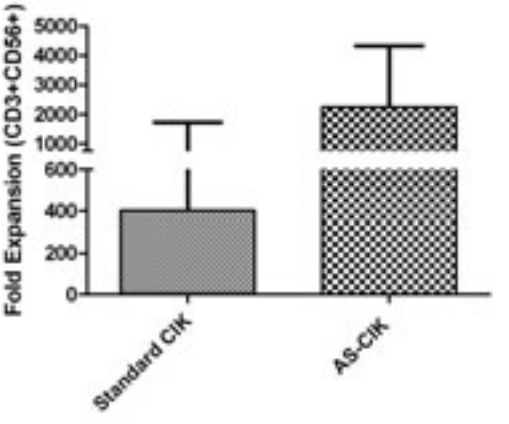

Figure 1. Fold Expansion (CD3+CD56+) of CIKs and AS-CIKs.

0637

THE INVOLVEMENT OF NCRS IN THE PRIMING STAGE OF NK CELL ACTIVATION BY TUMORS

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Background. CTV-1 tumor cells can specifically prime resting NK cells without triggering NK cell lysis. CTV-1 primed NK cells (pNK) have been used in a phase I trial for treatment of patients with AML. The pNK cells are able to lyse NK-resistant primary AML and tumor cell lines, even in the presence of inhibitory ligands (1). The CD69 surface molecule is upregulated on resting human NK cells by CTV-1 stimulation and can be used as a marker of successful priming. *Aim.* To assess the involvement of lectin-type and Ig-SF like NCRs in the NK cell priming process. *Methods.* CD56+ NK cells were purified from PBMCs of 6 normal donors by direct immunomagnetic separation. pNK cells were generated by coincubation with CTV-1 cells (ratio 1:2) for 16-20h. Matched pairs of NK/pNK cells (CD56+/CD3-) were immunophenotyped for CD69 (to assess NK activation), C-type lectin receptors (NKG2D, NKp80) and for Ig-SF like NCRs (NKp30, NKp44, NKp46). The proportion of cells expressing the specific Ag and its relative density of expression (median channel fluorescence-MedCF) were compared. To investigate the involvement of C-type lectins and Ig-SF like NCRs in the CTV-1 mediated NK priming, purified CD56+ NK cells from normal donors were preincubated for 30 minutes with cocktails of NK-receptor blocking antibodies before co-culture with CTV-1 cells. Four groups were tested: pNK1 - NK cells without added mAbs, pNK2 - NK cells pre-coated with anti-NKG2D, anti-NKp80 mAbs, pNK3 - NK cells pre-coated with anti-NKp30, anti-NKp44, anti-NKp46 mAbs and pNK4 - NK cells pre-coated with all Abs. After 24hours and 48 hours incubation period the CD69 percentage and MedCF expression of matched pairs of groups were compared as a measure of NK priming. *Results.* Within 24 hours co-incubation with CTV-1 CD69 was significantly up regulated in proportion (p=0.0061, 95%CI of mean of dif 17.17-61.75) and MedCF expression (p=0.013, 95%CI of mean of dif 6.68-32.44) compared with matched, non-stimulated NK cells. NKp80, NKp30 and NKp44 were unaffected by tumor priming while NKG2D and NKp46 were significantly downregulated. The blocking of C-type

lectins receptors significantly suppressed the proportion of pNK cells and the intensity of CD69 upregulation on pNK cells after coincubation with CTV-1 cells after 24 hours and 48 hours. In contrast, blocking of Ig-SF like receptors had no effect even when combined with the blockade of C-type lectins (Fig). CD69 is a known triggering ligand for human NK cells and we hypothesise that its presence and degree of expression is a marker of the pNK readiness to lyse tumor target cells. *Conclusion.* C-type lectin are the predominant NCRs involved in the process of NK cell priming by tumor cells while the Ig-SF like receptors have little or no role in the priming process.

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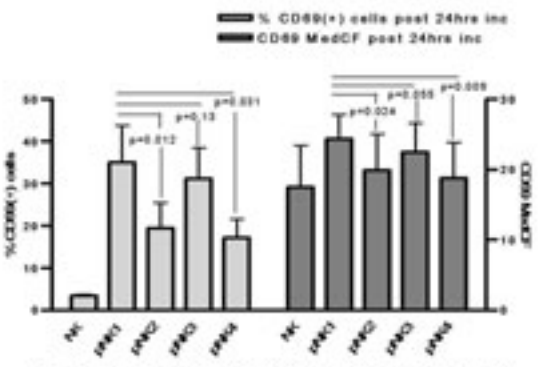

The proportion and intensity of CD69 expression on pNK cells after 24 hours coincubation with CTV-1 cells according to the blocked receptors group

Reference

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LARGE SCALE GMP-GRADE PRODUCTION OF IL-2 VERSUS IL-15-ACTIVATED ALLOGENETIC NK CELLS AGAINST ACUTE MYELOID LEUKEMIA: PRE CLINICAL RESULTS OF THE NK-ACT TRIAL

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Background. Allogeneic natural killer (NK) cells have the potential to provide anti-leukaemic effects without inducing graft versus host disease. Several lines of evidence suggest that allogeneic NK cell-based immunotherapy in a non transplantation setting may be a promising approach against acute myeloid leukaemia (AML). *Aims.* In the NK-ACT trial, we aimed at (1) developing a good manufacturing practice (GMP)-compliant procedure for large scale production of allogeneic activated NK cells for immunotherapy of AML, (2) comparing NK activation driven by interleukin (IL)-2 and IL-15. *Methods.* Three healthy volunteers were enrolled after written informed consent. All reagents were manufactured under GMP conditions. Mononuclear cells (MNC) were collected on day -1 using a Cobe Spectra cell separator and stored overnight at 4-8°C. Platelets were removed on day 0 and MNC were incubated with CD3 and CD19 reagents (Miltenyi Biotec). T- andcells were depleted from MNC on the CliniMACS system, (separation program DEPLETION 3.1). depleted MNC were split into 3 equal fractions, placed in VueLife FEP bags (AFC) in serum-free SCGM CellGro® medium (CellGenix) at $2x10^6$/ml and incubated overnight at 37°C under 5% CO_2 in medium alone, IL-2 (Proleukin, 1000UI/ml) or IL-15 (10ng/ml, CellGenix). On day 1, cells were harvested, washed 3 times in 0.9% NaCl and resuspended with 5% human albumin and 0.9% NaCl prior to analysis. The final product enriched in activated NK cells was evaluated for total nucleated cells (TNC), NK, T-cells, B-cells and monocytes content, viability and stability, microbiological testing, NK cell receptor repertoire expression and cytolytic activity against AML cell lines. *Results.* Leukapheresis products contained $12.05x10^9$-$14.34x10^9$ TNC with 58.1%-65.8% CD3+ T-cells, 8.8%-11.7% CD20+ B-cells, 14.1%-20.8% CD14+ monocytes and 5.2-8.1% CD3-CD56+ NK cells. Cell selection consistently resulted in a >3-log T- and B-cell depletion efficiency, yielding a final T- andcell content of 12.2-28.2x105 and 3.3-12.7x105, respectively. TNC and NK recovery were respectively 19.2%-26.5% and 81.2-82.9%. depleted MNC contained 24.2 to 26.2% NK cells, other cells were mainly monocytes. After overnight culture, no T-cell expansion occurred. TNC recovery from IL-2 and IL-15-activated products were comparable and ranged from 48% to 63.3%. NK cell recovery did not significantly differ between IL-2 and IL-15-activated products and ranged between 58.6 and 100%. IL-2 and IL-15-activated cell products contained between 27.5% and 47.2% NK cells. Viability was reproducibly over 80%. When compared to medium alone, both IL-2 and IL-15 proved efficient in activating NK cells, as attested by the upregulation of CD69 and a remarkable increase in NK cytotoxicity against K562 and the following AML targets: HL-60, KG-1, OCI/AML3, THP-1 (1.9 to 3.5-fold increase in CD107a+ NK cells). Microbiological cultures were negative. Final cell products proved stable upon storage for up to 6h at 4-8°C. *Conclusion.* We successfully generated large numbers of T/B cell depleted GMP-grade allogeneic NK cells suitable for immunotherapy of AML patients. Both IL-2 and IL-15 proved efficient in triggering NK activation. NK-ACT provides a solid basis to further assess the clinical efficacy of such activated NK cells in adults with high-risk AML in a non transplantation setting.

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IL-10 AND REGULATORY T CELLS LIMIT T CELL RESPONSES AFTER TRANSCUTANEOUS IMMUNIZATION

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The imidazoquinoline derivate imiquimod induces inflammatory responses and protection against transplanted tumors when applied to the skin in combination with a cognate peptide epitope (transcutaneous immunization, TCI). Investigating the influence of UV irradiation on the induction of a peptide-specific CTL-response we found that combining TCI with low dose UV-B boosted the CTL-response and induced potent memory formation. For UV-irradiation the induction of regulatory as well as NK-T cells and the release of suppressive cytokines like IL-10 and IL-4 has been described. As we were interested in the mechanisms behind supporting and inhibiting factors we depleted FoxP3+ regulatory T cells and found that specific CTL-responses were greatly enhanced. In *in vivo* studies it has been published that naturally occurring as well as induced Treg produce IL-10 to control immune responses. Here we can show that in our immunization protocol Treg mediated immune suppression is only partly dependent on the release of Treg-derived IL-10 which itself inhibits immune response formation as could be shown in experiments with IL-10-/- mice or application of an anti-IL-10-receptor-Ab. As the manipulation of NKT cells seems to play a substantial role in UV-induced suppression we immunized in a NK-T cell free system and induced increased immune responses showing suppressive capacities for this cell type. Understanding the structure behind UV-enhanced TCI could lead to further improvements and advanced vaccination protocols against tumors and persistent virus infection. arandalopez@uni-mainz.de

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MOLECULAR MECHANISMS INVOLVED IN T-CELL SUPRESSION AND REGULATORY T CELL GENERATION BY MESENCHYMAL STROMAL CELLS

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Introduction. In recent years, Mesenchymal Stem Cells (MSCs) have aroused the attention from scientific community for their capacity of suppress the T-cell proliferation and regulatory T-cells (Tregs) generation. These immunological properties of MSC attracted the interest of basic and clinical investigators, in light of its potential therapeutical use in different immunological diseases. Nevertheless, successful translation into clinical practice requires further dissection of the ways by witch MSCs modulate the signaling pathways activated on T-lymphocytes. *Aims.* Explore the molecular mechanisms underlying the induction of T-cell suppression and Tregs generation by MSCs. *Methods.*

Peripheral blood T-lymphocytes from 3 individuals were activated by anti-CD3/CD28 beads and cultured either in the absence or in the presence of MSC. Following a 5 day period, CD4+ lymphocytes were purified and profiled by whole genome microarrays. Real-time PCR was used for evaluations. MSCs inhibited the proliferation of activated T-lymphocytes, as compared to cells cultured alone. The proliferative suppression was clearly paralleled by a general transcriptional repression of components related to TCR signaling and to cell cycle progression. For instance, transcript levels of major components mediating TCR signaling, such as, CD3, LCK, Vav, ZAP70, LAT and GRB2 and controlling cell cycle progression trough the G1 phase, such as Cyclins D1 and E, and corresponding CDK4 and CDK2 kinases, were all repressed in lymphocytes cocultured with MSCs. Interestingly, the control of TCR signaling is not only involved in the suppression of T-cell proliferation but also in the induction of Treg, as continued TCR stimulation leads to a loss in Foxp3 inducibility. Moreover, the antagonism of the continued TCR stimulation over Foxp3 expression would be mediated by the PI3K/AKT and mTOR signaling pathways, as inhibition of these pathways in activated T-cells induces transcriptional changes driving the generation of Tregs phenotype. Strikingly, our results show that these pathways are clearly transcriptionally repressed on activated T-cells cocultured with MSCs; with the down-modulation of transcripts that include central components of these pathways, namely, the catalytic subunit of PI3K, PDPK1, AKT1 and 2, FKBP1A, among others. Moreover, we have evidences that upon co-culture with MSCs, canonical NF-kB pathway signaling on activated T-cell is inhibited and substituted by noncanonical signaling, and that this correlates with the acquisition of Treg-related genes. The general transcriptional repression of components of TCR, PI3K/AKT and mTOR signaling pathways in activated T-lymphocytes cocultured with MSCs, indicates that a molecular mechanism with a broader action would be implicated in the suppression of the proliferation and the concomitant induction of Tregs. In line with this, our results provides evidences that MSCs can modulate the expression of crucial components of the epigenetic machinery in activated T-cells leading to the general repression observed by us. *Conclusion.* Our results shown that several pathways related to T-cell activation, proliferation and involved with the induction of a regulatory phenotype, are modulated in lymphocytes, upon coculture with MSCs. These findings are useful to understand the molecular mechanisms involved in T-cell immunomodulaton by MSCs and help further studies to ensure the success on the clinical use of these cells.

0641

A UNIQUE IMMUNOTHERAPEUTIC MODALITY BY USING A LEUKEMIC PLASMACYTOID DENDRITIC CELL LINE (PMDC05) AS POTENT ANTIGEN PRESENTING CELLS

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Background. Although cellular immunotherapy based on antigen-specific cytotoxic T lymphocytes (CTLs) against tumors including leukemia and severe infections is a promising strategy, one of the pivotal issues is a hardship in constant supply of high quality antigen presenting cells for generating CTLs against tumor or pathogen-associated antigens. We established a leukemic plasmacytoid dendritic cell (pDC) line (PMDC05) with potent antigen presenting capacity from leukemia cells of a HLA-A*0201/*2402 patient with pDC acute leukemia. PMDC05 possessed a considerable antigen presenting ability to naïve T cells, which was enhanced by culturing with IL-3 or influenza virus and especially by LPS. *Aims.* In order to establish PMDC05-based tumor immunotherapy, we investigated whether PMDC05 could be efficiently used for generating CTLs specific for antigens of leukemia cells or pathogens. In addition, in order to elevate the efficiency of PMDC05-based anti-tumor immunotherapy, we evaluated the effectiveness of newly synthesized inhibiting agent for indoleamine-2,3-dioxygenase (new IDO inhibitor) for potentiating antigen presenting ability of PMDC05. *Methods.* PMDC05 cells, which was stimulated with 0.1 μg/mL LPS and loaded with 10 μg/mL WT1 peptides (HLA-A*2402-restricted, modified-type 9-mer peptide; CYTWNQMNL) or CMV pp65 peptides (HLA-A*2402-restricted, 9-mer peptide; QYDPVAALF) for 24 hours, were irradiated (60 Gy) and co-cultured with allogeneic $CD8^+$ T cells which were purified from peripheral blood mononuclear cells (PB-MNCs) of HLA-A*2402^+ healthy donor (PMDC05:$CD8^+$ Tcells = 1:10). 50 U/mL IL-2 was added to the co-culture at day 2, and IL-2 as well as 10 ng/mL IL-7 were added every 3 days thereafter. Induction of WT1 or CMV-specific CTLs was evaluated by flow cytometry analysis using HLA-A*2402 WT1 tetramer or CMV tetramer every week. In addition, PMDC05 cells were treated with new IDO inhibitor during the stimulation with LPS and IFN-γ and used as stimulator cells in mixed leukocyte culture (MLC) using PB-MNCs as responder cells. MLC was performed in both CFSE-based proliferation and ^{3}H-thymidine incorporation methods. *Results.* PMDC05 began to induce WT1 $tetramer^+CD8^+$ T cells at week 4 and the percentage of WT1 $tetramer^+$ T cells in $CD8^+$ T cells rose to more than 75% at week 7. Likewise, CMV $tetramer^+CD8^+$ T cells were amplified in $CD8^+$ T cells co-cultured with CMV peptide-pulsed PMDC05. By treateing PMDC05 cells with new IDO inhibitor, antigen presenting ability was increased in both CFSE-based proliferation and ^{3}H-thymidine incorporation methods. *Conclusions.* These data suggested that PMDC05 could be efficiently used for generating CTLs specific for tumor or pathogen-associated antigens and newly synthesized IDO inhibitor could be applicable for enhancing the antigen presenting ability of PMDC05. These findings revealed that PMDC05 cells in combination with new IDO inhibitor could be a promising strategy in cellular immunotherapy for tumors and severe infections.

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IMMUNOTHERAPY OF RECURRENT B-CELL MALIGNANCIES IN PEDIATRIC PATIENTS WITH THE TRIFUNCTIONAL ANTIBODY FBTA05 (ANTI-CD3 X ANTI-CD20)

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Treatment options for B cell malignancies in pediatric patients refractory to standard therapy are limited. Thus novel treatment approaches are urgently required. Bispecific antibodies, especially in combination with allogeneic stem cell transplantation (SCT) and donor lymphocyte infusion (DLI) represent a highly attractive therapeutic concept to direct immunity towards tumor cells. Seven children (three with relapsed diffuse large B-cell lymphoma (DLBCL), one with mature Burkitt lymphoma, two with mature B-cell acute leukemia and one with pre-B cell acute lymphoblastic leukemia) were treated in individual settings with escalating doses of FBTA05. All patients were extensively pretreated and presented refractory to standard treatment (radiation, chemotherapy) including rituximab. Five children received HLA-identical allogeneic SCT before FBTA05 treatment. In two patients FBTA05 application was followed by DLI. In one child with DLBCL FBTA05 and DLI were combined with lenalidomide in a second treatment cycle. For safety reasons dose escalation always started with 10 μg followed by 20 μg and 50 μg every third day. Thereafter weekly applications (100-300μg) were performed. Due to tumor progression before start of therapy daily application of FBTA05 were performed in three patients up to maximum doses of 200, 500 and 1000μg, respectively. Six of the seven children displayed a clinical response: five stable diseases and one complete response (CR). Remarkably, in this patient a CR even in the bone marrow was achieved without SCT and DLI. Overall survival is in the range of 85 up to 551 days (updated at time of presentation). Three out of the seven children died due to relapse or tumor progression. FBTA05 infusions were well tolerated by all children. Adverse events were restricted to fever and chills and could be managed by supportive treatment. Also the combination of lenalidomide and FBTA05 was well tolerated with nausea and increase of pre-existent leucocytopenia during the first cycle (10 and 15 mg daily), while a dose of 5 mg daily in the following cycles was well tolerated. In only one patient, human anti-mouse antibodies were detectable. Importantly, this patient could be safely treated with

two additional applications of FBTA05. The cytokine profile was characterized by transient increase of IL-6, IL-8 and IL-10. Plasma concentrations of FBTA05 strictly correlated with the corresponding dosing schedules with up to 0.19 µg/mL after daily escalating applications of FBTA05 up to 1,000 µg accompanied by the rapid clearance within few days. Graft versus host disease (grade III-IV) developed in two patients (in one case after DLI), but could be controlled by further immunosuppressive therapies. Based on these encouraging results, FBTA05 shall be further tested in children in a clinical study. Currently, a phase I/II study in combination with DLI in adult patients with low and high grade B-cell lymphoma after allo-SCT (STP-LYM-01-V01) is performed.

0643

A NOVEL SINGLE CHAIN P53TCR PREVENTS GVHD IN A HUMANIZED MOUSE MODEL OF ADOPTIVE T CELL TRANSFER

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Background. Adoptive cell therapy with T cells retrovirally transduced with tumor-associated antigen (TAA) specific TCRs is a promising approach for immunotherapy in patients with hematological malignancies. The TAA p53 is over-expressed in approximately 50% of human tumors. One barrier to the development of T cell-based immunotherapies is the absence of high-affinity tumor specific TCRs in the patient due to self tolerance. We have reported that HLA-A*0201 (A2.1) transgenic mice can be used to circumvent self-tolerance to universal human TAA and to generate efficient tumor-reactive CTL. We used A2.1 transgenic mice, in which the mouse CD8 molecule cannot efficiently interact with A2.1 to generate a high-affinity, CD8-independent p53(264-272) specific TCR. Retroviral expression of CD8-independent p53-specific TCR into T cells, allowed CD8+ T lymphocytes to acquire a broad tumor-specific CTL activity but also redirected CD4+ T cells into potent tumor-reactive, p53-specific T helper cells. However, a particular safety concern with TCR gene transfer, is the formation of mixed TCR heterodimers between the introduced TCR α and β chains with the endogenous TCR chains, resulting in the potential generation of autoreactive T cells. *Aim.* Our aim is to optimize p53TCR construct to prevent mixed dimers formation. *Methods.* To reduce the formation of TCR mixed dimers an additional inter-chain disulfide bond (Cys.) between the TCR α and β chain constant domains was introduced. We further improved the expression level of p53 TCR transgene using codon-optimization (Opt.) of the TCR sequence in retroviral vector containing the self-cleaving 2A virus-derived peptide element. To prevent the formation of mixed TCR dimers, we engineered a novel p53(264-272)-specific Opt.Cys single chain (sc) TCR construct. The safety of engineered Opt.Cys p53TCR constructs was assessed in a humanized mouse model of adoptive T cell transfer. *Results.* Mouse T cells transduced with p53(264-272)A2.1-Opt.Cys TCR showed higher expression levels of the introduced TCR as compared to p53(264-272)-specific WT TCR. Importantly, p53(264-272)A2.1-Opt.Cys and sc TCR transduced CTL recognized and killed a wide variety of malignant A2.1 tumor cells with altered p53 expression, but not p53-deficient A2.1 cells more efficiently as compared to CTL transduced with the WT p53(264-272)-specific TCR. However, when p53(264-272)A2.1-Opt.Cys TCR transduced mouse T cells are adoptively transferred into p53-deficient partially humanized (A2Kb) mice, under conditioning-induced lymphopenia, expansion of infused T cells following high dose IL-2 administration is associated with the development of lethal autoimmunity similarly to mice receiving p53(264-272)-specific WT TCR transduced T cells due to the formation of self-reactive TCRs. In contrast, mice receiving T cells transduced with p53(264-272)A2.1-Opt.Cys scTCR did not develop any sign of GvHD. *Conclusions.* Our data demonstrate that p53(264-272) TCR gene transfer-induced off-target autoimmunity observed in preclinical mouse model could be prevented by the use of p53(264-272) engineered scTCR, suggesting that sc p53TCR may represent a new and safe approach for TCR-based gene therapy of p53-associated hematological malignancies.

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AN HLA-DR-TARGETING IMMUNOCYTOKINE COMPRISING TETRAMERIC INTERFERON-ALPHA-2B HAS POTENT *IN VITRO* AND *IN VIVO* ACTIVITY WITH VARIOUS HEMATOLOGICAL CANCERS

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Background. IFNα2 has been used in therapy of a variety of hematopoietic neoplasias, including myeloma (MM), NHL, hairy cell leukemia (HCL), and CML. However, its therapeutic potential is limited by its short circulating half-life and systemic toxicity. Fusion of IFNα2 to a monoclonal antibody (MAb-IFNα) improves solubility and stability, and markedly increases circulating half-life. In addition to allowing less frequent and lower doses due to extended pharmacokinetics, targeting of IFNα2 to tumor sites using a tumor-directed mAb can reduce systemic concentrations and increase local concentration and tumor retention of IFNα2, thereby improving the therapeutic index. Increased tumor concentrations of IFNα2 can augment its direct anti-proliferative, apoptotic and anti-angiogenic activity, as well as prime an antitumor immune response. *Aims.* We evaluated the therapeutic potential of HLA-DR-targeting MAb-IFNα and identified specific hematological diseases that might be of benefit. *Methods.* We previously used the DNL method to generate stably-tethered immunocytokines (Rossi *et al.*, Blood 2009;114:3864-71; Rossi *et al.*, Cancer Res 2010;70:7600-9), which include 20-2b-2b (CD20-targeted mAb-IFNα comprising tetrameric IFNα2b and veltuzumab) and 20-C2-2b (bispecific CD20/HLA-DR-targeting dimeric IFNα2b). Here we describe the potent *in vitro* and *in vivo* anti-tumor activities of a third immunocytokine, C2-2b-2b, comprising tetrameric IFNα2b and the anti-HLA-DR mAb, hL243, against myeloma, lymphoma and leukemia cell lines. *Results.* C2-2b-2b bound HLA-DR+ cells with similar avidity to hL243 and exhibited high IFNα specific activity. *In vitro*, C2-2b-2b inhibited a panel of 20 hematopoietic cancer cell lines including NHL (Burkitt, mantle cell & follicular), leukemia (HCL, AML, ALL & CLL), and MM. In most cases, C2-2b-2b was more effective than CD20-targeted mAb-IFNα or a mixture comprising hL243 and IFNα. The responsiveness of the cell lines correlated with HLA-DR expression/density and sensitivity to IFNα and hL243. *In vivo*, a single 1 microgram dose of C2-2b-2b significantly ($P<0.0001$) improved survival in advanced Daudi (NHL) and CAG (MM) xenograft models, and single doses of ≥10 micrograms resulted in 70-100% long-term survivors (cures). In the advanced Daudi model, the group treated with a single 1 microgram dose of C2-2b-2b exhibited a median survival time (MST) of >130 days, compared to the saline control group MST of 42 days. C2-2b-2b was significantly ($P<0.0001$) more effective than molar equivalent treatments of hL243 + rIFNα2b (MST=52.5 days) or non-targeting mAb-IFNα (MST=45.5 days). Additionally, C2-2b-2b was superior to peginterferonalfa-2a used at four- and 20-fold greater concentrations (MST=71 and 91 days, respectively; $P<0.001$). C2-2b-2b depleted Daudi (91% depletion) and CAG (98%) cells from whole blood (*ex vivo*) more effectively than hL243 IgG or non-targeting mAb-IFNα. C2-2b-2b was less toxic to normal B cells (62%), monocytes (58%) and T cells (0%) compared to Daudi or CAG. C2-2b-2b depleted dendritic cells, with myeloid DCs (90%) being more sensitive than plasmacytoid DCs (48%). *Conclusions.* The DNL method provides a modular approach for producing defined multimeric immunocytokines, resulting in superior *in vivo* potency compared to the original cytokines due to improved pharmacokinetics, targeting and reduced systemic toxicity. These results suggest that C2-2b-2b might be useful in the treatment of various HLA-DR+ hematological malignancies.

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A SINGLE INJECTION OF AAV-8 VECTOR EXPRESSING IL-24 EFFICIENTLY SUPPRESSES TUMOR GROWTH MEDIATED BY MULTIPLE ANTI-TUMOR MECHANISMS IN MLL/AF4 POSITIVE ALL MODEL MICE

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Mixed-lineage leukemia (MLL)/AF4 positive acute lymphoblastic leukemia (ALL) is a very common leukemia in infant and is associated with high relapse rate and poor prognosis even after allogenic bone marrow transplantation (allo-BMT). The resistance to graft-versus-leukemia (GVL) effect may be responsible for the poor effect of allo-BMT on MLL/AF4 positive ALL. Interleukin 24 (IL24) selectively induces apoptosis in cancer cells without harming normal cells. It also exerts immunomodulatory and anti-angiogenic effects, as well as potent antitumor bystander effects, making it an ideal candidate for use in a new anti-cancer gene therapy. Here, we examined the feasibility of adeno-associated virus type 8 (AAV8) vector-mediated muscle directed systemic gene therapy of MLL/AF4 positive ALL using IL24. *In vitro* study using TUNEL assay showed that IL24 induced apoptosis against MLL/AF4 positive cell lines (SEM and RS4;11) with dose dependency of IL24 concentration. Western blotting analysis showed that down-regulation of expression of HOXA9 by IL24 treatment and synergic up-regulation of p53-Caspase8 apoptosis pathway in IL24 and TNFα treated MLL/AF4 positive cell lines were detected. Moreover, the activities of p53-Caspase8 apoptosis pathway of MLL/AF4 positive cell lines were significantly lower than those in MLL/AF4 negative cell lines (H9, MOLT4, and Raji) under single TNFα stimulation. To assess the *in vivo* effects of muscle targeted AAV8-mediated systemic delivery of IL-24, we established a MLL/AF4 positive ALL murine model in which the SEM cells expressing luciferase gene was inoculated into the caudal vein of SCID mice. Using this MLL/AF4 positive ALL murine model, we can detect the tumor growth and metastases by a real-time *in vivo* imaging analyze system (IVIS). After single injection of AAV8-IL24 (1.5x1011 vg/body) into the right quadriceps muscle of the model mice, tumor cell growth was monitored by IVIS. Suppression of tumor growth was observed in AAV8-IL24 injected mice compared to control GFP expressing AAV injected mice (4.9±1.7x105 vs. 11.7±2.0x105 photon/sec; p=0.01). Survival effect was also detected in AAV8-IL24 mice (median 49M vs. 63M, P=0.022). Finally, we confirmed the *in vivo* effects of AAV8-IL24 by treatment of MLL/AF4 transgenic (Tg) mice model. A single intramuscular injection of AAV8-IL24 significantly inhibited appearance of lymphoma or leukemia with suppression of angiogenesis in MLL/AF4 transgenic (Tg) mice. These results clearly demonstrate that AAV8-IL24 was effective for MLL/AF4 leukemia through down-regulation of HOXA9, anti-angiogenesis and up-sensitivity to TNFα, which is a key factor of graft versus leukemia effect, followed by up-regulation of p53-Caspase8 apoptosis pathway. Thus, gene therapy using AAV8-IL24 combined with allo-BMT may be the promising strategy of MLL/AF4 positive ALL.

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TGF-B1 MODULATES LPS-INDUCED CYTOKINE/CHEMOKINE PRODUCTION AND INHIBITS NF-κB, ERK AND P38 ACTIVATION IN DENDRITIC CELLS<

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Background. There is growing evidence that dendritic cells (DC) can be not only immunogenic but also tolerogenic, both intrathymically and in the periphery. Previously, we have found that, compared with immature DC (iDC), TGF-β1 treated DC (TGFβ-DC) are resistant to maturation stimulus-lipopolysaccharide(LPS) and might have correlation with the downregulation of Toll-like receptor(TLR)-4 expression. Moreover, we also have demonstrated that recipient-derived TGFβ-DC induce major histocompatibility complex (MHC)-specific tolerance in a murine bone marrow transplantation (BMT) model. However, the molecular mechanisms underlying the phenotypical and functional changes in TGFβ-DC upon LPS stimulation have not been clearly elucidated. *Aims.* To analyze whether TGF-β1 affected the production of cytokines/chemokines and proteins in the TLR4 signal transduction pathway following LPS stimulation. *Methods.* C57BL/6J murine bone marrow cells were cultured with different cytokines combination to generate iDC (GM-CSF only) and TGFβ-DC (GM-CSF+TGF-β1). Afterwards, they were stimulated by lipopolysaccharide (LPS) for 2 days to induce nuclear translocation of nuclear factor(NF)-κB and maturation of DC. The concentrations of murine IL-12p70, IFN-γ, IL-18 and IL-10 in culture supernatants were assayed by ELISA. The mRNA levels of CCL2, CCL3, CCL5, CXCL10 were detected by reverse-transcriptase polymerase chain reaction (RT-PCR). We used electrophoretic mobility shift assay (EMSA) to determine the NF-κB activity in DC. ERK1/2 and p38 mitogen activated protein kinases (MAPKs) protein expression were checked by Western blot analysis. *Results.* In the resting state, both two types of DC produced low levels of cytokines. LPS stimulation induced cytokine production, but with a distinctly different pattern for each subset. The production of IL-12p70 in TGFβ-DC was significantly less than that of iDC (115.4±15.2 pg/ml vs 517.0±29.7 pg/ml, P<0.01), but the production of Th2 cytokine-IL-10 was significantly higher (132.1±17.5 pg/ml vs 75.1±16.6 pg/ml, P<0.05). We also found that levels of Th1-inclined chemokines mRNA, CCL2, CCL3 and CXCL10 increased earlier and remained relatively higher in iDC than in TGFβ-DC after LPS stimulation. In contrast, CCL5 mRNA was expressed at comparable levels between the two subsets of DC. The results suggested that NF-κB DNA binding activity was significantly increased in iDC in response to LPS, but the addition of TGF-β1 to DC decreased NF-κB binding. Furthermore, TGF-β1 was effective in suppressing LPS-induced activation of ERK1/2 and p38 kinase, the level of phosphorylation of ERK1/2 and p38 kinase were lower than iDC. *Conclusions.* Our findings reveal that TGF-β1 modulates the secretion of inflammatory cytokines/chemokines in DC. These effects may result from interfering with the activities of key elements of TLR4 pathway, such as NF-κB, ERK1/2 and p38 MAPKs. This study strengthens the notion that TGFβ-DC are a unique type of tolerogenic DC exhibiting some distinct characteristics.

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SEQUENTIAL GENOMIC ANALYSIS OF UNTREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA DEMONSTRATES CLONAL EVOLUTION

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Background. The difference in clinicobiological behavior of chronic lymphocytic leukemia (CLL) can be related to genetic aberrations present either at diagnosis or acquired during disease course. This clonal evolution has been studied by means of conventional cytogenetic analysis (CCA) and fluorescence *in situ* hybridization (FISH). However, SNP-arrays enable to investigate clonal evolution at a higher resolution. *Aims.* This study aimed to characterize clonal evolution using different approaches and to identify a possible association with disease progression, i.e. therapy initiation. *Methods.* Patient selection met following inclusion criteria: (i) diagnosis of CLL, (iia) at least two available stored samples, (iib) second sample (S2) obtained at least one year after the first (S1), and (iii) patient untreated at both times of sampling. Sequential samples were investigated by CCA, FISH and Affymetrix cytogenetics whole-genome 2.7M arrays on peripheral blood or bone marrow samples. Clinical and biological data were available for all patients. *Results.* Fifty-three patients fulfilled the selection criteria. The median interval between sampling was 41 months (range 13-102 months). Male/female ratio and median age at diagnosis were 32/21 and 58.4 years (range 30.1-82.8 years), respectively. Patients presented with more advanced Binet stages at S2 vs. S1 (A/B/C in 34/12/7 vs. 47/5/1 patients, respectively). In addition, lymphocytosis was higher and lymphadenopathy, splenomegaly, thrombocytopenia, anemia and hypogammaglobulinemia were more frequent at S2. IGVH was unmutated in 15 and mutated in 32 patients. Treatment was initiated in 33 patients after a median duration of 51 months (range 19-173 months) after diagnosis and 1 month (range 0-54 months) after S2. Karyotyping revealed clonal evolution in 17/53 cases: acquired aberrations included unbalanced translocations (n=7), del(13)(q14) (n=4), del(11)(q22q23) (n=4), del(17)(p13) (n=2), del(6)(q) (n=2) and balanced translocations (n=2) and various other aberrations (n=5). In contrast, FISH using the CLL-"4 probe panel"revealed clonal evolution in only 11 cases. All cases with evolution by FISH, showed del(13)(q14) either as a new or additional subclone. The del(13)(q14) was accompanied by a new del(11)(q22q23) and del(17)(p13), in one case each. Whereas array analysis detected more gains at S1 vs. S2 (9009 vs. 7220 segments, respectively including copy number variations), losses were predominantly found at S2 (n=1293 vs. 2907 segments at S1 and S2, respectively). Recurrent losses were located in the regions 13q14.3, 11q22-23 and 17p13 (n=11/18, 3/7, 0/2 patients at S1/S2, respectively). Losses of 8p23-11.23, 15q11.2, 22q11.23, 9p21.1, 9p23-21.3 and 14q21.3-22.1 (n=9/5, 5/3, 1/3, 2/1, 2/2 and 2/1 patients at S1/S2, respectively) were also recurrent. In addition, the size of the losses was larger at S2 vs. S1 and in the cohort of patients with impending need vs. without need for therapy; i.e. the mean number of segments lost in patients without vs. with need for therapy at S1/S2 was 18/31 vs. 54/66 in general, 3/12 vs. 11/19 for 13q14.3, and 0/3 vs. 0/13 for 11q22-23, respectively. *Conclusion.* The present study confirms the occurrence of clonal evolution, especially losses in CLL. Further analysis is ongoing, in order to elucidate the clinical significance of clonal evolution in CLL.

0648

ROLE OF THE GLYCOSYLATION STATUS OF THE SURFACE IGM IN DISEASE PROGRESSION OF CLL

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The B-cell receptor (BCR) has a critical influence on the fate of both normal B-cells and of the majority of B-cell malignancies. This key molecule determines the fate of antigen-specific B cells, by integration of multiple signals from antigen and from the microenvironment. Our most recent data show that in CLL, the surface IgM (sIgM) exists in two different glycoforms which are expressed in variable proportions, presumably depending on the engagement with antigen *in vivo*. The two sIgM glycoforms exhibit different N-glycosylation patterns in the µ-constant region. One glycoform is a mannosylated form which is a characteristic of the immature ER-located Igs, and the other is the complex form which is normally expressed on normal B-cells. However, treatment of normal B cells with anti-IgM *in vitro*, converted the complex form of its sIgM to a high-mannose form. Also, the expression of the mannosylated form on CLL cells, could be reverted to the mature form following incubation *in vitro*. This novel observation that antigen engagement alters the glycosylation status of the sIgM, suggests that mannosylation of the µ-chain might be an index of antigen engagement. We also show that both glycoforms are able to signal following surface IgM engagement *in vitro* leading to enhanced tyrosine phosphorylation. The localization of candidate lectin-bearing cells, specially those surrounding the Ki67+ cells within the proliferation centers (PCs) of SLL/CLL lymph nodes, was investigated by immunohistochemistry. Preliminary analysis showed the presence of (CD68+) macrophages, known to express high levels of the mannose receptor (MR), within the PCs. This study gives a new insight into the CLL disease and also reveals a new potential therapeutic target.

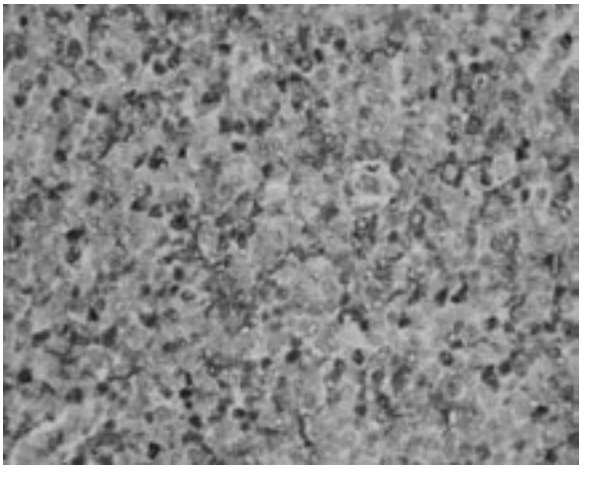

Figure 1. SLL lymph node double stained for CD38 and Ki67.

0649

SEROLOGICAL PROTEOME ANALYSIS (SERPA) FOR THE IDENTIFICATION OF TUMOR-ASSOCIATED ANTIGENS ELICITING ANTIBODY-RESPONSES IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Serological proteome analysis has been demonstrated to be a useful method to detect tumor antigens (Ag) eliciting antibody responses in human malignancies. Ag identified in tumor patients can provide information regarding intracellular molecules engaged in the transformation process and may be useful for the development of immune-based therapeutic strategies. *Aims.* aim of this study is to identify potential biomarkers predicting disease aggressiveness and specific targets for immune-based approach in CLL. *Methods.* Twenty-one untreated CLL pa-

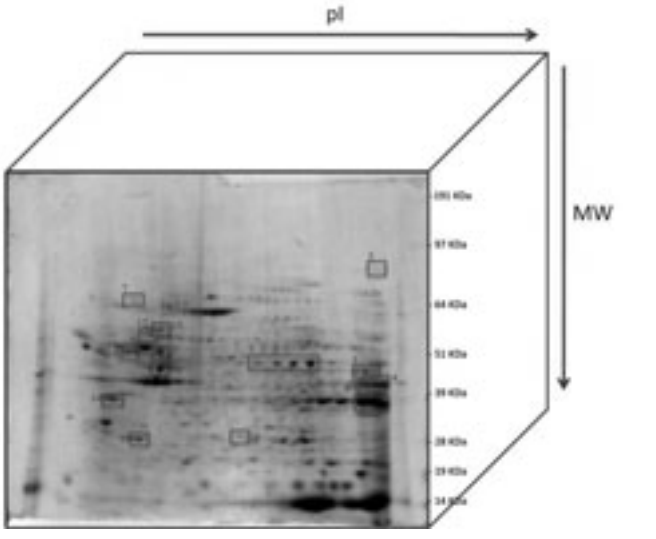

Figure 1.

tients were included in the study. Proteins extracted from leukemic cells were separated by 2-D electrophoresis (2-DE) and transferred onto membranes by electroblotting to obtain 21 2-DE proteomic maps. Each map was probed with the corresponding autologous serum by Western Blot (WB). To verify the CLL-specificity of antibodies recognition, 7 out of 21 maps obtained from CLL patients were also probed with sera collected from 7 healthy donors (HD). For identification, Ag spots in WB were aligned with proteins in 2-DE maps. The protein spots corresponding to the assigned Ag were excised from the gel, trypsin digested and analyzed by peptide mass fingerprint by MALDITOF Mass Spectroscopy (MS) with the software MASCOT. T cells isolated from the peripheral blood (PB) of 3 CLL patients with anti-ENOA antibodies were stimulated with autologous unpulsed and ENOA-pulsed dendritic cells (DC), and evaluated for their ability to secrete IFNγ through an ELISPOT assay. *Results.* Sixteen out of 21 CLL sera (76%) showed immunoreactivity against at least one Ag and produced an overall number of 45 Ag spots. By contrast, sera from HD were significantly less reactive ($p<.03$) and produced an overall number of 3 Ag spots. Eleven out of 16 (69%) reactive CLL sera recognized from 2 to 6 different Ag. All the Ag spots were characterized and consisted of 16 different proteins (Fig.1). Sera from 48% CLL patients exhibited reactivity against a protein which was identified by MS as α-Enolase (ENOA). ENOA recognition was CLL specific since none of the sera from HD showed reactivity against this protein. The IGHV mutational status was available in 20 CLL patients and 12 patients were mutated (M), while 8 patients were unmutated (UM). ENOA was recognized from sera of 7 out of 12 M patients (58%), but only from sera of 2 out of 8 UM patients (25%). The ability of ENOA to induce Ag-specific T cell responses was assessed in 3 patients. T cells isolated from the PB of CLL patients with antibody-based ENOA reactivity were stimulated with autologous ENOA-pulsed DC. The results showed that CLL-derived ENOA-pulsed DC stimulated autologous T cells to secrete IFNγ. This response was ENOA-specific because it was not induced by unpulsed DC or DC pulsed with an irrelevant protein, and also CLL-specific because IFNγ release was not induced when T cells from a HD were stimulated with autologous ENOA-pulsed DC. *Summary/Conclusions.* Our results indicate that ENOA is capable of eliciting CLL-specific humoral and cellular immune responses. Therefore, ENOA can be considered a promising biomarker and a potential target for immune-based approaches in CLL.

0650

BCR STIMULATION INDUCES A DIFFERENTIAL MICRORNA (MIR) PROFILING BETWEEN B LYMPHOCYTES DERIVED FROM CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS AND HEALTY DONORS

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Background. MicroRNAs (miRs) are small non-coding RNAs that modulate the expression of genes at the post-transcriptional level, playing a pivotal role in many physiological and pathological processes. In lymphocyte ontogenesis, an involvement of miR-181a and miR-150 in B- and T-cell development has been documented. Moreover, in chronic lymphocytic leukemia (CLL) several miRs are associated with disease pathogenesis and/or outcome. *Aims.* In order to investigate a potential role of miRs in BCR stimulation, we evaluated the expression of these small RNAs following IgM and IgD cross-linking in CLL cells, as well as in healthy B lymphocytes. *Methods.* Peripheral blood mononuclear cells isolated from 9 untreated CLL samples and 2 healthy donors were enriched in CD19+ B cells and subsequently stimulated with a F(ab')2 anti-human IgM or with a F(ab')2 anti-human IgD, at a final concentration of 10 μg/ml. Following 24 and 48 hours of incubation, total RNA was extracted from unstimulated (US) and stimulated (S) samples for miR profiling analysis, performed using the GeneChip miRNA Affymetrix arrays. An unsupervised clustering was applied to evaluate samples responsiveness to BCR ligation. To identify differentially expressed miRs between US and S cases, a t-test retaining only probesets with a p-value <0.05 and a fold change >1.5 was used. *Results.* An unsupervised approach highlighted that, after both IgM and IgD stimulation, healthy donors clustered apart from CLL cases, suggesting a differential miRs expression pattern in healthy and leukemic B lymphocytes in response to BCR engagement. Based on these findings, we performed a t-test to compare US and S cells: in agreement with the unsupervised analysis, this approach showed a homogeneous signature associated to stimulation in B cells isolated from healthy donors and allowed to identify specific sets of miRs differentially expressed following IgM and IgD ligation, respectively. In CLL, we observed the modulation of several miRs both at 24 and 48 hours of IgM cross-linking, while miR expression changes occurred exclusively at 48 hours after IgD stimulation, suggesting a delayed activation in this context. Remarkably, miRs selected in CLL S cases were different from those identified in healthy donors, confirming a distinct miR regulation in BCR signaling of these samples. *Conclusions.* Our study reveals a differential miR expression pattern following IgM and IgD ligation between CLL and healthy B lymphocytes, suggesting that distinct mechanisms regulate BCR signal transduction at the physiological and pathological level. Further investigations to combine miR and gene expression profiles obtained from the same samples are currently underway with the aim of identifying putative miR targets.

0651

CLONAL EVOLUTION IN CHRONIC LYMPHOCYTIC LEUKEMIA: ANALYSIS OF CLINICOBIOLOGIC CORRELATIONS IN 105 PATIENTS

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Background. Clonal evolution (CE) involving chromosomes 17p, 11q, 6q, and 12 was reported in 15-42% of Chronic Lymphocytic Leukemia (CLL) cases using conventional karyotyping or fluorescence *in situ* hybridization (FISH). The incidence of this phenomenon depends on the length of follow-up and on the number of probes used for interphase FISH analysis. Attention was recently devoted to 14q32 translocation involving the immunoglobulin heavy chain gene (IGH). This aberration was found in 6-19% of CLL patients at diagnosis and was associated with therapy-demanding disease and inferior outcome. The incidence of this aberration at CE is presently unknown. *Aims.* To analyze the incidence, characteristics and clinicobiological significance of CE including 14q32 translocations in CLL. *Methods.* 105 patients seen at our institution between 1995 and 2004 were analyzed sequentially by FISH with the following probes: 13q14/D13S25, 11q22/ATM, 17p13/TP53, #12-centromere and 14q32/IGH break-a-part probe. FISH analysis was performed at diagnosis or before 1st line treatment. FISH was repeated at 4-6 year intervals in patients receiving ≤1 line of treatment. In relapsed patients who started 2nd line treatment, FISH was performed sequentially before administration of the 2nd line and before each subsequent line of therapy. These 105 patients fulfilled the following criteria: diagnosis of CLL based on morphology and immunophenotyping; successful FISH analysis at diagnosis and during follow-up (cases with t(11;14)(q13;q32)/BCL1-IGH were excluded); clinical records available for review. *Results.* The median follow-up of the entire series was 73 months (range 12-180 months). CE was observed in 15/105 patients after 24-170 months (median 64). Recurring aberrations at clonal evolution were 14q32/IGH translocation in 7 patients;

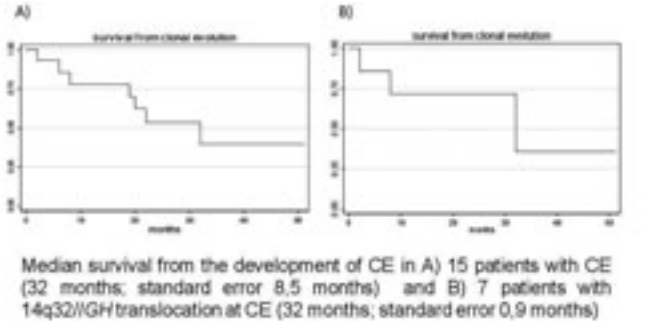

Figure 1. Survival from the development of CE.

17p- in 4 patients, 11q- in 2 patients, biallelic 13q- in 4 cases, hemizygous 13q- in 1 case, and 14q32 deletion in 1 patient. A 17p deletion was associated with 14q32/IGH rearrangement in 3/7 patients, one of whom also developed a biallelic 13q14 deletion. CE was detected in 15/58 pre-treated patients; to the contrary none of 47 untreated patients developed CE ($p<0.0001$). The 14q32/IGH rearrangement was detected after 1-4 lines of treatment (median 3 lines). In 3/7 cases with 14q32/IGH translocation BCL2 was the identified partner. In two cases the appearance of 14q32/IGH translocation was first detected in the bone marrow (BM) or in the lymph node (LN) and 13-58 months later in the peripheral blood (PB). ZAP70+ and high risk cytogenetics predicted for the occurrence of CE with borderline statistical significance ($p=0.055$ and 0.07, respectively). A shorter time to first treatment (TTT) and shorter time to chemorefractoriness (TTCR) was noted in 15 patients with CE ($p=0.033$ and 0.0046, respectively). Survival after the development of CE was 32 months (standard error 8,5). *Conclusions.* (i) 14q32/IGH translocation may represent one of the most frequent aberrations acquired during the natural history of CLL; (ii) The 14q32/IGH translocation may be detected earlier in BM or LN samples; (iii) CE including 14q32/IGH translocation occur in pre-treated patients with short TTT and TTCR; (iv) survival after CE is relatively short.

0652

ULTRA DEEP SEQUENCING OF IMMUNOGLOBULIN REARRANGEMENTS OF SEQUENTIAL SAMPLES FROM PATIENTS WITH B-CLL DEMONSTRATES CLONAL EVOLUTION

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Background and Aims. B-cell chronic lymphocytic leukaemia (CLL) is the most common leukaemia in the Western World. It is characterised by a chronic relapsing course and the development of chemotherapy resistance. There is mounting evidence that the immunoglobulin heavy chain (IgH) locus plays a central role in antigenic drive and that this may be important for disease maintenance and progression. Previous studies using next generation amplicon sequencing (NGAS) of the IgH locus have identified small sub-clones in patients with hypermutated IgH. In this study, we focussed on unmutated poor prognosis CLL and used NGAS to study clonal evolution of the IgH locus in sequential samples of the same patients. *Methods.* We amplified the clone-specific VDJ rearrangement in the immunoglobulin heavy chain using published Biomed consensus primers on sequential samples taken at diagnosis, after first treatment and at subsequent relapse on 4 CLL patients with an unmutated IgH. These products were sequenced on a 454-FLX (Roche Diagnostics). Resulting sequences were grouped using a Perl script to identify recurring reads. Reads of greater than 100 copies were analysed using Jalview and IGMT. Reads present less then 100 times were excluded from the analysis. *Results.* An average of 30000 reads were obtained for each sample. Within each sample we detected a dominant clone representing approximately 60% of all reads included in the analysis. In addition to this clone, multiple productive rearrangements were also identified when aligned to the germline using IGMT. These were present in a minority of reads. All subclones were clonally related to each other and the frequency of both dominant and additional subclones remained constant over time in the different samples despite treatment. Interestingly, one patient with an unmutated V1-69 dominant clone was found to have a hypermutated V1-69 subclone present at all three time-points. *Conclusions.* Together the data suggests that, in unmutated CLL, the leukaemic population consists of a mixture of clonally related dominant and minor IgH clones. The composition and proportion of these IgH clones remains remarkably stable over time. Further analyses of sequential samples are on-going.

0653

GENOMIC AND FUNCTIONAL ANALYSES OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS FOLLOWING IGD STIMULATION

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Background. Proliferation of chronic lymphocytic leukemia (CLL) cells may be influenced by antigenic stimulation and accessory signals from the microenvironment. Indeed, these factors induce different effects in distinct subgroups of patients, thus sustaining clinical heterogeneity of the disease. *Aims.* To investigate CLL responsiveness to B-cell receptor (BCR) stimulation, we evaluated the gene expression profile upon IgD cross-linking in different classes of patients, subdivided on the basis of their IGHV mutational status and clinical outcome. Microarray results were validated at the functional level using several *in vitro* assays. *Methods.* After 24 and 48 hours of incubation with a F(ab')2 anti-human IgD (10 µg/ml), unstimulated (US) and stimulated (S) CD19+ B cells isolated from untreated CLL patients underwent microarray analysis using the HGU133 Plus 2.0 Affymetrix arrays. Unsupervised clustering, t-test and Analysis of Variance (ANOVA) were performed. In addition, at 24 and 48 hours from the stimulus, antigenic expression was investigated by immunophenotypic analysis, cell cycle distribution changes were evaluated using the Acridine Orange (AO) technique, cell proliferation was measured by 3H-TdR uptake and, finally, apoptosis was analyzed by the Annexin-V and/or AO technique. *Results.* Unsupervised gene expression analysis showed that all CLL cells were responsive to stimulation, regardless of the clinico-biological features. T-test performed between US and S samples confirmed these findings and allowed to identify 290 differentially expressed genes - mostly involved in BCR signaling, cell adhesion, antigen processing and presentation, and MAPK cascade - after 24 hours of stimulation. At variance, at 48 hours, we selected 188 transcripts involved in regulation of transcription, chromatin organization, apoptosis and cell differentiation, suggesting that in CLL cells gene expression activation following IgD cross-linking occurs at later time points of stimulation. Furthermore, to assess the effects of IgD ligation in specific CLL subgroups, we used two different supervised approaches: t-test and ANOVA. Both analyses showed that the IGHV configuration and the status of the disease of the cases evaluated did not affect the responsiveness of cells to BCR engagement via sIgD. To validate the microarray results, we compared the antigen mean fluorescent intensity (MIF) ratio of US vs S cases of a set of selected transcripts encoding for B-lineage antigens involved in cell activation. This approach confirmed the downmodulation of CD79a, CD79b, CD27 and CD62L in all samples upon IgD ligation. Next, at 24 and 48 hours from the stimulus, cell cycle analysis and proliferation assay documented that IgD cross-linking induced a decrease of proliferative activity in CLL cells, irrespective of their clinico-biological characteristics; accordingly, at the same time points apoptosis increased significantly in S samples. *Conclusions.* Gene expression profile highlights that the majority of CLL are responsive to IgD stimulation, irrespective of the clinico-biological characteristics of the samples analyzed. In agreement with microarray results, *in vitro* experiments have shown a reduction of cell proliferation and a concomitant increase in the apoptotic rate of S cases, providing new insights into the mechanisms that regulate BCR engagement via sIgD in CLL.

0654

CORTACTIN EXPRESSION IS TIGHTLY CONNECTED TO B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA AGGRESSIVENESS

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Background. B-cell Chronic Lymphocytic Leukemia (B-CLL) is a disorder characterized by the accumulation of clonal CD5+ B lympho-

cytes due to uncontrolled growth and resistance to apoptosis. Several protein kinase pathways have been claimed to be involved in the regulation of apoptosis and cell survival of the neoplastic clone. We previously demonstrated that Src kinase Lyn displays anomalous properties in leukemic cells when compared to normal B lymphocytes. *Aims.* The protein cortactin is an ubiquitous actin-binding protein; it was originally identified as a substrate for Src kinases and overexpressed in several human tumors. Moreover its over-expression was correlated to cancer metastasis and poor prognosis of patients. Here we investigated the expression of cortactin in neoplastic B cells and its involvement in B-CLL pathogenesis and aggressiveness. *Methods.* Blood samples were collected from 106 patients that satisfied standard morphologic and immunophenotypic criteria for CLL B cells and from 15 healthy controls. Informed consent was obtained from all patients according to the Declaration of Helsinki. Untouched peripheral blood B cells were isolated from blood samples by negative selection using the RosetteSep for B cells isolation kit (StemCell Technologies; Vancouver, CND). The purity of B cells was at least 95% (CD19+), as assessed by flow cytometry. The expression level of cortactin was evaluated by real-time PCR, using GAPDH gene as calibrator, and by Western blotting analysis, using monoclonal Anti bodies against cortactin and β-actin as internal calibrator. Activity of matrix metalloproteinase 9 (MMP-9) in conditioned medium was evaluated by Zymography assay after 24h of culture with and without CXCL12/SDF1a chemokine; expression level of MMP-9 was also evaluated by real-time PCR. Migratory activity of B cells was induced by CXCL12/SDF1a chemokine and evaluated by chemotaxis test in a Boyden chamber. *Results.* We found that cortactin is overexpressed in B-CLL lymphocytes (1.10±0.12 SE) with respect to normal B cells (0.19±0.06 SE, Student's t-test $p<0.05$); when we investigated the correlation between level of cortactin and presence of somatic hypermutations (SHM) in the immunoglobulin heavy-chain variable region (IgVH) of B-CLL cells we found that unmutated patients expressed an higher levels of cortactin (SHM-: 1.46±0.27 SE) with respect to mutated ones (SHM+: 0.90±0.11 SE, *$p<0.05$, Student's t test) .We also evaluated the ability of neoplastic B cells to migrate after incubation with CXCL12/SDF1a chemokine and we observed that the overexpression of cortactin was associated with an increased B-CLL migration index ($r = 0.9$). Finally, the release of MMP-9 in cultured medium by neoplastic cells correlated with cortactin expression levels: in zymografy assay, patients without MMP-9 expression presented low cortactin level (0.46±0.20), while patients characterized by abundant expression of MMP-9 showed high cortactin level (2.43±0.04; $p<0.05$). *Conclusions.* The results that cortactin is overexpressed in neoplastic B cells and that this overexpression correlated with migration index after CXCL12 stimulus and MMP-9 release in cultured medium, suggest a role of cortactin in the aggressiveness of B-CLL neoplastic cells and support the cortactin as biomarker for diagnosis and prognosis of B-CLL.

0655

NON-CANONICAL WNT SIGNALLING AND VIMENTIN IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

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Background. CLL is the most common adult leukaemia in the western world, with over 3000 new diagnosis per year in the UK. CLL represents a clonal population of B-lymphocytes characterized by low proliferation rates and defective apoptotic pathways. Although CLL is an extremely heterogeneous disorder there is a cohort of patients who require urgent treatment and usually have a rapidly fatal disease. This cohort have an aggressive disease and often exhibit poor prognostic markers (high CD38, Binet B/C, poor risk cytogenetics (T12, 11q-, 17p), IGHV UM, ZAP70+), require immediate treatment and many die of CLL within three years despite therapy, leading to over 1000 deaths per annum in the UK. Deregulation of Wnt signalling has been reported to occur in CLL, which may contribute to the pathogenesis of the disease through up regulation of vimentin. Vimentin is an intermediate filament, important for cell migration, signalling and determining the rigidity of lymphocytes. *Aim.* To determine whether vimentin expression is higher in poor prognostic CLL patients and the role Wnt signalling plays in regulating vimentin. *Methods.* Expression of vimentin, Wnts and Wnt signalling in B-CLL cell lines and CLL patient samples was assessed using RQ-PCR, Western blotting and flow cytometry. Staining for vimentin was also performed in lymph node biopsies. Association of vimentin with Raf, pERK, 14-3-3 and PKC was assessed by immunoprecipitation and fluorescent microscopy. *Results.* We have screened 35 patients and confirmed a correlation between high vimentin levels and patients with poor prognostic markers (High CD38, Binet stage B/C, Poor risk cytogenetics and IGHV UM). Lymph node biopsies also revealed higher expression for vimentin in poor prognostic patients (n=13). In addition we have developed a flow cytometry based assay to measure vimentin and initial results indicate an excellent correlation with other poor prognostic markers. Stimulation of CLL cells with Wnt5a and Wnt4 indicate that non-canonical Wnt signalling increases vimentin levels. Immunoprecipitation and fluorescent staining indicate that vimentin co-localises with 14-3-3/Raf-1, ERK and PKC beta following stimulation. *Summary/Conclusion.* High vimentin expression is associated with progressive disease and a shortened time from diagnosis to initial treatment. Vimentin levels are controlled by non-canonical Wnt signalling and once expressed it forms dimers then tetramers, and eventually spindles. In addition, the inability of vimentin to disassemble has been linked to defective mitosis leading to chromosomal abnormalities, a phenomenon observed in these patients. Vimentin plays an important role in relaying intracellular signals, with phosphorylation and Ca2+ levels facilitating its interaction with signalling molecules such as PKC beta, 14-3-3/Raf-1 complex and pERK, pathways previously reported to be deregulated in CLL.

0656

PERIPHERAL BLOOD MINIMAL RESIDUAL DISEASE AND RESPONSE ARE INDEPENDENT PROGNOSIS FACTORS OF SURVIVAL IN CHRONIC LYMPHOCYTIC LEUKEMIA, WHEN USED IN ROUTINE PRACTICE AND REGARDLESS OF TREATMENT LINE

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Background. Eradication of minimal residual disease (MRD) has been shown to correlate with better outcome in chronic lymphocytic leukemia (CLL) in clinical trials. However the impact of peripheral blood (PB) MRD evaluation in routine practice is not established. *Aims.* To evaluate the impact of PB MRD detection on progression free survival (PFS) and overall survival (OS) in a large cohort of CLL patients and to determine its independent value when analyzed against the type of treatment, the line of treatment, the response status, the time of evaluation. *Methods.* 130 CLL patients were treated in a single institution. 76 patients (58.5%) received immunochemotherapy [fludarabine/rituximab (FR) +/- cyclophosphamide (FCR)], 23 (17.7%) fludarabine/cyclophosphamide, 14 (10.8%) fludarabine monotherapy and 11 (8.5%) alemtuzumab. Median follow-up was 33 months (0.5 - 115 months). MRD was determined in PB by 5 colors flow cytometry with limit of detection of 2x10-4, after 1st, 2nd, and 3rd or more line of therapy in 63%, 26.2% and 10.8% patients respectively. *Results.* 56.9% patients achieved a clinical complete response (CR), 14.6% patients a CR with incomplete marrow recovery (CRi), 26.9% patients a partial response (PR). MRD was negative (MRD-) in 56.2% patients. MRD negativity was achieved in 68.9% CR, 57.8% CRi and 31.4% PR patients. MRD negativity was correlated to significant improvement in both PFS and OS ($p<.001$) with 5 years PFS of 42.1% and 17.2% in MRD- and MRD+ population respectively. Nor the percentage of residual CD19+ B cells neither the delay of MRD assessment after the end of treatment (< or ≥ 60 days) influenced this result ($p>0.05$). Within the MRD- population, univariate analysis demonstrated prognosis correlation to line of therapy, type of treatment and response: 5yPFS was 51.3% versus 24.3% after 1st versus 2nd or more line of treatment ($p=.0324$); 61.1% versus 23.6% in "FCR"versus "other than FCR"treatment groups ($p=.0211$) and 62.7% versus 18.9% for CR versus not-CR patients ($p=.025$). The impact of MRD status on PFS was studied against line of therapy and response to treatment by multivariate analysis. Both MRD status and response, but not treatment line, were shown to be independent prognosis factors for PFS and OS. At the end of treatment, four categories of patients were defined: MRD-/CR, MRD+/CR, MRD-/not-CR and MRD+/not-CR categories with median PFS not reached, 42.2 months, 40.5 months and 14.8 months respectively. Interestingly, median PFS was not significantly different in MRD+/CR and MRD-/not-CR categories ($p=0.71$). We could then identify three prognosis groups of patients: favorable (MRD-/CR), intermediate (MRD-/not-CR or MRD+/CR) and unfavor-

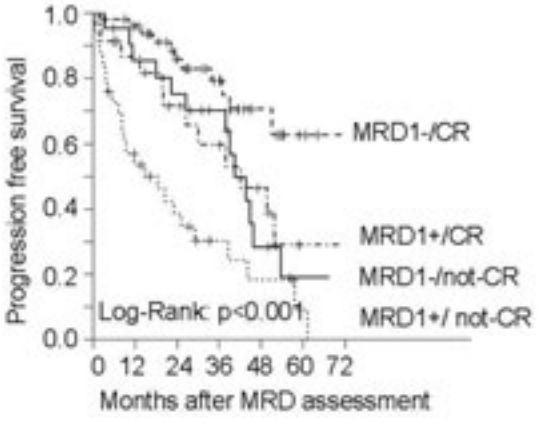

Figure 1.

able (MRD+/not-CR) (p<0.001). *Conclusions.* Although studied in a heterogeneous cohort with a limit of detection of 2x10-4, PB MRD status and clinical response were both found to significantly correlate to survival regardless of the line of treatment. Circulating residual B cells number and precise timing of the assessment did not seem to influence these results. PB MRD is clinically relevant in routine practice in CLL.

0657

A VALIDATED 8 GENE EXPRESSION SIGNATURE FOR THE PREDICTION OF OVERALL SURVIVAL AND TIME TO TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background. Chronic lymphocytic leukemia (CLL) is a common and heterogeneous disease. An accurate prediction of outcome is highly relevant for the development of personalized treatment strategies in CLL. Microarray technology was shown to be a useful tool for the development of prognostic gene expression scores. However, limited availability and high costs have so far prevented its widespread use. *Aim.* We set out to develop and validate a simple but powerful gene expression score that predicts overall survival (OS) in CLL patients and can easily be assayed in a routine diagnostics setting. *Methods.* 151 CLL microarray data sets (44 Affymetrix HG-U133 A&B and 107 Affymetrix HG-U133 Plus 2.0 chips) were correlated with OS using Cox regression and supervised principal component analysis to derive a prognostic score. This score was validated in an independent group of 149 CLL patients by quantitative real time PCR (qRT-PCR). An additional predictive value of the score was assessed using prediction error curves. *Results.* A prognostic score was developed based on the expression levels of eight genes (PS.8). PS.8 was predictive for OS and time to treatment (TTT) in univariate Cox regression in a heterogeneous validation data set (OS: p<0.001; TTT: p<0.001). The prognostic value of PS.8 could be demonstrated in patients without previous treatment, at first diagnosis, Binet A patients and subgroups defined by the traditional molecular markers. Multivariable Cox regression models were fitted to OS and TTT using PS.8, *IGVH*-status, 17p13 deletion, 11q22-23 deletion, age (< 65 years vs. ≥ 65 years) and sex as covariates. PS.8 had a highly significant association with both endpoints. For OS, 17p13 deletion, age and PS.8 were the only significant covariates. In the multivariate model for TTT PS.8 is dominating all other covariates and is the only covariate significantly associated with TTT. Prediction error curves showed that PS.8 achieved superior prognostic accuracy compared to models based on fluorescence *in situ* hybridization analysis and *IGVH*-status. *Conclusion.* We developed a prognostic score for OS using the largest gene expression profiling data set available for CLL patients with long-term follow-up. This score (PS.8) is based on the expression levels of eight genes and was validated on a different technical platform (qRT-PCR) in an independent group of 149 patients. PS.8 showed additional prognostic value for OS and TTT and improves the prognostic accuracy of previously used molecular markers. Specifically, PS.8 was able to add information in several subgroups defined by the established molecular markers and in Binet A patients. In addition, this eight gene score can be determined fast and cost effectively and is suitable for routine diagnostics. Prospective confirmatory trials are now required to assess the relevance of PS.8 to guide individualized treatment choices to improve the clinical outcome of CLL patients.

0658

LEVELS OF TRANSCRIPT EXPRESSION OF P66SHC ADAPTER, A NOVEL REGULATOR OF B-CELL SURVIVAL, PREDICTS OUTCOME AND CHEMOREFRACTORINESS IN CLL

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Background. p66shc is a novel regulator B-cell survival that induces apoptosis by uncoupling B-cell receptor signaling. p66Shc expression is impaired in chronic lymphocytic leukemia (CLL), and levels in unmutated (U)-CLL are lower than in mutated (M)-CLL (Capitani *et al.*, 2010). *Aims.* Aims of this study were to investigate the prognostic significance of p66shc in relation to time to first treatment (TTFT), overall survival (OS) and refractoriness to Fludarabine-based combinations and/or Alkilators. *Methods.* p66shc transcript levels were measured by Real-time PCR (Capitani *et al.* 2010) in a series of 143 patients with CLL defined according to the IWCLL-2008 criteria. cDNA from purified B-cells pooled from 11 healthy donors was used as a standard and given the arbitrary value of 1. The best cut-off to distinguish low vs high p66shc levels was determined by ROC analysis and the Youden's index (death as state variable). p66shc levels were investigated for any association with i) clinical and biological prognostic parameters at diagnosis ii) TTFT (of patients diagnosed in stage 0) and OS (of all patients) and iii) refractoriness and time to refractoriness to Fludarabine and/or Alkilators. *Results.* Rai stage was 0 in 94/143 (65,7%), I-II in 37/143 (25,9%), III-IV in 12/143 (8,4%) patients Sixty-five/143 (54,6%) CLL used U-IGHV, 60/143 (43%) scored CD38+ve (cut-off>30%). 81/137 (59,1%) scored ZAP70+ve (cut-off>20%), 103/143 (73,6%) had del13q or normal FISH, 18/143 (12%) had 12 trisomy, 13/143 (9,5%) del11q, 8/143 (5,5%) del17p. TP53 disrupture by mutations and/or deletion was found in 12/143 (8,5%). Interquartile Median p66shc level was 0,335 (range 0,202-0,600). Based on the best cut-off (0,375), 63/143 (44,1%) patients had high p66shc (p66hi) and 80/143 (55,9%) low p66shc (p66lo) levels. A significant association was found between p66lo status and U-IGHV (18/63, 28,6% p66hi vs 47/80, 58,8% p66low, p<.001), CD38+ve (17/60, 28,3% p66hi vs 43/79, 54,4% p66low, p=.002), ZAP70+ve (29/60, 48,3% p66hi vs 52/77, 67,5% p66low, p=.02), overall lack of del13q only or normal FISH (53/61, 86,9% p66hi vs 50/79, 63,3% p66lo, p=.002), trisomy 12 (3/61, 4,9% p66hi vs 15/79, 17,3% p66low, p=.01), del17p (0/61, 0% p66hi vs 8/79, 10,1% p66low, p=.01) or overall TP53 disrupture (1/61, 1,6% p66hi vs 11/78, 14,1% p66low, p=.009). p66lo status also associated with shorter TTFT of stage 0 CLL (median 245 in p66hi vs 65 months in p66low, p<.001). By multivariate Cox-analysis (covariates: CD38+ve, ZAP70+ve and U-IGHV), p66lo (95%-CI 1,1-2,9, HR=1,7, p=.04) scored as independent prognostic factors for TTFT. p66lo status also associated with shorter OS (median OS not reached in p66hi vs 112 months in p66low, p<.001). By multivariate Cox analysis (covariates: U-IGHV and del17p), p66lo (95%-CI 1,1-7,9, HR=2,9, p=.03) scored as independent prognostic factors for OS. Remarkably, p66lo associated with shorter time-to-fludarabine-refractoriness (median not reached in p66hi vs 125 months in p66low, p=.02) and time-to-alkilator-refractoriness (median 245 in p66hi vs 60 months in p66low, p=.008). Interestingly, 10/16 (62,5%) fludarabine-resistant and 19/32 (59,3%) alkilator-resistant patients scored p66lo in the absence of TP53 disrupture. *Conclusions.* These data suggest that p66shc play an important (independent) role in defininig outcome and chemorefractoriness risk in CLL patients.

0659

THE IMPACT OF SPECIFIC MUTATIONS IN TP53 GENE ON THE RESULTS OF ALEMTUZUMAB THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background. A poor prognosis in chronic lymphocytic leukemia (CLL) is associated particularly with the presence of del(17p) and/or p53 mutation. The recommended therapeutic approach for patients with TP53 loss or mutation involves primarily a use of monoclonal antibody alemtuzumab. It remains unclear whether the type of TP53 mutation influences the results of alemtuzumab therapy. *Aim.* The aim of the study was to evaluate the efficacy of alemtuzumab therapy in CLL patients with p53 defects *Patients and Methods.* We analyzed the data of CLL patients with p53 defects, which were identified at our institution by the p53 yeast functional assay coupled to sequencing together with complementary I-FISH analysis detecting del(17p), and were treated by alemtuzumab. The response rate, progression-free survival (PFS) and overall survival (OS) after alemtuzumab administration were evaluated. Patients were divided according to the presence of p53 mutation in vs. outside DNA-binding motifs (DBM), structurally well defined part of the p53 DNA-binding domain. *Results.* Forty-nine CLL patients (23 females, 26 males) were included in the analysis, receiving in total 58 courses of alemtuzumab. The average age at diagnosis was 59 years (range 26-67). The average number of therapies prior to alemtuzumab was 2 (0-6). 41 patients were treated with a fludarabine based regimen prior to alemtuzumab. The Rai stage of patients at the start of alemtuzumab therapy was as follows: Rai I 14%; Rai II 1%, Rai III 40%; Rai IV 45%. All patients had unmutated IgVH locus. The frequency of chromosomal aberrations (Döhner's hierarchical model) was as follows: del(17p) n=40 (69%); del(11q) n=3 (5%); trisomy 12 n=2 (3%); del(13q) n=5 (9%); and normal karyotype n=8 (14%). The average cummulative dose of alemtuzumab was 702mg per course (29-1280). The median OS assessed from the time of the first alemtuzumab administration was significantly reduced in patients harboring missense mutation located in the p53 DBM (n=27) in comparison with those having missense mutations outside DBM or non-missense mutations (n=31) (471 days vs. 1056 days; p=0.03) (Figure).

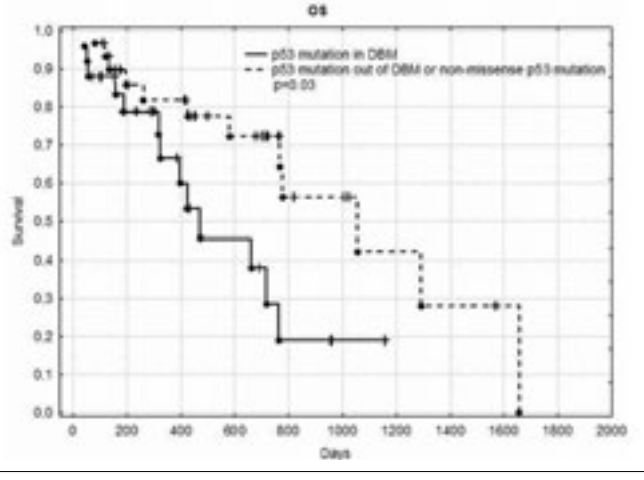

Figure 1.

On the other hand, we did not find any difference in PFS between these patients' groups, after the administration of alemtuzumab (221 days vs. 222 days; p=ns). The overall response rate (ORR) was similar for both groups (63% vs. 58%). The patients' groups did not differ significantly in the other parameters (chromosomal aberrations; alemtuzumab cumulative dose). *Conclusion.* The TP53 mutations located in DBM do not seem to have an effect on ORR or PFS after the therapy of CLL patients with alemtuzumab compared to remaining p53 mutations. However, the survival is obviously substantially reduced in patients with DBM mutation. It may be a consequence of (a) a more severe progression and/or (b) a worse response to the subsequent therapy.

Acknowledgements: Supported by grants IGA NS9858-4/2009, IGA NS10439-3/2009, IGA NT11218-6/2010, GACR P301/10/0590, MPO FR-TI2/254, MSM0021622430, the European Research Initiative on CLL (ERIC) and the Czech Leukemia Study Group for Life (CELL).

0660

MONOCYTE COUNT AT DIAGNOSIS IS AN INDEPENDENT PREDICTOR OF TIME TO FIRST TREATMENT IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background. The survival of chronic lymphocytic leukaemia (CLL) cells in-vivo is critically dependent upon interactions between the malignant cells and non-malignant stromal cells and is mediated by pro-survival chemokines and cytokines. Cultured monocytes are capable of sustaining the survival of CLL-cells *in-vitro* and monocyte-derived soluble CD14 promotes the survival of CLL cells and has prognostic value. We hypothesised that an elevated monocyte count in the peripheral blood at diagnosis would be an adverse prognostic factor in CLL. *Aims.* To determine the prognostic value of the monocyte count in patients with newly diagnosed CLL in terms of time to first treatment (TTFT) and overall survival (OS). *Methods.* We conducted a retrospective cohort study. All newly diagnosed CLL cases identified between January 2000 and December 2010, were examined. We stratified patients based on a monocyte count ≤ or > 0.8 x10^9/L which represents our laboratory upper limit of normal and Kaplan Meier analysis was used to assess TTFT and OS for the entire cohort. Data were also analysed to exclude the confounding effects of known prognostic markers including Binet stage, CD38, IgVH mutational status and ZAP-70. *Results.* We identified 234 patients (M:F;150:84). The median age was 66 years (range 35 -91). Binet stage at presentation was as follows: stage A 191 (82%), stage B 23 (10%) and stage C 20 (8%). Mean duration of follow up was 54 months. The median monocyte count at presentation was 0.61 x10^9/L (range 0.0 - 5.6 x10^9/L). 74 (32%) patients presented with a monocyte count > 0.8 x10^9/L of whom 45% commenced treatment within the study period versus 33% amongst those with a monocyte count ≤ 0.8 x10^9/L. Patients with a monocyte count >0.8 x10^9/L had a significantly shortened TTFT (41 vs 99 months; p=0.002, Figure 1). The prognostic importance of monocyte count was associated with Binet stage, but was independent of CD38, IgVH and ZAP-70. An elevated monocyte count was associated with a trend towards reduced OS, but this did not achieve significance (95 vs 109 months, p=0.094). *Conclusions.* Monocytosis at presentation in CLL is an independent adverse prognostic factor and is associated with a significantly shortened time to first treatment. This is a routinely and inexpensively measured parameter that warrants further investigation in prospective studies.

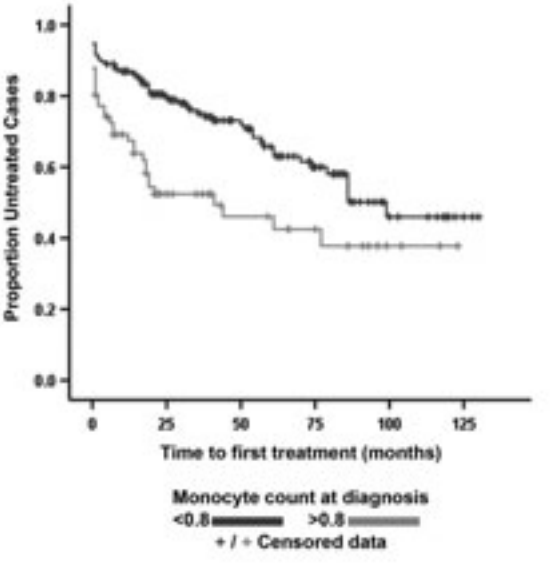

Figure 1. Kaplan Meier curve. p=0.002.

0661

BCL2L12, A NOVEL MEMBER OF THE BCL2 FAMILY, IS DRAMATICALLY ELEVATED IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS AND CONSTITUTES AN UNFAVORABLE BIOMARKER IN CLL, PREDICTING POOR OVERALL SURVIVAL

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Background. BCL2L12 is a recently identified gene belonging to the *BCL2* family, members of which are implicated in hematological malignancies, including chronic lymphocytic leukemia (CLL). The clinical course of individual CLL patients is extremely heterogeneous, with survival ranging from months to decades. Notably, an important subset of patients presenting with low grade CLL will develop a more aggressive and life-threatening disease. Since these patients might potentially benefit from early treatment, it is critical to reliably predict patients' prognosis at diagnosis, especially those in an early disease stage, thus allowing personalized, risk-adapted therapy. Hence, there is high need for biomarkers accurately identifying those patients in early stages that will progress from those who will remain indolent. *Aims.* The purpose of this study was to analyze the mRNA expression of the novel apoptosis-related *BCL2L12* gene in patients with CLL, and to examine its prognostic and predictive value, and potential clinical application as a novel molecular biomarker for CLL. *Methods.* Total RNA was isolated from peripheral blood of 65 CLL patients and 23 healthy donors. An ultra-sensitive quantitative real-time PCR (qRT-PCR) methodology for *BCL2L12* and *BCL2* mRNA quantification was developed using SYBR® Green chemistry. After preparing cDNA by reverse transcription, relative quantification analysis was performed using the comparative CT ($2^{-\Delta\Delta CT}$) method. Moreover, analysis of *IGHV* mutational status, CD38 expression, and detection of apoptosis by double staining with annexin V-FITC and propidium iodide were performed. *Results. BCL2L12* mRNA expression was significantly higher in CLL patients than in healthy donors ($p<0.001$). Moreover, ROC analysis demonstrated that *BCL2L12* expression had significant discriminatory value, distinguishing very efficiently CLL patients from non-leukemic population (AUC=0.833, $p<0.001$). *BCL2L12* expression was also shown to predict the presence of CLL, as demonstrated by both univariate and multivariate logistic regression analyses ($p=0.001$ and $p=0.003$, respectively). Finally, high *BCL2L12* mRNA levels were associated with advanced clinical stage ($p=0.028$) and shorter overall survival ($p=0.043$) of CLL patients. *Conclusions. BCL2L12* mRNA is overexpressed in the majority of CLL patients and constitutes a powerful predictor of the presence of the disease. In addition, high *BCL2L12* mRNA expression is associated with advanced stage of the disease and predicts poor overall survival in CLL patients.

0662

T REGULATORY CELLS (TREG) IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) TREATED WITH HIGH DOSE METHYLPREDNISLONE (HDMP) AND RITUXIMAB (RTX)

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Background. Regulatory T cells were found to be lower in healthy population than in CLL patients. Treg were particularly increased in untreated CLL patients with advanced stage and higher Treg frequencies predicted shorter time to the first treatment. It has not been evaluated if Treg values predict survival in treated patients. Aim of the study was to evaluate T reg dynamics during treatment and their impact on survival. *Methods.* Two-center, single arm, open-label, prospective study was conducted to evaluate the efficacy of dose-dense HDMP in combination with Rtx in pretreated CLL patients with clinically or biologically high-risk disease. T regulatory cells, defined as CD4+CD25+CD127-FoxP3+ cells, were calculated as percentage of total CD4+ T lymphocytes and were assessed by 4-colour flow cytometry in peripheral blood at screening, after three treatment cycles and two months after the last treatment cycle. All patients provided informed consent. *Results.* 29 patients with CLL were enrolled. Median age was 59 years (range 45-76), 22 (76%) patients had Rai III-IV stage, 17 (59%) had bulky (> 5 cm) lymphadenopathy. 25 (86%) patients had unmutated IgVH, 13 (45%) had 17p del and/or p53 mutation, 11 (38%) had 11q del, and one (3%) patient had trisomy 12, 10 (34%) were fludarabine refractory. Overall response rate (ORR) in 26 evaluable patients was 62%, all patients achieved partial response (PR). The median (range) Treg frequency at screening was 3.7% (0.06%-10.46%) and then decreased to 1.1% (0.25%-7.03%) after three treatment cycles and to 2.475% (0.21%-3.66%) at the end of therapy ($p<0.001$ and $p=0.006$ compared to Treg at screening, respectively). Treg frequency at screening did not correlate with response to treatment ($p = 0.661$) or with other prognostic factors such as bulky lymphadenopathy ($p = 0.829$), fludarabine refractoriness ($p = 0.109$), adverse cytogenetics ($p = 0.676$). The median follow-up for all patients was 22 months (range: 1-37). The median progression free survival (PFS) was 12 months (95% CI: 8-16) and the median overall survival (OS) was 31 months (95% CI: 20-42). More prominent Treg decrease between screening and 3 treatment cycles and between screening and the end of therapy predicted better PFS ($p = 0.036$ and $p=0.041$, respectively) by linear regression method. In univariate analysis, patients with higher Treg frequency reduction between screening and three treatment cycles had a trend towards better OS ($p=0.084$) In multivariate analysis, only response to treatment ($p=0.01$) had a significant impact on PFS and fludarabine refractoriness ($p=0.022$) and response to treatment ($p=0.01$) predicted OS. *Conclusion.* HDMP and Rtx combination effectively reduces T regulatory cells in pretreated high risk CLL patients. However, Treg reduction during treatment was not an independent predictive factor for either PFS or OS. Further evaluation of T lymphocyte subsets and their impact on survival is being performed. (ClinicalTrials.gov identifier: NCT005 58181; supported by EEA and Norway grant No. 2004-LT0040-IP-1EEE.).

0663

IMMUNOHISTOCHEMICAL EXPRESSION OF TCL1 AND ZAP70 IN CHRONIC LYMPHOCYTIC LEUKEMIA AND SPLENIC MARGINAL ZONE LYMPHOMA PATIENTS: CORRELATIONS WITH CLINICAL AND LABORATORY CHARACTERISTICS

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Background. Chronic lymphocytic leukemia (CLL) and spenic marginal zone lymphoma (SZML) are both B-lymphoproliferative disorders that have possible common pathogenetic mechanisms. CLL follows a variable course with survival ranging from months to decade, while a small fraction of SZML patients display more aggressive behavior. TCL1 oncogene, a target of chromosomal translocations and inversions at 14q31.2 and zeta-associated protein (ZAP 70), a member of the Syk family of tyrosine kinases, are associated with adverse outcome in CLL patients, while the expression and role of these genes in SMZL are not fully clarified. *Aim.* To evaluate TCL1 and ZAP70 expression in CLL and SMZL patients and correlate them with clinical and laboratory characteristics. *Methods.* 55 patients diagnosed with SLL and SMZL were included in the present study, based on the availability of tissue specimens. Their baseline clinical and laboratory features were recorded. All patients underwent bone marrow (BM) aspiration and biopsy at diagnosis. Analysis of the IgVH somatic mutations as well as FISH for 11q- and 17p- were performed at diagnosis in CLL patients. TCL1 and ZAP70 were studied in paraffin embedded tissue using Envision kit. Analysis was performed in bone marrow sections in all CLL patients and among 22 SMZL patients while in 2 SMZL on spleen sections. *Results.* 55 patients (31 CLL and 24 SMZL) patients were included. Among 31 CLL patients with a median age of 58.5 years (42-71), 19 were male, 23 were staged A and 8 B (Binet). IgVH mutation analysis performed in 20 patients, 13 of them were unmutated. FISH analysis for 17p- and 11q- was made in 20 and 16 patients respectively. One patient had 17p- deletion and 2 presented 11q-. All patients underwent immunohistochemical analysis of ZAP 70 and 20 of them were ZAP 70 (+), while 18 underwent TCL1 analysis and 9 of them were TCL1 (+). The clinical-laboratory characteristics of TCL1 (+) patients were as follows: 6/9 were male, median absolute lymphocyte count (ALC) was 79.78×10^9/l ($5\text{-}152 \times 10^9$/lt) with rapid duplication time in 2 patients, Binet stage was A in 8/9, 7 had intermediate or diffuse pattern

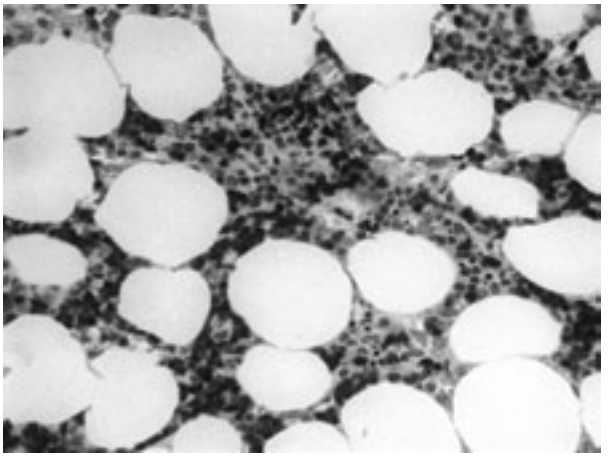

Figure 1. TCL 1 expression in CLL.

of bone marrow infiltration. 3 were unmutated, no patient presented 17p-,1 had 11q- and 7/9 had ZAP70 (+). The only correlation of borderline significance was between ZAP (+) and TCL (+). Among 24 SMZL patients with median age 61 years (45-78), 10 were male,10 had elevated LDH and 3 patients were classified as high/intermediate IPI. ZAP 70 and TCL1 were evaluated in 21 patients. 20/21 were negative and only one was found positive to both antigens. *Conclusions.* Immunohistochemical detection of TCL1 and ZAP 70 is feasible in CLL. TCL1 (+) and ZAP70(+) correlation is statistically significant in CLL patients. TCL1 positivity may be associated with other unfavorable CLL markers such as BM infiltration pattern and ALC. Expression of TCL1 and ZAP70 is rare in SMZL patients.

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LIPOPROTEIN LIPASE AS A PROGNOSTIC MARKER AND IGVH MUTATION SURROGATE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Chronic lymphocytic leukemia (CLL) is a highly heterogeneous condition clinically and biologically. In the era of rapidly improving treatment options, there is an increasing demand to predict outcome early in the course of the disease, a task not fulfilled by clinical stage. Among the numerous markers introduced recently, lipoprotein lipase (LPL) has been reported to be informative as a prognostic marker, related closely to IgVH mutation status in CLL. *Aims.* Our objective was to test LPL in the clinical practice as a predictor of outcome and correlate its expression with other prognosticators. *Methods.* Using peripheral blood samples of 73 unselected CLL patients, lipoprotein lipase mRNA levels were determined by quantitative PCR. LPL/ ABL expression levels were normalized to healthy controls. A cutoff of 1.77 was established by ROC analysis in order to distinguish between positive and negative clones. IgVH mutation status was analysed by a multiplex PCR method and BIOMED-2 standardized primers. Greater than 4% sequence divergence from the germline was considered mutated. For CD38 evaluation a 7% cutoff was applied. *Results.* LPL positive patients showed significantly shorter survival than negative ones (median survival 175 months vs not reached, $p<0.0001$). A close relationship was found between LPL expression and IgVH mutation status with 22 of 24 (92%) LPL positive cases being unmutated and 23/32 (72%) LPL negative cases mutated. When CD38 was employed as an IgVH surrogate, the sensitivity and specificity were 75% and 65%, respectively. A linear relationship was detected between LPL and CD38 ($p<0.003$). Interestingly, within the good prognostic group having 13q-, LPL positivity identified a subgroup with shorter survival (median survival 136 months vs not reached, $p<0.009$). *Conclusions.* as measured by PCR from unselected peripheral blood, LPL determination proved to be a powerful predictor of outcome refining prognosis in the good-risk category of CLL. As an IgVH mutation surrogate, LPL outperformed CD38 in this series. These findings justify further study and eventually, the routine application of LPL in the diagnostics of CLL.

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HUMAN AND MOUSE DOCK10 SPLICING ISOFORMS WITH ALTERNATIVE FIRST CODING EXON USAGE ARE DIFFERENTIALLY EXPRESSED IN T AND B LYMPHOCYTES

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Background. Dedicator of cytokinesis (DOCK) proteins are a family of guanosine nucleotide exchange factors (GEF) for Rho GTPases, constituted by 11 mammalian members. Diverse Rho GTPases play crucial roles in lymphocyte development, activation, differentiation and migration. We have previously reported the cloning of a full length human DOCK10 cDNA [Yelo *et al*, Mol Immunol 2008; 45: 3411-8], whose expression is distinctively induced by IL-4 in normal peripheral blood B lymphocytes and chronic lymphocytic leukemia (CLL) B cells. Here, we analyzed DOCK10 mRNA diversity produced as a result of alternative splicing. *Aims.* To define the DOCK10 protein-coding transcripts in the human and the mouse and to gain insights into regulation of their expression. *Methods.* Full-length cDNA clones of DOCK10 were obtained from total RNA of both normal human peripheral blood mononuclear cells (PBMC) (10 clones from 10 individuals, total 100 clones) and mouse spleen (10 clones), by high fidelity RT-PCR. B and T lymphocytes from normal individuals and CLL B cells were isolated using negative selection and cultured in the presence of 10 ng/ml of IL4 (BD Pharmingen). Expression of the DOCK10 isoforms was measured by quantitative PCR (Q-PCR) and western blotting. Panels of human and mouse tissues were analyzed for mRNA expression. Protein and mRNA expression were also studied in a panel of human lymphoid, myeloid and epithelial cell lines. *Results.* Alternative first coding exon usage led to two main protein-coding transcripts, which we named DOCK10.1 and DOCK10.2. Full-length cDNA clones of both isoforms were obtained from both normal human PBMC and mouse spleen, for the first time for human DOCK10.1, mouse DOCK10.1, and mouse DOCK10.2. Human and mouse DOCK10.1 clones corresponded to the protein coding assemblies provided by NCBI as Reference Sequences (RefSeq) for DOCK10. Our analysis especially focused on human cDNA clones, of which 63% were alternatively spliced forms involving diverse exons and introns (17% in frame, 46% frame shifts). The most frequent variations were shortened versions of exons 5, 12, 18, 35, 43, and 45. Deletions of entire exons were also found, e.g., exons 8, 28, 31, and 40. The consensus rules for intron-exon boundaries were fulfilled in all the subvariants. DOCK10.1 expression was enriched in normal T cells, and DOCK10.2 expression was enriched in normal and CLL B cells. Some lymphoid cell lines express one isoform preferentially over the other. Both isoforms were up-regulated in response to interleukin-4 (IL-4) in B cells, both normal and CLL, but not in T cells. *Summary/Conclusions.* Two DOCK10 isoforms, named DOCK10.1 and DOCK10.2, arise from the use of alternative first exons. Whether the frequent shortened and truncated isoforms play specific functional roles or are just transcriptional noise remains to be elucidated. The two isoforms are expressed in human and mouse tissues, mainly in lymphocytes and lymphocyte-rich organs. DOCK10.1 is enriched in T cells and DOCK10.2 in B cells. IL4-treatment up-regulates both isoforms in B cells, but not in T cells. Our data suggest that cell-specific mechanisms regulate expression of the alternative first exon variants of DOCK10 in vertebrates. antonio.parrado@carm.es

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HAPLOTYPE FOR POLYMORPHISMS C1236T, C3435T AND G2677T/A IN MDR1GENE IS ASSOCIATED WITH MAJOR MOLECULAR RESPONSE IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH STANDARD-DOSE OF IMATINIB

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Background. Chronic myeloid leukemia (CML) is a clonal expansion of hematopoietic progenitor cells, resulting in myeloid hyperplasia, leukocytosis, neutrophilia, basophilia and splenomegaly. Imatinib Mesylate (IM) used in the treatment of CML, interacts with membrane efflux transporters such as MDR1. Polymorphisms in this gene have been associated with changes in its functionality and may be involved in the response to IM treatment. *Aims.* The aim of this study is to investigate the relationship of MDR1 gene polymorphisms (C1236T (rs1128503), C3435T (rs1045642) and G2677T/A (rs2032582)) with response markers to treatment with IM in patients with CML. *Methods.* 118 patients in chronic phase of CML, both genders with age range 18 to 80 were studied. All patients were initially treated with a standard dose of IM (400 mg/day) and divided in two groups according to response. The first group ("responder") comprised 70 patients who had a complete cytogenetic response within 18 months of treatment. The second group ("non responder") 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 Leukemia Net. Patients with cytogenetic patterns other than the Philadelphia chromosome and patients with mutations in the BCR-ABL1 gene were excluded from this study. Major molecular response (MMR) was defined as a reduction of BCR-ABL1 transcripts levels to ≤ 0.1% in the peripheral blood standardized on the International scale. MDR1 gene polymorphisms were determined on all patients using RFLP PCR. *Results.* The frequencies of MDR1 variant alleles were 41.1% (1236T), 37.3% (3435T), 32.2% (2677T) and 2.1% (2677A), respectively for C1236T, C3435T, G2677T/A polymorphisms. No subject had the MDR1 2677AA genotype. The genotypes frequencies for C1236T, C3435T and G2677T/A polymorphisms were similar in the two groups (responder and non responder; p>0.05). In the responder group, the frequency of 1236CT/2677GT/3435CT haplotype was higher in patients with MMR (51.7%) than in patients without MMR (8.3%, p= 0.010). Furthermore, carriers of 1236CT/2677GT/3435CT haplotype had a 11.8 fold greater odds ratio (95%CI: 1.43-97.3, p=0.022) of achieving molecular response compared with all who had others haplotypes in logistic regression. *Conclusions.* The 1236CT/2677GT/3435CT haplotype in the MDR1 gene is positively associated with major molecular response to treatment with IM.

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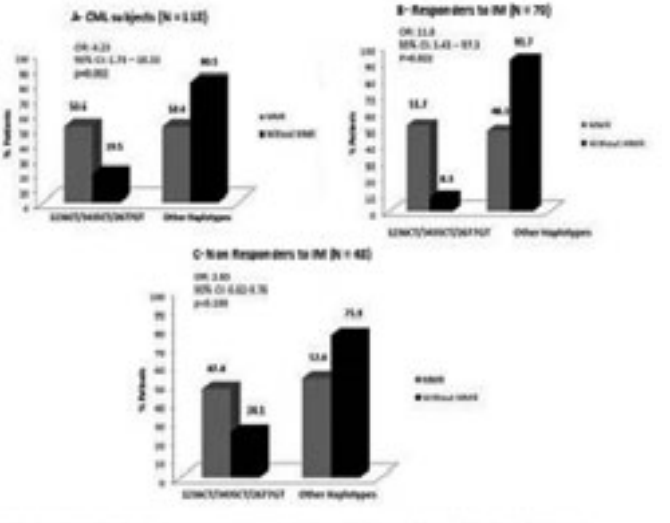

Frequencies of 1236CT/3435CT/2677G haplotype according to major molecular response to imatinib mesylate (IM) treatment. A) CML subjects, B) Responders to IM, and C) Non Responders to IM.

Figure 1.

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CONNECTING DIAGNOSTIC RISK SCORES TO INDIVIDUAL PATIENT RESPONSE UNDER NILOTINIB THERAPY

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Introduction. At diagnosis early chronic phase CML (ECP-CML) patients are typically categorized in low, intermediate or high-risk groups using the Sokal or Hasford scoring systems, that provide an indication of the patient survival probability if treated with chemotherapy or interferon respectively. Given that tyrosine-kinase inhibitors have become the first-line treatment method for these patients, one can wonder how individual patient response to drugs like imatinib or nilotinib relates to the standard scoring systems at diagnosis. *Methods.* Combining a computational model of hematopoiesis and CML with individual serial Q-RT-PCR data for disease burden (BCR-ABL) under nilotinib therapy of 73 patients, we estimated for every patient independently the self-renewal probability of CML cells (eCML), the fraction of CML cells responding to therapy (z NILO) and the impact of TKI on the self-renewal probability of CML cells (eNILO) under therapy. These estimates were obtained from the data on response to treatment, and independent of the calculation of the prognostic categories. As such they allow to one to correlate scores with the values of these three model parameters. *Results.* The results show that two model features, i.e. self-renewal probabilities for CML cells (eCML) and treated CML cells (eNILO), provide the best partitioning of the response data. The first feature differentiates between two clusters: One cluster (A) with eCML>0.7 and another (B) with eCML<0.7, that partition patients into two clusters. Within each group, the second feature differentiates between patients responding optimally or suboptimally to therapy, making it possible to introduce again two types of subclusters. Examining the relationship between the cluster a patient belongs to based on response dynamics and its Sokal or Hasford score shows only a slight correlation: Most low-risk patients are in cluster A, whereas most high-risk are in cluster B. However patients from all prognostic categories can be found in most clusters and those in the best group are not necessarily patients in the low-risk Sokal group. *Conclusions.* Through our computational model of hematopoiesis and CML we can classify patients' response to therapy based on two important features: the fitness of the CML cell before therapy and the fitness-change induced by treatment. Together these features allow one to categorize a patient response to TKI treatment and correlate it with prognostic scores calculated at diagnosis. Our results show that the correlation appears to be weak, requiring on the one hand that new prognostic tools based on TKI therapy should be designed and on the other hand that one might take early therapy-response markers into account to improve patient treatment in the long run.

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THE GENE EXPRESSION EVALUATION OF THE SEPT5 IN PATIENTS WITH CML, AT THE DIAGNOSIS, HEALTHY BLOOD DONORS AND CELL LINES OF HUMAN AND MURINE

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Background. SEPT5 is a member of nucleotide binding proteins, called septins that were firstly described in yeast as cell division cycle regulatory proteins. This gene was reported in patients with acute myeloid

leukemia translocated with MLL gene and with high expression in myeloid leukemia cell line and in adult human brain and heart in association with alpha granules of human blood platelets. In a recent study using SSH libraries, we compared the gene expression pattern between granulocytes of health control and CML patients, and we identified this gene expressed only in CML patients. Although several studies in literature, there is not a clear relationship between the expression of this gene and the development of CML, making it an interesting target for functional studies with regard to their function in differentiation, proliferation and apoptosis of granulocytic cells and myeloid progenitors. *Aims.* Functional analysis of SEPT5 gene expression in the granulocytic lineage of CML patients and the relationship between this gene and the development and progression of CML. *Methods and Patients.* The evaluation was made in peripheral blood granulocytes and mononuclear cells of CML patients and healthy blood donors, and in the cell lines K562, BaF3/BCR-ABLp210 and BaF3T315I using real time PCR. *Results.* To validate the result found in the SSH library, the gene expression of SEPT5 was evaluated by real time PCR using the same samples used in the library construction. These results confirmed our previous results showing that the SEPT5 expression is increased in the granulocytes of CML patients. The same results was observed when we studied the expression comparing individually 11 patients at diagnosis and 05 health controls, suggesting that this protein could be increased in all human cells that have the translocation BCR-ABL. However, using cell lines of murine BaF3/BCR-ABLp210 and BaF3T315I, the expression of this gene was absent, suggesting that this gene had a very low expression in the translocated cells of this model and that could be involved in the human CML. *Conclusion.* Despite major advances in the treatment of CML, the treatments available are not capable of inactivating all the signaling pathways activated by BCR / ABL. Our results demonstrate that SEPT5 could be involved in the development of CML and the importance of the study of possible pathways that could culminate in its high expression or the triggering of other unknown pathways involved in the development of CML by this gene. This work was supported by FAPESP.

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GENE EXPRESSION MARKERS OF RESPONSE IN CHRONIC MYELOID LEUKAEMIA: DYSREGULATION OF THE AURORA KINASES AND MGMT

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Background. The t(9;22)(q34;q11) translocation, resulting in the BCR-ABL1 fusion gene and subsequent constitutively activated tyrosine kinase oncoprotein is both the hallmark and initiator of Chronic Myeloid Leukaemia (CML). Although initially indolent and generally responsive to first line tyrosine kinase inhibitor therapy (Imatinib), heterogeneity of response is observed in a proportion of patients, leading to primary and secondary resistance, suggesting that other genomic lesions play a role in determining outcome. Aurora Kinase A and B (AURKA, AURKB) have roles in regulating cell division and their dysregulation is frequently observed in solid-tumours and is associated with chromosomal instability. The MGMT gene encodes a DNA repair protein and its expression is known to be epigenetically silenced by promoter hypermethylation in glioblastomas, leading to poor treatment response. *Aims.* To ascertain whether the differential expression of AURKA, AURKB and MGMT correlated to treatment response, and additionally to investigate whether promoter CpG methylation played a role in MGMT down-regulation. *Methods.* Gene expression of AURKA, AURKB and MGMT (normalised against GUSB) were determined using RT-qPCR on 22 normal peripheral blood (PB) samples, 21 diagnostic CML samples (in chronic phase) and 42 CML remission (MMR) samples. qPCR results were expressed as delta-CT relative expression ratios (RER), calibrated against the BV173 cell line, and assayed for significance using the Kruskall-Wallis test. Diagnostic gene expression levels were stratified into low & high (maximal chi-square) and correlated against Imatinib treatment efficacy (400mg/day; time to complete cytogenetic response (CCyR); Kaplan-Meier). MGMT promoter hypermethylation at 5 CpG sites in the 5' UTR was investigated using pyrosequencing on bisulfite-treated DNA from paired samples from 3 CML patients. *Results.* Compared with normal and remission samples, gene expression of AURKA and AURKB were significantly up-regulated (median RER: 0.14, 0.13, 0.28; $p<0.001$ and 0.04, 0.06, 0.34; $p<0.01$, respectively) and MGMT was down-regulated (0.42, 0.36, 0.15; $p<0.01$). Patients with low relative MGMT expression (RER ≤0.20) were shown to have a significantly increased time to CCyR compared to those with high expression (>0.20) (median 10mnth and 6mnth, respectively; $p=0.046$). For AURKA there was a trend for those with higher expression (>0.17) to achieve CCyR later (6mnth and 10mnth; $p=0.076$); for AURKB there was no difference. Pyrosequencing showed no evidence of MGMT promoter hypermethylation at the investigated CpG sites (<3% methylation, comparable to the negative control). *Summary/Conclusions.* Although both AURKA and AURKB were significantly over-expressed in diagnostic CML samples, only AURKA was associated with a slower treatment response at a level that approached significance. Down regulation of MGMT lead to a significantly slower response to Imatinib, but there was no evidence that epigenetic silencing by promoter CpG hypermethylation was functionally involved; although caution must be taken when drawing conclusions from such a relatively small sample number. In summary, both AURKA and MGMT gene expression levels were found to be candidates as prognostic indicators for treatment response in CML, possibly reflecting the dysregulation of their roles in maintaining genomic stability. Down-regulation of MGMT may occur through mechanisms other than promoter hypermethylation.

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A RAT MODEL TO PREDICT ALTERATIONS IN BONE GROWTH AND METABOLISM IN CHILDREN WITH CML ON IMATINIB

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Background. Chronic myeloid leukaemia (CML) is a rare malignancy in paediatric patients (pts) and constitutes only approximately 2% of all childhood leukaemias. The diagnostic hallmark of CML is the Philadelphia chromosome (Ph+; t(9;22)(q34;q11)) resulting in the BCR-ABL fusion protein a constitutively active, oncogenic tyrosine kinase. Since 2001 the tyrosine kinase inhibitor (TK-I) imatinib mesylate (IMA; Glivec®, Novartis, Basle, Switzerland) has been established as front line therapy for adult pts with CML and has been fully licensed for treatment of CML in children since the year 2003. However, also related tyrosine kinases necessary for bone "remodelling" like PDGF-R, and c-FMS are blocked by IMA. First reports in adult pts described disturbances in bone and mineral metabolism (hypophosphatemia; inhibition of osteoclasts) as so far unexpected side effects after prolonged periods of treatment of CML. Up to know, in children the efficacy of IMA has been evaluated only in prospective trials with small pts numbers pointing to growth retardation in paediatric pts while on imatinib treatment. *Aim.* In an ongoing study we investigated the influence of IMA on the growing skeleton in juvenile rodents. *Methods.* Starting at an age of 4 weeks (w) [development milestones: weaning 3w; puberty 7w; adolescence 9w] juvenile male Wistar rats were chronically exposed to IMA via the drinking water. Over a ten week period, groups A and B were exposed daily to IMA at 500 mg/l (A) and 1000 mg/l (B), respectively, while group C received 1000 mg/l intermittently (3 days 'on' treatment, 4 days 'off'). Animals were sacrificed after 2w, 4w and 10w of IMA exposure to analyze the following parameters: bone length, bone mass density, bone metabolic parameters: amino-terminal propeptide of type I procollagen (PINP), C-terminal cross linking telopeptide of type 1 collagen (CTX-I), osteocalcin (OC) and tartrate resistant acid phosphatase (TRAP). In addition serum levels of IMA, testosterone and growth hormone (GH) were monitored. *Results.* IMA was well tolerated at all concentrations. Weight gain of rats was delayed in groups B and C while no change in animals overall growth, development and behaviour was observed. However, rats exhibited significantly reduced longitudinal bone length with reduced trabecular but normal cortical bone density. Femoral length in group C paralleled reduced length in group A, while length in group B was mostly impaired. Biochemical markers for bone resorption revealed no significant differences for CTX-I but decreased levels of TRAP of group A and B during exposition time and a "push up effect"in group C which declined to control range after puberty. Markers for bone resorption indicated reduced levels for OC and reduced levels of PINP prepubertal of group B and C increasing to levels of control group during puberty. Furthermore, testosterone levels were reduced permanently while GH measurement exhibited increased levels at the 4thw of IMA exposure. *Conclusion.* Here we could demonstrate alterations of bone metabolic parameters in the juvenile still growing bone confirming published data in children. Side effects of TK-I treatment observed in children can be predicted and mimicked in juvenile rodent models.

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DIFFERENTIAL ROLE OF THE JAK2-STAT5 SIGNALING MODULE IN BCR-ABL-DEPENDENT TRANSFORMATION

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Background. Activation of the transcription factor STAT5 is a critical event for the initiation and maintenance of Chronic Myeloid Leukemia (CML) that is causally linked to the expression of the tyrosine kinase Bcr-Abl. *AIM.* A possibility to inhibit STAT5 activation in CML could be the use tyrosine kinase inhibitors (TKIs) targeting the JAK2 kinase that was shown to be the upstream activator of STAT5 in different physiological and pathological setting. *Methods/Results.* Using conditional JAK2 mouse knock-out models, we show that JAK2 is dispensable for myeloid and lymphoid Bcr-Abl-driven leukemia and initial myeloid transformation, whereas JAK2 is required for initial lymphoid transformation. These observations speak for an uncoupling of STAT5 activation from JAK2 at different stages of transformation depending on the oncoprotein. We further show that JAK2 TKIs induce apoptosis in JAK2-deficient cells irrespective of the presence of JAK2. This is likely caused by the direct 'off-target' inhibition of Bcr-Abl. Using a combination of siRNA and pharmacological interference excluded a requirement for tyrosine kinases other than Bcr-Abl for STAT5 activation and indicating a direct phosphorylation of STAT5 by Bcr-Abl. This finding was further supported by enzymatic studies that indicated STAT5 phosphorylation with similar efficiency than the known Bcr-Abl substrate CrkL. *Summary.* Our results excluded a dominant role of JAK2 in Bcr-Abl dependent transformation and withdraw the rationale of a clinical use of JAK2 TKIs in CML.

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HIGH-RESOLUTION MELTING CURVE ANALYSIS FOR SCREENING FOR MUTATIONS IN THE BCR-ABL KINASE DOMAIN OF CML PATIENTS

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Background. Chronic myeloid leukaemia (CML) is characterized by the BCR-ABL fusion oncogene. The oncogene encodes for a tyrosine kinase that can effectively be inhibited by tyrosine kinase inhibitors (TKIs). Nevertheless, some patients acquire resistance to treatment as a result of mutations in the BCR-ABL kinase domain. Mutations impair the binding of TKIs to the kinase domain. Thus the detection of mutations in the kinase domain is important to explain sub-optimal responses or the loss of response as a result of resistance to TKIs. Mutations in the BCR-ABL kinase domain are usually detected through sequencing. Recently, high-resolution melting curve analysis (HRM) has been suggested as a method to screen the BCR-ABL kinase domain for single base mutations prior to sequencing. There have also been a few reports on the possible impact of indels in the kinase domain and gene amplification on patient prognosis. However it is not known whether HRM can be used to identify indels or duplications associated with the kinase domain. *Aim.* The aim of this study was to determine whether HRM analysis can be used to screen the BCR-ABL kinase domain for mutations including indels and duplications prior to sequencing. *Methods.* Informed consent was obtained from 33 CML patients on TKI treatment. Of these, 13 patients were suspected of having mutations due to sub-optimal response or a loss of response with TKIs. Sequencing of the kinase domain was performed according to Branford and Hughes (2006). HRM analysis was performed using the Melt-Doctor HRM Reagent Kit and primers from Poláková *et al.* (2008) on the ABI 7500 Fast. *Results.* Thirteen samples were found to contain mutations in the BCR-ABL kinase domain, including three samples with single base changes, one with a previously described 35 base pair insertion and nine with duplications of which seven contained indels not previously described. There was an overlap in Tm range for samples with mutations compared to samples without mutations, irrespective of the mutation type and so difference plots were used to identify samples with mutations. Samples with duplications were identified as different variants in the difference plot, from those with only single base mutations. Compared to this, samples with either one or two single base mutations were found in the same variant group in the difference plot. *Conclusions.* HRM analysis was successfully used to screen for mutations in the BCR-ABL kinase domain. Samples with mutations were not readily identifiable from shifts in Tm but were indicated as variants in the difference plot. An insertion in the BCR-ABL kinase domain as well as duplications of the kinase domain, were also detected by HRM analysis. Based on this study, HRM analysis can be used to screen the kinase domain for mutations including single base changes, indels and duplications, prior to sequencing.

0673

IDENTIFICATION OF SARI (SUPPRESSOR OF AP-1, REGULATED BY IFN) DOWN-REGULATED BY BCR-ABL IN K562 CELLS: A NOVEL TARGET FOR TREATMENT OF CML

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Backgrounds. SARI (Suppressor of AP-1, Regulated by IFN) is a novel basic leucine zipper containing type I IFN-inducible early response gene that exerts cancer-selective growth inhibitory effects. The constitutive SARI expression was detected in multiple lineage-specific normal cells, whereas its expression was not detected in their tumorigenic counterparts. However, little is known so far about SARI expression in patients with CML (chronic myelogenous leukemia), the regulation of SARI expression in CML cells. *Aims.* To investigate whether Bcr-Abl would play a role in down-regulation of SARI expression in CML-derived cell line K562. *Methods.* Forty-six Chinese patients with CML and forty healthy Chinese volunteers were recruited and informed consent at Union hospital of Wuhan in this study. SARI expression in the peripheral blood mononuclear cell of CML patients and healthy Chinese volunteers was assayed by using Real-time quantitative PCR. *In vitro*, in respective experiment, K562 cells were incubated with the Bcr-Abl inhibitor STI571 (Imatinib, Gleevec) (0.5, 1.5, 2.5μM) at 37°C and 5% CO2 for 6, 12, 24hours followed by detection of SARI expression using Real-time quantitative PCR. Further, the correlative downstream pathways were identified by using signal pathway inhibitors. K562 cells were incubated with PI3-kinase inhibitor LY294002 (20μM), MEK inhibitor PD98, 059 (50μM), JAK inhibitor Ag490 (50μM) at 37°C and 5% CO2 for 24 hours in respective experiment, then SARI expression were detected by Real-time PCR. All experiments were repeated three times. Statistical analysis was performed using SPSS 17.0. *Result.* Compared with healthy volunteers, expression of SARI mRNA in PMBCs of CML patients presented a lower level ($p<0.001$). *In vitro*, after exposure of K562 cells to STI571, the SARI expression was higher than those in control K562 cells (without STI571 treatment). SARI expression was enhanced in K562 cells as early as 6hours after treated with STI571 (1.5μM) and SARI expression was up-regulated in a time-dependent manner. In addition, after treated with JAK inhibitor Ag490, PI3K inhibitor LY294002 and MEK inhibitor PD98, 059 respectively, SARI mRNA expression was up-regulated by Ag490 ($p<0.05$)and PD98, 059 ($p<0.001$), but not by LY294002 compared with control K562 cells (without any treatment). *Conclusion.* The suppression of SARI expression is implicated in CML pathogenesis. Bcr-Abl mediates the down-regulation of SARI mRNA expression in K562 cells. Moreover, Bcl-Abl down-stream signal pathways, including JAK signal pathway and MEK signal pathway are involved in the down-regulation of SARI mRNA expression in K562 cells. These findings suggest that SARI is a potential gene target in CML therapy.

0674

THE ROLE OF CELL DIVISION CYCLE 6 OVEREXPRESSION IN CHRONIC MYELOID LEUKEMIA CELLS

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Background. The cell division cycle 6 (Cdc6), a protein in eukaryotic cells, is an essential regulator of DNA replication and plays important roles in the activation and maintenance of the checkpoint mechanisms in the cell cycle that coordinate S phase and mitosis. Recent documents indicated the deregulation of CDC6 expression in human cells posed a serious risk of carcinogenesis. Down-regulation of CDC6 was observed in prostate cancer. Up-regulation of Cdc6 was found in cervical cancer, lung cancer and brain cancer. Chronic myeloid leukemia (CML) is a form of leukemia characterized by the

increased and unregulated growth of predominantly myeloid cells in the bone marrow and the accumulation of these cells in the blood. And the cause of CML was confirmed the central importance of BCR-ABL. But up to date, the relationship of Cdc 6 and CML is still obscure. *Aims.* Our Aim is to investigate the expression of Cdc6 and its role in CML cells. *Methods.* The expression of Cdc6 in normal bone marrow mononuclear cells, CML primary cells and K562 cells (CML cell line) was detected by real time quantitative RT-PCR and Immunofluorescence assay. The effects of Cdc6 gene silencing by siRNA on cell proliferation and apoptosis in K562 cells were evaluated by CCK-8 assay and Flow Cytometry. The effects of specific inhibitors Imatinib, LY294002, PD98059 and AG490, which separately target BCR/ABL, PI3K, MAPKK and JAK, on the expression of Cdc6 in K562 cells were determined by real time quantitative RT-PCR and Western Blot. *Results.* Cdc6 expression was significantly up-regulated in CML primary cells and K562 cells, compared to normal bone marrow mononuclear cells. Cdc6 gene silencing by siRNA effectively inhibited DNA replication and induced apoptosis in K562 cells. Imatinib, a BCR/ABL inhibitor, induced down-regulation of Cdc6 expression in K562 cells. LY294002 and AG490, but not PD98059, decreased the expression of Cdc6 in K562. *Conclusions.* These data indicated Cdc6 overexpression contributed to the high proliferative activity and the low apoptosis, and regulated by BCR-ABL signal transduction through downstream PI3K/Akt and JAK/STAT pathways in CML cells. It was also suggested that Cdc6 protein may be an attractive target for the development of effective anti-cancer strategies in adult chronic myeloid leukemia patients.

0675

EXPRESSION OF TRANSKETOLASE-LIKE GENE 1 (TKTL1) CHANGES DURING ACCELERATION OF CHRONIC MYELOID LEUKEMIA

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Background. Development of resistance or progression to blastic phase in chronic myeloid leukemia (CML) occurs in a significant proportion of patients under tyrosine kinase inhibitor (TKI) therapy. Overexpression of transketolase-like gene 1 (TKTL-1) on mRNA and protein level has been linked to tumour progression, metastasis and poor patient outcome in many solid tumours. A TKTL1 knockout in tumour cells results in reduced activity of the pentose phosphate pathway (PPP), lower lactate production and G0/G1 arrest. Therefore TKTL1 is regarded as a potential target for drug therapy. Until today little is known about TKTL1expression in CML. *Aims.* We sought to evaluate TKTL1 gene expression in different CML phases. *Patients and Methods.* 108 peripheral blood samples of 79 CML patients (pts) (median age 56 years, range from 17 to 84) were investigated. 49 samples were collected from pts in chronic phase (CP), 22 from pts in major molecular response (MMR), 22 from accelerated phase (AP) pts. and 15 in blast crisis (BC) pts. A control group consisted of 21 healthy individuals. TKTL1 mRNA expression levels were determined by quantitative reverse transcription PCR (qRT-PCR) using LightCycler® technology and normalized against beta-glucuronidase (GUS) expressio n. TKTL1 expression has also been determined in granulocytes isolated by Ficoll gradient centrifugation from blood and in immature CD34+ and CD34-/CD33+ cells isolated from bone marrow by MACS beads technology. *Results.* A significantly lower TKTL1 expression was found in the CP group compared to the group of healthy donors (TKTL1/GUS ratio 2.5% vs 8.7%, p<0.01, Table). Intermediate expression levels were observed in AP (1.1%). Lowest expression levels were observed in the BC group (0.9%). No significant difference could be found in the MMR group compared to healthy individuals (6.4% vs 8.7%, p=0.25). In addition, this evaluation revealed no significant difference of TKTL1 expression at baseline in CP patients with subsequent favourable outcome (MMR within one year) and those with subsequent progression (AP or BC) during therapy. Further experiments showed significantly higher TKTL1 expression in mature granulocytes in comparison to immature CD34+ and CD34-/CD33+ cells. *Conclusions.* TKTL1 expression levels appear to decline in the course of CML with lowest levels during BC. This might be due to the suppression of matured cells by the clone of immature blasts with a lower expression of TKTL1. However, in this study TKTL1 expression levels at diagnosis failed to give a predictive information about disease progression.

Table 1.

Summary of TKTL1 expression levels and P values

	TKTL1/GUS ratio (%) Median (range)	P value (Mann-Whitney test)		
Healthy volunteers	8.7% (2.8-35.2%)	<0.01		
CP	2.5% (0.1-40.6%)		<0.01	0.25
AP	1.1% (0.02-23.4%)	0.26		
BC	0.9% (0.05-5.3%)			
Patients in MMR	6.4% (0.83-49.1%)			
CP patients with favourable outcome (MMR)	2.7% (0.2-18.4%)	0.78		
CP patients with subsequent acceleration	2.3 (0.1-40.6%)			

0676

IMATINIB DISCONTINUATION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA WHO HAVE RECEIVED FRONT-LINE IMATINIB THERAPY AND ACHIEVED COMPLETE MOLECULAR RESPONSE

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Background. Imatinib (IM) is an effective treatment in patients with chronic myeloid leukemia (CML). Complete molecular response (CMR) was only attained in 10-20% of chronic phase (CP) CML patients at 24 months, and the proportion was substantially increased by continuing IM therapy. Mahon and colleague showed that 41% of patients who achieved and maintained CMR for 2 years on IM therapy remained CMR after IM discontinuation. However, they included patients with front-line treatment of interferon, who didn't represent current patient population. Thus, the aims of this study were to investigate feasibility of IM discontinuation in patients with CML, who have treated with front-line IM therapy and achieved CMR, and also to evaluate the prognostic factors associated with loss of CMR in terms of time to loss of CMR (TTLC). *Methods.* We consecutively enrolled CP CML patients, who had their IM therapy discontinued after achieving CMR during at least one year on IM therapy at 2 Korean institutions from June 2009 to September 2010. CMR was defined by undetectable levels of BCR-ABL transcript by RQ-PCR with at least 10,000 ABL transcripts per volume cDNA. After discontinuation, BCR-ABL/ABL ratio was monitored by RQ-PCR monthly during the first 6 months and every 2-3 months thereafter, and relapse was defined by detectable levels of BCR-ABL transcript in two successive assays. *Results.* Fourteen patients were included. The median age was 60 (range, 29-78) years, and 10 patients were women. All patients were CP and received IM as an initial treatment. Sokal score at diagnosis was low in 1 patient, intermediate in 6, and high in 7. IM was started at a dose of 400mg/day. The median interval from IM initiation to CMR was 19.2 (range, 7.0-71.9) months. After achieving CMR, IM therapy was continued for a median of 32.3 (range, 12.1-72.4) months. And, the median duration of IM therapy was 56.4 (26.2-82.0) months. After IM discontinuation, molecular relapse occurred in 9 patients at 1.1 to 6.9 months. With a median follow-up of 13.4 (2.5-20.4) months, TTLC at 1-year was 31.4% (95% CI, 18.5-44.3). In the univariate analysis of factors affecting molecular relapse, high-risk of Sokal score (p=0.004) and more than 24 months of interval between IM initiation and CMR (p=0.008) were associated with frequent molecular relapse. Duration of IM after CMR was not significantly related with molecular relapse, but there was a trend of lower molecular relapse in patients with at least 32 months. IM was resumed in all patients with molecular relapse. 6 of 9 patients showed decrease in their BCR-ABL transcript levels and 3 achieved a CMR after IM rechallenge. *Conclusion.* IM discontinuation in patients with CML who have received IM as initial treatment and achieved a CMR might be feasible, as one-third of patients remain CMR at 1-year. Baseline Sokal score and achieving a CMR within 24 months after IM initiation are associated with lower rate of molecular relapse after IM discontinuation. Thus, treatment strategies that may increase the rates of CMR in early should be investigated, and further studies are warranted.

0677

BCR-ABL MUTATIONS IN IMATINIB RESISTANT PATIENTS WITH CML AND IMPACT OF SECOND GENERATION KINASE INHIBITORS ON BASELINE MUTATIONS TREATMENT - A STUDY ON BEHALF OF LATIN AMERICAN LEUKEMIA NET (LALNET)

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Background. Mutations within the BCR-ABL domain are the most frequent mechanism of imatinib (IM) resistance. The second generation inhibitors (SGI) are indicated for imatinib intolerance or resistance and the initials trials showed similar response rates in IM resistant patients after IM failure, independent of mutation status, except T315I. *Aims.* The aim of this work was to report the frequency of BCR-ABL mutations in chronic myeloid leukemia (CML) patients with imatinib resistance in a Latin American population and to evaluate the clinical impact of SGI treatment in this group of patients. *Methods.* A total of 187 resistant patients with BCR-ABL mutations were analyzed. After imatinib resistance, 120 received SGI (87 dasatinib, 28 nilotinib and 5 bosutinib). Mutations were detected by direct sequencing from bone marrow or peripheral blood samples, collected during imatinib (129), dasatinib (29), nilotinib (21) or other treatments (8). The median follow-up from mutation detection was 12 months. Overall survival (OS) was calculated from date of mutation detection until last follow-up or death, whereas the progression-free survival (PFS) from date of mutation detection until progression to accelerated phase or blast crisis, last follow-up or death. For the statistical analysis was used log-rang test using SPSS 14.0 software. *Results.* the median age of patients at diagnosis was 42 years. 73% were in CP, 19% in AP and 8% in BC. According to Sokal score, patients were stratified in low risk (27%), intermediate (37%) and high (36%); 32% had used Interferon. The median time from diagnosis until Imatinib treatment was 28 months (1-310) and from Imatinib start until mutation detection was 38 months (1-100). Mutations region: P-loop (57 / 30.5%), nucleotide contact site (47 / 25.1%), catalytic domain (46 / 24.6%), A-loop (8 / 4.3%) and others (29 / 15.5%). The most frequent mutations detected were: T315I (29/15.5%), F359V (24/12.8%), M244V (17/9.1%), G250E (16/8.6%), E255K (11/5.9%), M351T (9/4.8%), Y253H (7/3.7%). Twelve patients presented more than one mutation. During Dasatinib treatment mutation T315I was detected in 8/29 patients, F317L in 6/29 and E255K in 6/29. During Nilotinib treatment 7/21 patients presented mutation F359V and 4/21 T315I. Overall survival in the total group was 44% (95% CI: 30-58%) with a median time of 12 (1-91) months. There was no difference in survival comparing P-loop mutations and others (P= NS). In the group where mutations were detected during imatinib treatment, OS and PFS were superior in patients that received a SGI in comparison with other treatments after resistance (57% versus 43% and 40% vs 18%, P=0.001 and P=0.004, respectively). Twenty-four/55 died from disease progression; 5 from infections; 3 from graft versus host disease; 1 hemorrhage; 1 cardiac failure; 1 second neoplasia and 20 were not reported. *Conclusions.* the frequency of T315I, M244V, E255K were similar to previous reports. F359V was the second most frequent mutation detected in our population. Treatment with SGI clearly had a positive impact on patients' survival after imatinib failure.

0678

HIGHER ADHERENCE RELATED TO COMPLETE MOLECULAR RESPONSE (CMR) IN CHRONIC MYELOID LEUKEMIA PATIENTS USING IMATINIB MESILATE

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Background. Tyrosine Kinase Inhibitors are currently the treatment of choice for Chronic Myeloid Leukemia (CML). Treatment adherence is an important factor to obtain good therapeutical results. The WHO definition of adherence is: "the extent to which a person's behavior - taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider". *Aims.* to evaluate the adherence to Imatinib Mesilate (IM) in patients with CML and to identify factors can affect adherence to treatment. *Methods.* a prospective and observational analysis was performed, between March 2009 and December 2010, with 86 patients taking IM, in first line of treatment at Hematology and Hemotherapy Center, University of Campinas, Brazil. Adherence ratios were monitored for almost one year (308 to 433 days). The adherence was calculated using the mean medication possession ratio (MPR), calculated as total days' dose of IM divided by the number of the days in the observation time. Quantitative PCR were measured in peripheral blood pre and post analysis time and each 12 weeks during the interval. Statistical analysis began with descriptive analysis and we applied Spearman's correlation and t-Test or ANOVA, what it was adequate, considering significant p-value 0.05 (SPSS 14.0 software). *Results.* a total of 86 patients were evaluated. The median age was 49 (20-82) years. The majority of patients were male (59%) and the median of IM treatment time was 39 months (6-106). Considering all patients, the mean and median of adherence were 89% and 96% respectively and 24% of patients were completely adherents with 100% of MPR. Adherence decreased with longer term therapy (p= 0.02) and longer duration of disease (p= 0.002). The adherence was superior in patients enrolled in clinical trials (p=0.007). CMR rates were superior in patients more adherent (p=0.01 - ANOVA and p= 0.02 - Bonferroni). On the other hand, adherence was inferior in patients using higher dose (600/800mg) of IM (p=0.01). There was not significant difference in adherence regarding sex, age, socioeconomic status, marital status, Sokal and instruction level. *Conclusion.* higher adherence in CML patients using IM is related with superior CMR, IM lower doses and clinical trial participating. However, the poor compliance was associated with longer term therapy and longer duration of disease.

0679

LONG-TERM ESTIMATE OF QUALITY-ADJUSTED LIFE EXPECTANCY FOR NILOTINIB AND IMATINIB AS FIRST-LINE TREATMENT FOR NEWLY DIAGNOSED PATIENTS WITH PH+ CHRONIC MYELOID LEUKEMIA IN THE CHRONIC PHASE (CML-CP)

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Background. In the ENESTnd study, the 12-month rates of major molecular (MMR) and complete cytogenetic (CCyR) responses were significantly higher in newly diagnosed Ph+ CML-CP patients initiating nilotinib (300 mg BID) than imatinib (400 mg QD) (MMR: 44% v. 22%, respectively; CCyR: 80% v. 65%, respectively; all P<0.001). Nilotinib patients had a significant reduction in progression to accelerated phase or blast crisis v. imatinib patients (P = 0.01). *Aims.* Model the long-term quality-adjusted life expectancy (LE) of newly-diagnosed Ph+ CML-CP patients initiating first-line therapy with nilotinib or imatinib in the United Arab Emirates (UAE). *Methods.* A state-transition Markov model was developed. A central model feature was the rate at which patients discontinue initial therapy, which was (1) based on direct observations from ENESTnd (for the first 12 months of the model) and, (2) dependent on patients' 12-month ENESTnd treatment responses and modeled from the long-term IRIS outcomes. After initial treatment discontinuation, patients were modeled to receive an additional tyrosine kinase inhibitor (TKI), and prognosis was modeled using published lit-

erature. Uncertainty was assessed using intervals comprising 95% of 1,000 model replications generated via Monte-Carlo simulations (95% credible intervals [CI]). *Results.* Initiating TKI therapy with nilotinib resulted in an estimated mean (95% CI) LE of 28.03 (26.73-29.34) years and quality-adjusted LE of 25.14 (23.42-26.73) years. The corresponding estimates for imatinib first-line therapy were 24.43 (22.70-25.97) years and 21.56 (19.87-23.33) years, respectively. The differences in mean (95% CI) LE and quality-adjusted LE between nilotinib and imatinib were 3.68(2.04-5.35) years and 3.58 (2.03-5.14) years, respectively. *Conclusions.* First-line nilotinib was more efficacious than imatinib in the ENESTnd trial at 12 months. As trial data collection is ongoing, modeling can be used to estimate the long-term value of these treatments. The present model suggests that nilotinib is likely to substantially increase quality-adjusted LE v. imatinib.

0680

IMPROVEMENT OF IMATINIB-RELATED CHRONIC LOW-GRADE NONHEMATOLOGIC ADVERSE EVENTS (AES) IN PHILADELPHIA-POSITIVE (PH+) CHRONIC MYELOID LEUKEMIA-CHRONIC PHASE (CML-CP) PATIENTS SWITCHED TO NILOTINIB

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Background. Many CML patients treated with imatinib experience low-grade AEs that require chronic treatment and may impact quality of life (QOL). Nilotinib is approved for adults with Ph+ CML-CP or accelerated phase who are intolerant or resistant to imatinib and for patients with newly diagnosed Ph+ CML-CP. Cross-intolerance between imatinib and nilotinib is rare (4% in the nilotinib registration study). *Aim.* Assess the potential improvement of chronic low-grade nonhematologic AEs in patients with Ph+ CML-CP when switched from imatinib to nilotinib therapy. *Methods.* This is an on-going, multicenter, open-label study. To be eligible, patients with Ph+ CML-CP must have received imatinib 400 mg daily for ≥3 months before screening, experienced an imatinib-related Grade 1 or 2 nonhematologic AE persisting >2 months or recurring more than 3 times and persisting despite best supportive care. At enrollment, patients are switched to nilotinib 300 mg twice daily. The primary endpoint is change in imatinib-related nonhematologic AEs using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 after 3 months (end of cycle [EOC] 3) of nilotinib. Secondary endpoints include response to nilotinib and patient-reported outcomes as measured by QOL questionnaire and the MD Anderson Symptom Inventory (MDASI)-CML. The study was conducted in accordance with Declaration of Helsinki; patients provided written informed consent. This preliminary analysis was performed on 23 patients enrolled as of the data cut-off date of November 30, 2010. *Results.* The median duration of nilotinib was 3.6 months. There were 105 baseline imatinib-related nonhematologic AEs (72 Grade 1; 33 Grade 2), persisting for a median 24.8 months. Fifteen patients completed EOC 3 by cut-off. Of the 75 imatinib-related AEs reported among these patients, 45 AEs were improved (30, 7, and 3 AEs resolved by months 1, 2, 3, respectively; 5 AEs decreased from Grade 2 to 1), 27 were unchanged, and 3 increased in severity. At study entry, 20 patients had achieved major molecular response (MMR, 3-log reduction of Bcr-Abl); 3 additional patients achieved MMR by cut-off. Eleven patients completed QOL questionnaires; 82% reported QOL improvements at EOC 3 compared to baseline. Mean reduction from baseline in MDASI-CML severity score and interference score were 1.47 & 1.86 (EOC 1) and 1.45 & 1.33 (EOC 3), respectively, indicating improvement in symptoms. Nilotinib dose was reduced in 7 patients for nilotinib-related nonhematologic AEs. Fourteen Grade 3 AEs were reported in 6 patients; 10 (increased bilirubin and lipase, dehydration, hypokalemia, hypophosphatemia, worsening of arthralgia, joint pain) were suspected to be nilotinib-related. No patient had a Grade 4 AE. Most AEs were managed by brief dose interruption. Two patients discontinued nilotinib (1 hyperglycemia, 1 upper abdominal pain/oral pain/headache). The maximum QTcF change from baseline was 37 msec; QTcF prolongation >500 msec did not occur. *Conclusions.* Switching to nilotinib improved nonhematologic imatinib-related, low-grade AEs in >50% of CML-CP patients in this analysis. Overall improvement in symptoms was observed and 82% of patients experienced improved QOL, including 5 of 6 patients with Grade 3 AEs. Longer-term evaluation and enrollment continue.

0681

PATIENT REPORTED OUTCOME RESULTS FROM A PHASE III RANDOMIZED TRIAL COMPARING NILOTINIB AND IMATINIB IN PATIENTS WITH NEWLY DIAGNOSED CML-CP

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Background. Nilotinib has been shown to be a more potent inhibitor of BCR-ABL than imatinib. A randomized Phase III study was conducted comparing these two therapies in adult patients with newly diagnosed Philadelphia Chromosome positive (Ph+) chronic myelogenous leukemia (CML) in chronic phase (CP). Major molecular response by 24 months was significantly improved with nilotinib 300mg BID (71%) and nilotinib 400 mg BID (67%) compared to imatinib 400 mg QD (44%); $p < 0.0001$ for both comparisons. Adverse event (AE) profiles differed among the treatment arms, and patients treated with nilotinib 300mg BID had the lowest rate of discontinuation due to AE. *Aims.* To evaluate the patient-reported outcomes, including health-related quality of life (HRQL) and functioning, within the phase III trial. *Methods.* A total of 846 patients were randomized to receive nilotinib 300mg BID (n=282), nilotinib 400mg BID (n=281), or imatinib 400mg QD (n=283). HRQL was assessed in this open-label study using the Functional Assessment of Cancer Therapy-Leukemia (FACT-Leu) and the Short Form 36 Health Survey (SF-36). The FACT-Leu consists of four general subscales measuring physical, social/family, emotional, and functional well-being and a 17-item leukemia-specific subscale. The FACT-Leu Total score is the sum of all five subscales. The SF-36 assesses eight domains (physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional, and mental health) that can be combined to create a mental component score (MCS) and a physical component score (PCS). Both questionnaires were administered at baseline, and at 3, 12, and 24 months. *Results.* Questionnaire completion rates at baseline were 86-92%, and 64-70% at 24 months. High baseline scores reflected that these newly diagnosed patients (median age 47) were in relatively good health and their CML was mostly asymptomatic. FACT-Leu subscale and Total scores were similar across treatment arms at baseline and at follow-up assessments, with differences between treatment arms less than 2 points. At 24 months compared to baseline, FACT-Leu subscale scores were maintained or improved for 87% of patients receiving nilotinib 300mg BID, with 13% experiencing worsening. At each time point

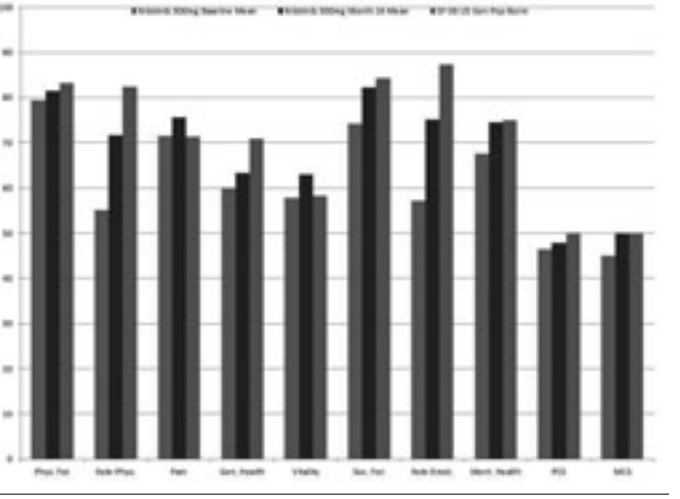

Figure 1. SF-36 scores compared to the US general population.

across treatment arms, differences among the PCS and MCS scores of the SF-36 were less than 1.5 points. Compared to the SF-36 norms for 45 -55 year old individuals in the US, mean nilotinib 300mg BID scores were similar to population means for most of the domains at 24 months (Figure). *Summary/conclusions.* Nilotinib 300mg BID demonstrated superior efficacy including significantly lower rates of progression to AP/BC, lower rates of discontinuation due to AE compared to imatinib, and maintains health-related quality of life and functioning in patients with newly diagnosed CML-CP, at a level nearly comparable to general population normative values. These findings provide a patient perspective and underscore the significant advances in the treatment of Ph+ CML, where patients can achieve deep clinical responses and maintain a near-normal life.

0682

SOCIO-DEMOGRAPHIC AND CLINICAL DETERMINANTS OF PATIENT-REPORTED SYMPTOM SEVERITY IN CHRONIC MYELOID LEUKEMIA SURVIVORS IN TREATMENT WITH IMATINIB OVER THE LONG RUN

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Background. The treatment of chronic myeloid leukemia (CML) has changed dramatically over the last decade with the advent of targeted therapies but there is lack of data concerning the long term impact on quality of life and symptoms from the patients' perspective. *Aims.* The aim of this study was to investigate the predictive value of socio-demographic and clinical treatment related data of self-reported symptom severity in CML patients undergoing Imatinib (IM) over the long run. *Methods.* A large multicenter case-control survivorship study including 26 centers was set up to investigate the main research objective and included 422 CML patients who started IM, as first line therapy, in the early chronic phase of the disease. Patients were receiving IM treatment (regardless of dose) for at least three years and were in complete cytogenetic response (CCyR) at the time of study entry. Patient reported symptoms were measured with a previously devised nine items checklist for CML patients undergoing IM treatment. This checklist had a one-week time recall period and all items were rated on a four point likert-type scale (i.e. "not at all", "a little", "quite a bit"or "very much"); these measurement characteristics were selected to be consistent with other symptom scales/items included in other psychometric robust measures, widely used in similar long-term studies. The selection of the core symptom domains was based on an extensive literature review, published data on side effects of IM as well as from patients input. Items investigated the following issues: abdominal discomfort, diarrhea, edema, fatigue, headache, muscle cramps, musculoskeletal pain, nausea and skin problems. An overall symptoms index was derived by averaging all symptoms standardized scores. A regression analysis was performed for symptoms index as response variable and a number of potential key covariates were investigated including: gender, age, toxicity, performance status, living arrangements, education, time to achieve a first CCyR, haemoglobin, comorbidity and IM current dose. *Results.* Fatigue was the most prevalent symptom with 82% of patients reporting it with any level of concern. Nausea and abdominal problems were the less frequently reported symptoms. A univariate analysis identified the following variables predicting symptom severity: gender (P<0.001), age (P=0.002), living arrangements (P=0.007), comorbidity at diagnosis (P<0.001), assuming other drugs not related to the diagnosis of CML (P=0.008) and having had a previous diagnosis of cancer (P=0.036). The final model retained the following variables predicting a lower reported symptom severity: being male (P<0.001), living with a spouse or partner (P=0.027) and not reporting comorbidity at the time of diagnosis (P=0.002). The total variance explained with this model was, however, small being 13% thus suggesting that other unexplored factors might have an important role in determining patient-reported symptom severity. *Conclusions.* This is the first evidence based data investigating possible clinical determinants of patient-reported symptoms in CML patients suggesting that gender, comorbidity and living arrangements are of importance. Further possible determinants need to be investigated in future analyses.

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A NEW SYMPTOM MEASURE IN CHRONIC MYELOID LEUKEMIA

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Background. Symptom burden is the combined impact of symptoms from disease and treatment on daily functioning. While the severity of symptoms experienced by patients with Philadelphia chromosome positive chronic myeloid leukemia (CML) receiving kinase inhibitor (KI) therapy may be less than with more intense forms of cancer therapy, these symptom are experienced for years rather than days or months. This may lead to overall equal or greater symptom burden and resultant functional impairment for these patients. A major barrier to effective symptom management in CML is inadequate assessment. In addition, patient-reported outcomes (PROs) can be endpoints in clinical trials to establish treatment benefits. *Aims.* We aimed to develop a short, easily-understood, valid, and reliable PRO measure of CML symptoms for research and practice. *Methods.* After providing institutional review board-approved informed consent, 127 patients with CML completed the 13 symptom severity and 6 interference items of the core MD Anderson Symptom Inventory (MDASI) plus 7 CML-specific symptom items (Table 1), generated from patient and expert input, measured on a 0-10 scale (0 = none, 10 = worst imaginable). 85 patients completed the same items 2 weeks later. Patients also answered a single quality-of-life (QOL) question. Demographic and disease information was collected on all patients. Multivariate analysis examined relationships among items. Psychometric procedures determined reliability and validity of the MDASI-CML. *Results.* Mean subject age was 50.4 years (standard deviation [sd] = 13.7). 54% of the subjects were female, 76% were white, 62% were employed, 99% had an ECOG performance status < 2, and 98% were in chronic phase. 94% were receiving KI therapy (of total receiving KI therapy, 48% were receiving imatinib, 28% were receiving dasatinib, 16% were receiving nilotinib, and 9% were receiving an investigational KI). Mean overall quality of life rating was 8.2 (best = 10, sd = 1.9). Selected symptom severity and interference scores are in Table 1. All items were retained as clinically significant and non-redundant. The reliability index (Cronbach α) and test-retest reliability of the 20 symptom items were 0.94 and 0.93 respectively and of the 6 interference items were 0.94 and 0.91 respectively. The MDASI-CML discriminated between patients who were employed versus those medically disabled and with good versus poor QOL. Symptom severity explained 87% of the variance in interference, with the core symptoms explaining 83% and the CML-specific symptoms explaining 76%. Patients receiving imatinib reported significantly (p = 0.048) more severe CML-specific symptoms (mean = 1.83, sd = 1.60) than patients receiving dasatinib or nilotinib (means = 1.27 and 1.03 respectively, sd = 0.99 and 1.30 respectively). *Summary/Conclusions.* We have validated an analytic tool, the MDASI-CML, for quantifying CML symptoms. The MDASI-CML is being used to assess side effects in treatment trials and to monitor symptoms in clinical care. Additional research on the longitudinal symptom burden, including differences based on type of KI therapy, is needed.

Chronic myeloid leukemia - Clinical 2

0684

LOSS OF MAJOR MOLECULAR RESPONSE (MMR) IS MORE ACCURATE THAN LOSS OF COMPLETE MOLECULAR RESPONSE (CMR) FOR RESTARTING IMATINIB AFTER IMATINIB DISCONTINUATION IN CP-CML PATIENTS WITH LONG LASTING CMR

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Background. We have reported the results of imatinib discontinuation in 100 CML (Chronic Myelogenous Leukemia) patients in complete molecular response (CMR) for more than 2 years under imatinib therapy (STIM study). The molecular relapse was defined by two consecutive positive values of the BCR-ABL/ABL ratio. Using this criterion, 59% of the patients had to restart imatinib therapy and achieved a second CMR (Mahon *et al.* Lancet Oncology 2010). However, we identified patients experiencing occasional positive values without a confirmed molecular relapse. We then asked whether the loss of major molecular response (MMR) could be a more accurate criterion for restarting imatinib in an independent cohort of patients. *Patients and Methods.* Patients were retrospectively analysed. CP-CML patients were eligible if they were in CMR (CMR 4.5 log) under imatinib therapy for more than 2 years. Those patients were not enrolled in the STIM study because the study was not initiated or closed or because they experienced one positive value of the BCR-ABL/ABL ratio during the 2 years follow-up. The criterion for restarting imatinib was the loss of MMR. We were then able to calculate molecular relapse free survival using different end-points such as loss of CMR (only one BCR-ABL positivity), loss of CMR using the STIM definition and loss of MMR. *Results.* 25 CP-CML patients were included in the analysis. Median follow-up is 54.8 months (33-102.7). Sex ratio (M/F) was 53% with a median age of 55.7 years (32.7-76.7). Sokal score distribution was 39.1%, 34.7% and 26% for low, intermediate and high values respectively. 14 out of 25 patients received interferon therapy prior to imatinib. Median duration of imatinib therapy and median duration of CMR prior to discontinuation was 58.7 months (30.1-117.6) and 33.2 months (13.6-72.8). One patient had a CMR duration less than 24 months. 11 out of 25 patients (44%) had a least one BCR-ABL positive value after the achievement of CMR. 9 patients (36%) restarted imatinib including 7 patients after the loss of MMR and two patients after the loss of CMR (these two patients were censored at the time of treatment initiation for the loss of MMR analysis). We next compared different end-points in order to evaluate the best criterion for restarting imatinib after discontinuation. Median relapse free survival was 4.8 months, 13.7 months and not reached using loss of CMR, loss of CMR according to the STIM study and loss of MMR criteria (p=0.035). At 48 months, 32%, 40,9% and 78.7% of the patients were relapse free using the same criteria respectively. Of note, among the 18 patients experiencing a persistent MMR after imatinib discontinuation, 7 (38.9%) lost their CMR according the STIM criteria and did not restart imatinib. *Conclusions.* Using the loss of MMR, 78.7% of the patients are relapse free (and treatment free) at 48 months following imatinib discontinuation. We were able to identify patients with long lasting MMR despite a loss of CMR suggesting that a proportion of these patients were able to control their tumour burden without the need of imatinib therapy.

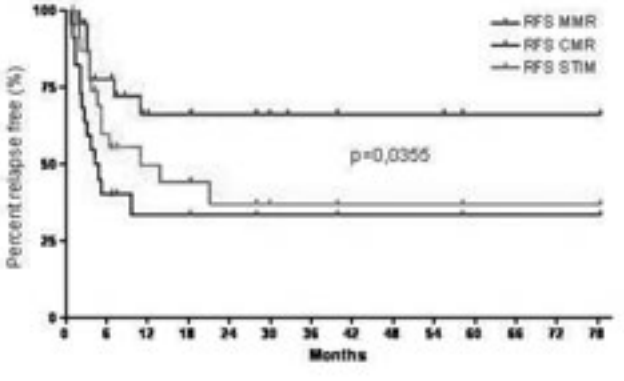

Figure 1. Kaplan Meier analysis of relapse free survival usi.

0685

OPERATIONAL CURE OF CHRONIC MYELOID LEUKEMIA AFTER TREATMENT WITH INTERFERON-ALPHA: A RETROSPECTIVE LONG-TERM STUDY FROM NORTHERN MORAVIA REGION

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Background. Tyrosine kinase inhibitors (TKIs) are today the drugs of choice in the treatment of patients with chronic myeloid leukemia (CML). However, their potential to cure the disease is limited as survival of CML stem cells is probably not dependent on Bcr-Abl kinase activity. Interferon-alpha (INF), on the other hand, may have significant impact on CML stem cells due to wide range effect on cytokines, cell cycle and immune system. *Aims.* To analyze long-term results of CML patients treated exclusively with INF (as single-agent therapy or in combination with hydroxyurea or cytarabine) and impact of achievement of complete cytogenetic response (CCyR) and BCR-ABL negativity in nested reverse-transcriptase polymerase chain reaction (RT-PCR) on overall survival (OS) of patients. *Methods.* Retrospective analyses of all consecutively patients with CML in chronic phase treated with INF in the region between 1989 and 2006. INF dosage was adjusted according tolerability in order to maintain leukopenia 2-4x109/l between 3MU three times weekly and 10 MU daily. BCR-ABL negativity was assessed by standard nested RT-PCR. INF treatment was stopped in actual patient after one year of gradual dose reduction only after two years duration of BCR-ABL negativity. OS was calculated according method of Kaplan and Meier, log-rank test, Mann-Whitney test and Cox regression analysis were used for statistic evaluation. Patients were censored for OS evaluation at the time of allogeneic stem cell transplantation or initiation of treatment with TKIs. *Results.* Treatment results of 118 patients with CML in first chronic phase (67 males and 51 females at median age 50 years; range 18-71) were analyzed. Median follow-up of all patients was 82.6 months (range 12.4-212.6). 61 patients were treated with INF for more than 12 months. Their mean OS calculated from the first dose of INF was 137 (95% CI 117.6-156.4) months. 18 (29.5%) of them reached CCyR in median time of 16.7 months (95% CI 3.7-47.3). Nine of these patients (14.8% of patients treated more than one year; 7.6% of the whole cohort) achieved BCR-ABL negativity in nested RT-PCR and 6 of them remain in operational cure without any treatment for 3-9 years; while two continue with INF therapy and one died due to unrelated cause. Percentage of peripheral blasts, splenomegaly, anemia (Hgb ≤110g/l) and Sokal score had significant impact on OS in univariate statistic assessment but only Sokal score remained significant in multivariate analysis. *Conclusions.* Despite numerous adverse effects and treatment failures a significant group of CML patients can gain long-term profit from INF. Unlike the patients with the sole CCyR of whom majority lost CCyR despite continuing INF therapy and later required treatment with TKIs, patients who achieved BCR-ABL negativity in nested RT-PCR had excellent long-term outcome and high probability of operational cure.

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0686

THE NEW TYROSINE KINASE INHIBITORS FOR FIRST-LINE TREATMENT IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA - SYSTEMATIC REVIEW AND META-ANALYSIS

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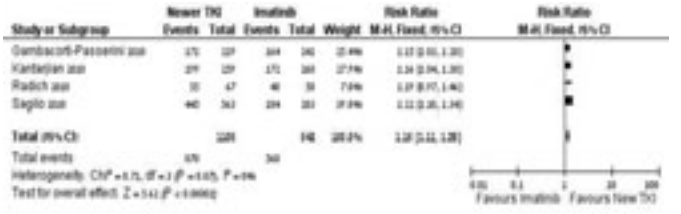
Background. Imatinib is considered first line treatment in chronic phase (CP) chronic myelogenous leukemia (CML) patients. Despite the excellent results obtained from the IRIS trial, 30% to 35% of patients discon-

Table 1. Complete cytogenetic response at 12 months.



tinue imatinib for various reasons. One way to overcome this is to use second generation tyrosine kinase inhibitors (TKIs). These newer agents show a high rate of response in patients with imatinib failure or intolerance. Several trials evaluated response rate and long-term outcomes with second and third generation TKIs compared to imatinib for first-line treatment in patients with CP-CML. *Aims.* We aimed to evaluate the efficacy and safety of the newer TKIs (second and third generation) vs. imatinib for first line treatment of patients with chronic phase CML. *Methods.* Systematic review and meta-analysis of randomized controlled trials comparing treatment with the newer TKIs to imatinib as first line treatment in patients with CP-CML. The Cochrane Library, MEDLINE, conference proceedings and references were searched until February 2011. Two reviewers appraised the quality of trials and extracted data. Outcomes assessed were: complete cytogenetic response (CCyR), major molecular response (MMR), progression to accelerated phase (AP) / blastic crisis (BC), all-cause mortality and toxicity, all at 12 months. Relative risks (RR) were estimated and pooled. *Results.* Our search yielded four trials including 2120 patients, two of them published as abstracts. These trials examined the effect of nilotinib, dasatinib and bosutinib vs. imatinib. Data from the four trials, including 1950 patients were available for analysis of CCyR at 12 months. Treatment with the newer TKIs significantly improved CCyR in all patients (RR 1.18, 95% CI 1.11-1.25, 4 trials) without any differences when the analysis was conducted according to intention to treat or per protocol. Also, treatment with the newer TKI improved CCyR in patients at high risk, (RR 1.33 95% CI 1.11-1.60, 2 trials, 333 patients). There was a significant advantage in favor of the newer TKIs in terms of MMR at 12 months (RR 1.82, 95% CI 1.26-2.64, 4 trials, 2014 patients). Importantly, progression rate to AP/BC was significantly lower with the newer TKIs compared to imatinib (RR 0.32, 95% CI 0.17-0.59, 4 trials). However there was no difference in all-cause mortality (RR 1.17, 95% CI 0.56-2.46, 3 trials) and in the number of adverse events requiring discontinuation of treatment (RR 1.57, 95% CI 0.84-2.93, 4 trials). *Conclusions.* The newer TKIs are superior to imatinib in terms of CCyR, MMR and progression to AP/BC with no further toxicity. A survival advantage could not be shown, probably due to a short follow-up period and the excellent prognosis of CML patients treated with imatinib. Thus, the newer TKIs may be considered as an optional first line treatment in CP-CML patients.

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HIGH BCR-ABL EXPRESSION LEVELS AT DIAGNOSIS AND AFTER 3 AND 6 MONTHS OF TREATMENT ARE ASSOCIATED WITH AN UNFAVORABLE RESPONSE TO IMATINIB

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Background. Imatinib mesylate (IM) has shown remarkable efficacy for the treatment of Chronic Myeloid Leukemia (CML) patients (pts) in the chronic phase of the disease. However, a growing number of pts either fail IM or develop intolerance to the drug. *Aims.* To identify biological parameters predictive of IM response (at diagnosis or during the first months of therapy) in order to recognize pts with a more aggressive disease that should receive alternative treatments. *Methods.* We examined the outcomes of the first 192 CML pts accrued to the observational SCREEN (Sicily and Calabria CML REgional ENterprise) multicenter non-sponsored study, recruiting newly-diagnosed CML pts receiving IM 400 mg daily. Median follow-up was 37 months (range 12-72). Complete hematological (CHR), cytogenetic (CCyR) and major molecular responses (MMR) were rated according to the European Leukemia Net 2006 guidelines. Peripheral blood samples were used for BCR-ABL determination by quantitative real-time polymerase chain reaction according to the International standardized Scale (IS) using either GUS (at diagnosis) or ABL (at every other time-point) as reference genes. Pts were stratified according to clinical and molecular responses or BCR-ABL transcript levels at diagnosis and analyzed for their outcome on an intention to treat basis. *Results.* At 12 months, cumulative incidences of CHRs, CCyRs and MMRs were 100%, 80% and 46.5%. At 24 months, incidences of CCyR and MMR increased to 92.7% and 72%, respectively. According to the ELN criteria, 106 pts (55.3%) achieved an optimal response; 45 pts (23.4%) had a suboptimal response; 35 pts (18.2 %) failed IM because of either primary (22 pts) or secondary (13 pts) resistance. Only 6 pts (3.1%) were intolerant to IM. Kaplan-Meyer estimates for overall, progression-free, event-free (EFS) and failure-free survival (FFS) at 60 months were 98.6%, 96.1%, 80.5% and 69.5%. When we clustered all subjects in optimal responders (ORs) and suboptimal/resistant (S/R) pts and correlated response to therapy with various molecular characteristics we found that high BCR-ABL^{IS} transcripts at diagnosis (using GUS as a reference gene) predicted response to IM (p=1.076e-9). Moreover, high BCR-ABL^{IS} transcripts at diagnosis significantly correlated with a lower probability of obtaining a CCyR (p=0.000861) and lower rates of EFS (p=0.0001864) and FFS (p=0.00005935). As WBC counts were not significantly different between ORs and S/R pts (p=0.2065), increased amounts of BCR-ABL transcripts were probably representative of the aggressiveness of the leukemic clone. We also observed that pts displaying >10% BCR-ABL^{IS} after 3 months of IM or >1% BCR-ABL^{IS} after 6 months of therapy had a significantly lower probability of achieving a CCyR (p=0.00000000388 and p=2.631e-16, respectively). *Conclusions.* Approximately 40% of newly-diagnosed CML pts will either fail IM or obtain a suboptimal response. High levels of BCR-ABL^{IS} transcripts at diagnosis allow the rapid identification of CML pts that are unlikely to benefit from IM. Furthermore, failing to achieve BCR-ABL^{IS} transcript levels <10% after 3 months or <1% after 6 months of IM significantly reduces the probability of subsequently obtaining a CCyR.

0688

HIGH IMATINIB PLASMA LEVEL IS CRITICALLY IMPORTANT FOR COMPLETE CYTOGENETIC RESPONSE MAINTENANCE IN CML PATIENTS

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Background. The treatment of patients with chronic phase (CP) Ph-positive chronic myeloid leukemia (CML) with imatinib has resulted in high rates of cytogenetic and molecular responses. However some patients lose achieved complete cytogenetic response (CCyR). Recent data have shown that the achievement of CCyR to imatinib therapy is related with more than 1000 ng/ml Imatinib plasma level (IPL). *The Aim* of our study was to elucidate the role of IPL in maintenance of CCyR

Table 1.

Imatinib plasma level (C_{trough}) in CP CML patients with CCyR and loss of CCyR

Imatinib Dose	Patients	n	Age Median [min-max]	Male/ Female	Imatinib Treatment Duration (months)	C_{trough} of Imatinib (ng/ml)		
						Median [min-max]	Mean	P value
400 mg QD	Loss of CCyR	24	52,8 [22-77]	14/10	38,6 [12,1 – 66,9]	645 [259-1702]	651±66	<0,0001
	CCyR	183	54,2 [18-75]	106/77	24,3 [12,7 -90,3]	1232 [375-2003]	1162±30	
600 mg QD	Loss of CCyR	27	53,7 [32-72]	12/15	36,5 [12,5 -93,6]	791 [178-2631]	878±91	<0,0001
	CCyR	82	53,4 [19-76]	40/42	33 [12,6 – 119,7]	1688 [328-2789]	1709±61	

by comparison IPL in CML patients with stable CCyR and with loss of CCyR. *Methods.* IPL were detected in 316 CP CML patients with Imatinib treatment duration more than 12 months. The age of patients was 18-78. Male/female ratio was 172/144. Imatinib doses were 400 mg QD (n=207) and 600 mg QD (n=109). Blood samples were collected in 21-27h after the last Imatinib dose intake. All patients gave informed consent before blood sampling. Imatinib concentrations (C trough) were determined by a validated LC/MS/MS method. *Results*. The obtained results were analyzed in 4 groups CP-CML patients: CML patients in imatinib treatment with 400 mg QD with CCyR and loss of CCyR, with 600 mg QD with CCyR and loss of CCyR (Tab.1). The mean of IPL in CML patients treated with Imatinib 400 mg QD with CCyR was 1162±30 ng/ml vs 651±66 ng/ml in patients with loss of CCyR (p<0,0001). The mean of IPL in CML patients treated with Imatinib 600 mg QD with CCyR was 1709±61ng/ml vs 878±91 ng/ml with loss of CCyR (p<0,0001). *Conclusions.* The IPL in CML patients achieved stable CCyR significantly higher than in patients with loss of CCyR. High level of IPL is important for maintenance of CCyR. Probably the achievement of CCyR in cases with low level of imatinib concentration in plasma is associated with low stability of achieved CCyR.

0689

COST EFFECTIVENESS OF NILOTINIB VS. IMATINIB AS FIRST-LINE TREATMENT FOR NEWLY DIAGNOSED PATIENTS WITH PH+ CHRONIC MYELOID LEUKEMIA IN THE CHRONIC PHASE (CML CP): SWEDISH PERSPECTIVE

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Background. The ENESTnd study showed that, vs. imatinib (400 mg QD), nilotinib (300 mg BID) results in superior major molecular and complete cytogenetic responses and time to disease progression as first line (FL) therapy in newly-diagnosed patients with Ph+ CML-CP. *Aims.* To assess, from a Swedish societal perspective, the direct and indirect costs and quality adjusted life years (QALYs) of FL imatinib vs. nilotinib in newly-diagnosed Ph+ CML-CP. *Methods.* A literature-based Markov model was developed to estimate the lifetime QALYs and costs of Ph+ CML-CP patients initiating therapy with nilotinib or imatinib. A central model feature is the discontinuation rate from FL therapy, which was based on ENESTnd for the first 12 months and, thereafter, on the rate observed in the IRIS study, stratified by initial 12-month FL responses. Patients discontinuing FL therapy were modeled to receive one additional tyrosine kinase inhibitor (TKI). Prognosis after FL discontinuation was modeled using published studies. Clinical outcomes and drug exposure were obtained from the ENESTnd study. Non-TKI-drug costs and productivity loss were assumed to increase as disease progressed. Quality of life varied by disease stage and response. *Results.* Compared to FL imatinib (Table), FL nilotinib results in increases in net discounted FL drug therapy costs, decreases in other direct medical costs and productivity loss, and gains in discounted survival and QALYs. The discounted incremental cost/LY and cost/QALY are estimated at 16 028 € and $18 163 €, respectively. In probabilistic sensitivity analysis, 95% of model replications cost ≤44 500 €/QALY gained. *Conclusions.* FL nilotinib is cost-effective in Swedish patients with Ph+ CML-CP who are initiating TKI therapy.

Table 1.

	Imatinib	Nilotinib	Difference
*FL Drug Costs**	477 660 €	581 751 €	104 091 €
*Other Direct Medical Costs**	180 320 €	152 603 €	-27 718 €
*Productivity**	513 327 €	564 436 €	51 109 €
*Net Cost Difference**			25 265 €
*Life Expectancy (years)**	17,30	18,82	1,52
*QALY**	14,39	15,78	1,39
*Cost / LYG**			16 668 €
*Cost / QALY**			18 163 €

Notes: 1 Euro (€) = SEK 8,71; *All results discounted at 3%/year.

0690

THE EUROPEAN POPULATION-BASED CML-REGISTRY - OBJECTIVES AND FIRST RESULTS

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Background. Comparatively little is known about the epidemiology and the presenting features of chronic myeloid leukemia outside of clinical studies. *Aims.* Thus, a European CML registry was established with the aim to assess epidemiology, management and outcome of CML, with particular reference to the incidence, the stage of disease at time of diagnosis and the initial clinical management. *Methods.* All European countries were invited to collaborate. Regions had to be defined to allow for the estimation of incidence, and all newly diagnosed patients had to be recorded and reported. A follow-up of these patients is planned. The registry is strictly non-interventional and national laws and regulations are fulfilled. In late 2009 the registry was started. *Results.* Up to now 25 study groups from 24 European countries have registered 846 CML patients (evaluable: 833 pts.). At time of diagnosis 93.6% were in chronic, 4.1% in accelerated and 2.3% in blastic phase. The EuroScore risk group distribution was: 32.9% low, 50.2% intermediate and 16.9% high risk. Chromosomal abnormalities in Ph^+ cells were seen in 7.9%. The majority of the patients showed a WHO-score of 0 (52.3%) or 1 (36.8%). There are considerable differences between the European countries, e.g. the proportion of high risk patients is considerably higher in the eastern and northern countries of Europe and the UK. Data about the clinical management are not yet available. *Summary.* It is considered a major achievement that 25 study groups of 24 different European states are collaborating to establish a European population-based CML-Registry. The data indicate that patients recruited for clinical trials may not be fully representative of all CML patients.

0691

ANALYSIS OF BCR-ABL TYROSINE KINASE DOMAIN ABNORMALITIES IN IMATINIB-TREATED CHRONIC PHASE-CHRONIC MYELOID LEUKEMIA PATIENTS BASED ON 2009 EUROPEAN LEUKEMIA NET RECOMMENDATIONS

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Background. Abnormalities in BCR-ABL kianse domain (KD), such as point mutation, 35 base pair insertion (35INS), and deletion of exon 7 (Del ex7) have been found in chronic myeloid leukemia (CML) patients. Among such abnormalities, point mutations have been known as the clinically most relevant mechanism of imatinib resistance with 40~80% of detection frequency in imatinib resistant patients. 2009 European Leukemia Net (ELN) guideline recommends mutation screening of patients with suboptimal response and treatment failure during imatinib therapy. However, there has not been much information about BCR-ABL KD abnormality status in suboptimal responder. In addition, 35 INS and Del ex7 have not provided their clinical relevance yet al-

Table 1. Table 1. Abnormalities in BCR-ABL kinase domain.

	Months after IM Tx	Optimal N=228	Suboptimal N=52	Failure N=22
Point mutation	3 mons.	0 of 20 (0%)	0 of 1 (0%)	0 of 1 (0%)
	6 months	6 of 97 (6%)	1 of 8 (13%)	1 of 2 (50%)
	12 months	4 of 91 (4%)	1 of 8 (13%)	2 of 12 (17%)
	18 months	1 of 20 (5%)	3 of 35 (9%)	3 of 7 (43%)
	Total	11 of 228 (5%)	5 of 52 (10%)	6 of 22 (27%)
35INS or Del ex7	3 months	3 of 20 (15%)	0 of 1 (0%)	0 of 1 (0%)
	6 months	11 of 97 (11%)	0 of 8 (0%)	0 of 2 (0%)
	12 months	12 of 91 (13%)	0 of 8 (0%)	0 of 12 (0%)
	18 months	2 of 20 (10%)	9 of 35 (26%)	1 of 7 (14%)
	Total	28 of 228 (12%)	9 of 52 (17%)	1 of 22 (5%)

though they have been found frequently in CML patients including suboptimal responders. *Aims.* We analyzed abnormalities in BCR-ABL KD from CP-CML patients with suboptimal response or treatment failure to imatinib, and investigated their mutation status and clinical relevance of the abnormalities. *Methods.* This study included 151 CP-CML patients registered in Seoul St. Mary's hospital since 2002. Serial samples of patients at 3, 6, 12, and 18 months were collected from the Korea leukemia bank in the form of cryopreserved cells or isolated RNAs depending on availability of each sample. Abnormalities in BCR-ABL KD were analyzed using direct sequencing. The 2009 ELN guideline was employed for definition of patient's response including optimal response, suboptimal response and treatment failure at each time point. *Results.* We analyzed total 302 serial samples (228 optimal, 52 suboptimal and 22 failures samples) from 151patients at different time points including 3, 6, 12 and 18 months after initiation of imatinib treatment. KD abnormalities were found with difference in each response group. Point mutations were found in 5% of optimal responders, 10% of suboptimal responders and 27% of treatment failures with higher frequency at 6 months in all 3 response groups. Other abnormalities in BCR-ABL KD, including 35INS and Del ex7, were detected in 12% of optimal responders, 17% of suboptimal responders and 5% of treatment failures with higher frequency at 18 months in suboptimal responders and failure group. Interestingly, patients with other abnormalities in BCR-ABL KD showed significantly ($p<0.024$) higher ratio of turning out suboptimal response; Among 18 optimal patients with 35INS or Del ex7 by 12 months, 72% (n=13) turned out suboptimal responders at 18 months while 38% (n=15) became suboptimal responders among 39 optimal responders without any abnormalities in BCR-ABL KD by 12 months. *Conclusions.* Patients with suboptimal response or treatment failure showed tendency of much higher chance of BCR-ABL KD point mutation in comparison with optimal responders, suggesting that mutation screening is important for patients with suboptimal response as well as treatment failure on the basis of 2009 ELN guideline. Especially, optimal responders harboring 35INS or Del ex7 turned out suboptimal response at 18 months than optimal responders without those abnormalities. However, solid clinical relevance of 35 INS and Del ex7 requires long-term follow up with large cohort.

0692

PLEURAL EFFUSION IN PATIENTS WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CML-CP) WHO RECEIVED FIRST-LINE DASATINIB IN THE DASISION TRIAL: PATIENT CHARACTERISTICS, MANAGEMENT, AND OUTCOMES

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Background. In the randomized phase 3 DASISION trial of dasatinib vs imatinib in newly diagnosed CML-CP, dasatinib continued to show superior efficacy (higher, faster, and deeper response rates) and acceptable safety/tolerability at 18 months of follow-up (Shah *et al.* Blood 2010; 116: abs 206). Whereas fluid retention was more frequent among imatinib-treated patients, pleural effusion was observed only in dasatinib-treated patients. *Aims.* To perform retrospective and exploratory analyses of patients with drug-related pleural effusion in DASISION, including assessing if pleural effusion impacted efficacy and describing how pleural effusions were managed. *Methods.* In DASISION, after informed consent, patients were randomized 1:1 to receive dasatinib 100 mg once daily (QD; n=259) or imatinib 400 mg QD (n=260). The primary endpoint was confirmed complete cytogenetic response (cCCyR) rate (defined as complete cytogenetic response [CCyR] on two consecutive assessments) by 12 months. Patients with baseline pleural effusion were excluded. Chest x-rays were performed at baseline and at 6 months, or more frequently if indicated clinically. Pleural effusions were graded according to CTCAE v3.0 criteria: grade 1, asymptomatic; grade 2, symptomatic, ≤2 therapeutic thoracenteses; grade 3, symptomatic requiring supplemental oxygen, >2 therapeutic thoracenteses; grade 4, life-threatening, hemodynamic instability. *Results.* After 18 months' median treatment duration, pleural effusion had occurred in 31/258 dasatinib-treated patients (12%; 3% grade 1, 9% grade 2, <1% grade 3). Median age was higher in patients with pleural effusion (n=31; 59 years; range 28-82) compared with those with no pleural effusion (n=227; 44 years; range 18-84). In patients with vs without pleural effusion, median dasatinib dose by last follow-up was 93 vs 100 mg/d and Hasford risk score was low in 23% vs 35%, intermediate in 68% vs 45% and high in 10% vs 20%, respectively. In patients experiencing pleural effusion, median time to effusion was 33 weeks, with 87% of effusions occurring more than 8 weeks after start of treatment. Pleural effusions were managed by dose modification (therapy was interrupted in 24 patients and reduced in 13 patients) and/or medical intervention (13 patients received diuretics, 11 received corticosteroids, eight received both diuretics and corticosteroids, and three had therapeutic thoracentesis). Four patients (1.5%) discontinued therapy due to pleural effusion. Within available follow-up, most effusions (84%) have not recurred. Based on consensus from an expert panel, a pleural effusion management algorithm will be presented. Pleural effusion did not seem to impact efficacy: 94% and 65% of patients who had a pleural effusion achieved a CCyR and major molecular response (MMR), compared with 84% and 56% of patients without pleural effusion, respectively. In patients with or without pleural effusion, peripheral lymphocytosis (defined as absolute blood lymphocyte count > 3.6 x 109/L occurring on ≥2 occasions after >4 weeks of dasatinib treatment) occurred in 42% vs 19%. *Conclusions.* After 18 months of treatment in patients with CML-CP receiving first-line dasatinib, pleural effusion was predominantly mild to moderate in severity, managed by dose modification and/or medical intervention, more commonly associated with lymphocytosis, and did not seem to affect the achievement of CCyR and MMR.

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OUTCOME OF PATIENTS WITH CHRONIC PHASE CML TREATED WITH DASATINIB OR NILOTINIB AFTER FAILURE OF SECOND PRIOR TKIS

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Background. The TKIs Nilotinib and Dasatinib offer additional therapeutic options for patients with CML who are resistant or intolerant to Imatinib. Preliminary results suggest that some patients may respond

to a second TKI used as third line therapy, but little is known about the long term benefit of such an approach. *Aim* of this collaborative Italian study was to verify the response and the clinical outcome in patients with CML treated with a third TKI after sequential failure of the previous ones. *Methods.* We evaluated 82 patients with CML, resistant/intolerant to Imatinib and treated with Dasatinib or Nilotinib, then switched to a third- line TKI after treatment failure; 34 patients were treated with dasatinib after imatinib/nilotinib failure and 48 with nilotinib after imatinib/dasatinib failure. *Results.* A total of 82 patients were treated with sequential TKIs; 62 (75.6%) patients had received interferon-a before starting Imatinib. At the start of nilotinib as second line, 30/34 (88.2%) patients were in CP, 2 in AP, and 2 in BP. 9 patients had developed mutations before starting treatment. In the resistant patients 5 new mutations were identified. At the start of dasatinib as second line, 41/48 (85.4%) patients were in CP, 6 in AP, 1 in BP. 10 patients (20.8%) had developed mutations before starting treatment. In the resistant patients 8 new mutations were identified. At the start of the third TKI, 68/82 (82.9%) patients were in CP, 9 in AP, and 5 in BP. Of these, 9 patients on dasatinib and 8 on nilotinib had mutations before starting treatment. The best response to the third line treatment with TKI was 13 (15.8%) MMR, 14 (17.1%) CCyR, 11 PcyR (13.4%), 6 (7.3%) mCyR, 25 (30.5%) CHR and 13 (15.8%) No Response (NR). In the dasatinib group, 14 (41.2%) patients discontinued treatment because of toxicity versus 24 (50%) patients in the nilotinib group. Two new mutations (F317L, E255V) emerged with dasatinib and two new mutations (Y253H, G250E) emerged with nilotinib as third line therapy. After a median follow up of 14 months, 58 patients are continuing therapy;70 patients (85.3%) are still alive for a median overall survival of 46 months (57 CP, 7 AP, 6 NA). Discussion About one third (28.4%) of patients derived benefit from the use of three sequential TKIs; patients with better response to third TKI were the same patients with a better response to the Imatinib and 2TKIs therapy. In this subset of patients, 6 developed mutations that were sensitive to the sequential treatment. The lack of a durable cytogenetic remission could be explained by the emergence of new kinase domain mutations and a change of therapy resulted in an adequate response. *Conclusions.* Although allogeneic SCT is the treatment of choice in all patients failing 2 TKI, alternative strategies are required for ineligible patients. The use of a third TKI after failure of two previous TKIs induces response in some patients. Longer follow up of a larger series of patients is needed to determine the long term impact of the response.

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GENETIC MECHANISMS OF TYROSINE KINASE INHIBITOR RESISTANCE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background. Additional chromosome abnormalities (ACA), mutations of the BCR-ABL tyrosine kinase domain (TKD) and BCR-ABL splice variants may cause resistance for first- and second-generation tyrosine kinase inhibitors (TKI) in chronic myeloid leukemia (CML) and Philadelphia positive acute lymphoid leukemia (Ph+ ALL). *Aims*. The aim of the study was to investigate three potential resistance mechanisms during first- and second-generation tyrosine TKI treatment in CML and in Ph+ ALL. *Methods.* Karyotyping and BCR-ABL TKD mutation screening were performed in 71 imatinib resistant CML and 6 Ph+ ALL patients. Exon 7 deletion (Δexon7) was screened by sequencing in all patients at the time of TKI failure and by fragment analysis in selected samples of CML patients and healthy controls. 56 out of 77 patients received second generation TKI. *Results.* ACA were present in 30/65 (46%) imatinib resistant patients. In 27/77 (35%) imatinib resistant patients, 15 different BCR-ABL TKD mutations were detected. Mutations were found in 25% (12/47) of chronic-phase, 33% (5/15) of accelerated-phase, 71% (5/7) of blast crisis CML and 100% of ALL patients. In second generation TKI resistance, the spectra of mutations has changed and fewer types of mutations were detected. In nilotinib-resistant patients, Y253H, T315I, F359V/I; in dasatinib-resistant patients, L248M, E279K and T315I mutations were detected. T315I was found more frequently on dasatinib than on imatinib therapy (p=0.005). The presence of ACA predicted shorter survival during first and second generation TKI therapy, while TKD mutations only influenced survival during second generation TKI therapy. The detection rate of Δexon7 was highly dependent on the sensitivity of detection method (23% by sequencing and 70% by fragment analysis) and on the expression levels of ABL or BCR-ABL. Δexon7 was detected more often in samples at diagnosis and resistance compared to samples collected at times of imatinib sensitivity. In addition, Δexon7 was detected on ABL not involved in BCR-ABL translocation in 100% of control and 70% of CML samples. *Summary*. Our report represents a systematic analysis of the clinical outcome and dynamics of BCR-ABL TKD mutations and ACA during sequential TKI therapies. Our results suggest that the frequency and type of TKD mutation depends on disease phase and TKI applied. For patients with imatinib resistance, mutation and ACA screening may play a role in identifying patients with poorer prognosis. Screening for BCR-ABL TKD mutations is recommended in TKI resistance before changing TKI, because the presence of different mutations may influence the selection of TKI and the therapeutic response. ABL Δexon7 is likely to be unrelated to TKI-resistance since it was abundantly detected in imatinib naive CML patients on BCR-ABL and the alternative splicing is independent from BCR-ABL translocation. In case of sequencing, we recommend using a primer annealing in ABL exon 7, because Δexon7 may lead to a sudden deterioration of sequence quality.

0695

EFFICACY OF NILOTINIB VERSUS HIGH-DOSE IMATINIB IN EARLY CP CHRONIC MYELOID LEUKEMIA PATIENTS WHO HAVE SUBOPTIMAL MOLECULAR RESPONSES TO STANDARD-DOSE IMATINIB (RE-NICE MULTICENCENTER STUDY)

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Background. Achievement of major molecular response (MMR) is a significant prognostic factor in CML as it has been shown to be associated with longer duration of complete cytogenetic response (CCyR) and long-term progression-free survival. In IRIS study, patients who achieved both CCyR and MMR showed higher progression-free survival rates, compared to those who had CCyR without MMR and those who did not achieve CCyR. Compared to standard dose of imatinib, higher doses of imatinib are expected to yield higher CCyR and MMR rates, and second-generation tyrosine kinase inhibitor, nilotinib also produces high CCyR and MMR rates in patients with CP CML who are resistant to imatinib. *Aims.* In this study, the efficacy of nilotinib and high-dose imatinib was investigated in suboptimal molecular response patients who received first line imatinib therapy at a daily dose of 400 mg. *Methods.* Early CP CML patients who have achieved CCyR but no MMR after at least 18 months and up to 24 months (≥ 18 to ≤ 24 months) on first line imatinib therapy at a daily dose of 400 mg were enrolled in this clinical trial, and informed consents were obtained prior to participation. In nilotinib arm, patients received oral dose of 400 mg BID (800 mg/day), and patients received 800 mg/day administrated as 400 mg BID in imatinib dose-escalation arm. To assess the drug efficacy, cytogenetics and RQ-PCR analysis were performed at regular intervals, and baseline mutational analysis was conducted for every patient with subsequent mutational analyses performed in patients demonstrating either lack of response or disease progression. Primary endpoint is to evaluate the cumulative MMR rates by 12 months, and secondary endpoints are to evaluate the cumulative CMR rates and time to and duration of MMR and CMR during further 24 month follow-up. Progression-free survival and safety profiles will also be assessed as secondary endpoints. *Results.* A total of 21 patients were randomized into nilotinib arm (n = 10) or imatinib arm (n = 11). With a median follow-up of 6 months (range, 1 - 24 months), all patients have maintained CCyR without progression to advanced disease, and progressive decrease in BCR-ABL transcript levels was observed in all patients. Cumulative MMR rates by 12 months were significantly higher in nilotinib arm compared to imatinib dose-escalation arm (68.90% vs. 22.10%, P = 0.0274), and patients treated with nilotinib also showed faster molecular response rates, with 5 patients achieving MMR within 3 months of nilotinib therapy. (Figure 1) Although toxicity was observed more frequently in imatinib dose-escalation arm, no patient required dose reduction or discontinuation of therapy due to toxicities in both randomized groups. *Conclusions.* These preliminary results demonstrate that BCR-ABL transcript levels in suboptimal molecular responders progressively decrease in both

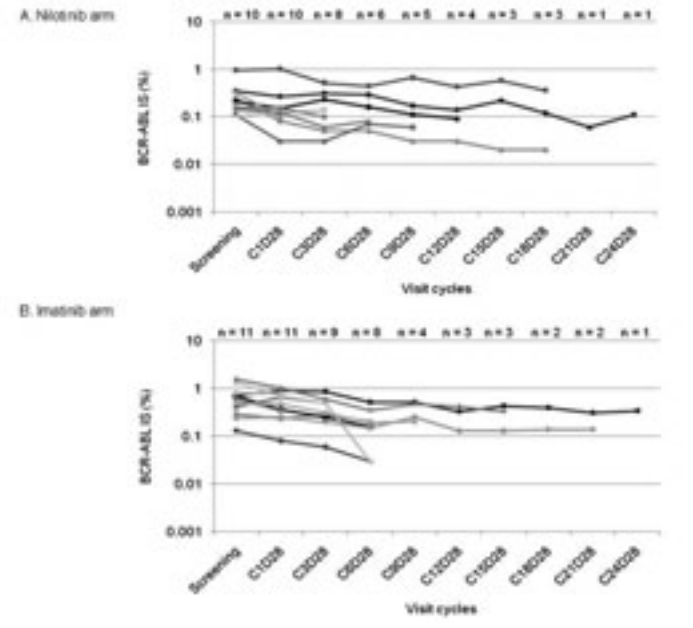

Figure 1. Molecular follow-up in 21 patients.

nilotinib and imatinib arms at a daily dose of 800 mg with higher and faster molecular responses in nilotinib arm. Through further clinical investigation on a large patient population and longer period of observation, the efficacy of early intervention of suboptimal molecular response using nilotinib or dose escalation of imatinib will be assessed.

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OUTCOMES OF CHRONIC MYELOID LEUKEMIA PATIENTS WITH SUBOPTIMAL RESPONSE TO IMATINIB

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Background. Most of the patients with chronic myeloid leukemia (CML) treated with imatinib response favourably. Nevertheless, some patients fail to achieve optimal responses or loose the response. Monitoring endpoints correlate with long-term outcomes, and guidelines have been established to judge response using cytogenetic, polymeresa chain reaction (PCR), and mutation testing. The European Leukemia Net (ELN) guidelines classified patients according to dynamic responses in time. Patients with suboptimal response criteria are a heterogeneous group of patients with different outcomes where the best therapeutic options have not been established. *Aims.* Analyze the evolution of CML patients with suboptimal response critera (ELN), and identify patients with different outcomes. *Patients and Methods.* We studied 80 CML patients in chronic phase treated with imatinib with a median follow up of 66 months. The follow up was done following the ELN recommendations. We have classified the patients in two groups according to the moment when suboptimal response was identified. Group 1: Lack of cytogenetic response at 3 months, less than partial cytogenetic response at 6 months or partial cytogenetic response at 12 months; group 2: included patients with complete cytogenetic response who had not achieved mayor molecular response. *Results.* Responses to treatment in our patients were: failure 16%, suboptimal response 37% and optimal response 47%. We have identified 30 patients with suboptimal criteria at any time during treatment. Of the 30 patients with suboptimal response, 8 (27%) corresponded to group 1 and 22 (73%) to group 2. The evolution of these patients until last follow up or treatment change was: failure 62% vs 9% (p=,007), and achievement of late mayor molecular response 37% vs 54% (p>,005) for group 1 and 2 respectively. We have found no correlation among failure and classical prognostic factors (Sokal-Index, mutations at the TK domain or imatinib plasma levels). *Conclusions.* Suboptimal response criteria fail to identify patients with similar outcomes. In our experience, patients with early suboptimal response (group 1) seem to behave as failure, while a high percentage of patients with late suboptimal response (group2) achieve an optimal response later on.

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CML PATIENTS TREATED WITH TKI'S WHO OBTAIN MAJOR MOLECULAR RESPONSE ARE HIGHLY PROTECTED FOR TRANSFORMATION AND THIS PROTECTION SEEMS TO BE INDEPENDENT OF THE 18-MONTH CUTOFF

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Background. The RELMC Registry is a continuous, 17-hospitals-based cancer registry whose aim is to describe the treatments received by patients with CML in Spain, their outcomes, and the variables that influence treatment choices. *Patients and Methods.* 491 CML patients in chronic phase (CP). Two subgroups according to treatments received: 275 were newly diagnosed and 216 received IM as second line. *Results.* They are depicted in figure 1. Late CP group: 216 patients (M 126/F 90) with a median age of 51 y and Sokal I L*H (47%/43%/10%) and Hasford Index* H (56%/40%/4%). 25 patients received BMT and 145 interferon before IM. 15 patients with transformation before IM. Median follow up from diagnosis and IM treatment were 9,2 and 6,4 years respectively. Patients were treated with different schemes of treatment, always including Imatinib as first TKI (see Table). Major molecular response: MMR was lower in patients who changed to 2nd generation TKI's; Pearson's: 19,4(a)p=0,001. Sokal and Hasford index didn't have influence in MMR. 40 patients achieved MMR before 18 months (median: 11,8 months), 116 after 18 (median: 52) and 40 didn't achieve MMR, with a follow up of 57 months. Transformations and Transformation free survival (TFS) 49 patients (22%) progressed to AP or BC (39) or died of non-CML related causes (10). Sokal and Hasford index influenced TFS (p=0,001), but not treatment schemes (p=0,2). Patients with MMR before 18 months had less transformations or deaths 2/40 (5%), than patients after 18 months 13/116(11%) and patients without MMR 20/40 (50%); Pearson's 38,3;p<0,0001. This variable also influenced TFS. Newly diagnosed group: 275 patients (male 164 female 111) with a median age of 52 y and Sokal L*H (36%/50%/14%) and Hasford Index* H (46%/49%/5%). Median follow up from diagnosis and IM

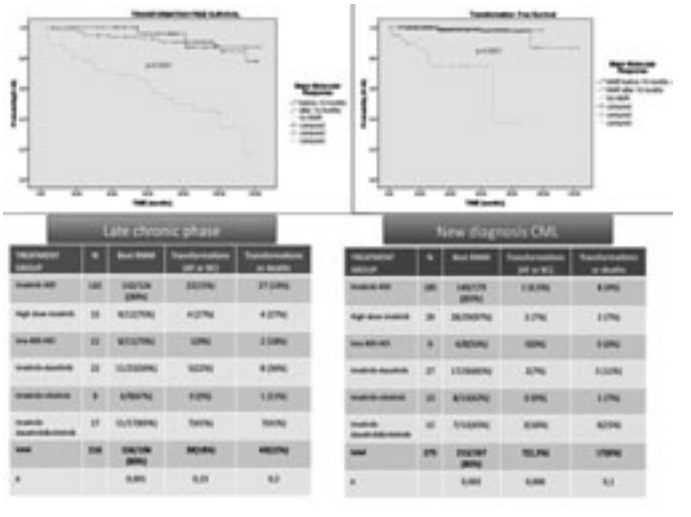

Figure 1.

treatment were 3,8 and 3,7 years respectively. Patients were treated with different schemes of treatment, always including Imatinib as first TKI (see Table). Major molecular response: MMR was lower in patients who changed to 2nd generation TKI's; Pearson's= 20;p=0,001. Hasford Index influenced the rate of MMR (Pearson's: 6,4 p=0,04. 103 patients achieved MMR before 18 months (median: 13 months), 111 after 18 months (median: 40 months) and 56 didn't achieve MMR (follow up: 12,7 months). Transformations and Transformation free survival (TFS) 17 patients (6%) progressed to AP or CB (7) or died of non-CML related causes (10). Sokal and Hasford index influenced TFS (p<0,001), but not treatment groups (p=0,2). Patients with MMR before 18 months had less transformations or deaths 1/102 (1%), than patients after 18 months 3/111 (3%) and patients without MMR 10/56 (18%); Pearson's: 23, p<0,0001. This variable also influenced TFS. *Conclusions.* Imatinib-based regimes produced a high molecular response rate (≈ 80%), especially in patients with high IM dose upfront. Rescue therapy with 2ndG TKI obtained a MMR in the range of 55%. Sokal and Hasford high-risk patients showed worse outcome regardless of treatment chosen. Patients with a MMR were highly protected from transformation to AP or BC regardless of the 18 month cut-off.

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SECOND ATTEMPT OF IMATINIB DISCONTINUATION IN CP-CML PATIENTS WITH A SUSTAINED SECOND COMPLETE MOLECULAR REMISSION

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Background. Imatinib is able to induce sustained responses including complete molecular responses (CMR) in patients with chronic phase chronic myeloid leukemia (CP-CML). Recent results from the STop IMatinib trial suggest that imatinib may be safely discontinued in patients with long-lasting CMR (Mahon *et al.* Lancet Oncol. 2010). Actually, 60 % of patients had a recurrence of the disease i.e a molecular relapse which was defined as positivity of BCR-ABL transcripts in quantitative RT-PCR (sensitivity 4.5log), confirmed by a second analysis point, indicating an increase at two successive assessments. All patients who relapsed responded to reintroduction of imatinib. So, we explored the possibility of a second attempt of imatinib discontinuation for the patients who achieved again a prolonged CMR. *Patients and Methods.* The recommendation was to stop again the treatment using the same criteria, i.e, in patients with a second CMR during at least 1 year after imatinib reintroduction. 15 patients were included in this pilot study. The kinetic of BCR-ABL quantification was identical between the first and the second discontinuation of imatinib. At diagnosis, the Sokal risk group was low, intermediate, high or unknown in respectively 15, 2, 2, and 1 patient. All patients were treated with imatinib 400 mg per day. The median time on imatinib prior to the 1st discontinuation was 49 months (range: 32-105) and the median duration of 1st CMR was 31 months (range: 27-61). The 1st molecular relapse occurred with a median of 2.6 months (range: 0.9-8.4) and a second CMR (CMR2) after imatinib re-challenge was obtained after a median of 5.3 months (0-18.9). *Results.* The median duration of CMR2 was 19 months (range: 3-28). In 10 out of 15 patients (66%) a molecular relapse was observed 2 months in median after imatinib discontinuation (1-14). Among them, 8 were re-treated with a tyrosine kinase inhibitor (imatinib, n=9; dasatinib n=1). 2 patients with a non confirmed molecular relapse remained free of treatment with a follow up of 12 and 15 months respectively. In 5 patients a prolonged CMR2 after the second episode of imatinib discontinuation was observed with a median follow-up of 24 months (6-49). *Conclusions.* It is possible to safely attempt to discontinue imatinib for a second time after a sustained CMR2. However, 66% of our patients experienced a second molecular relapse and restarted TKI therapy. At the other hand, 34% of the patients remained free of treatment with either a non confirmed molecular relapse (13%) or a sustained second CMR (20%). A larger number of patients and with a longer follow up will be presented.

0699

PREGNANCY OUTCOMES IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA RECEIVING TYROSINE KINASE INHIBITORS

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Background. Tyrosine kinase inhibitors (TKI) therapy has improved a survival rate and a life quality for the majority of patients with chronic myeloid leukemia (CML). Therefore pregnancy management in CML patients is one of the important issues. We should be informed about the outcomes and therapy risks on TKI therapy by analyzing the existing data. *Aim and Methods.* We provide an information about pregnancy outcomes in CML patients on TKI therapy (imatinib, nilotinib, dasatinib) collected into the Russian CML Registry and pregnancy cases observed in Hematology Research Center, Moscow. *Results.* The outcomes for 32 pregnancy cases in CML patients on imatinib therapy and about 4 pregnancies on TKI2 (in females and in partners of males) are shown in Table 1. There were 13 pregnancy cases in partners of CML males on imatinib. All of them were in chronic phase (CP), imatinib dose was from 400 to 600 mg daily, no treatment interruption at conception. There were 19 pregnancy cases on imatinib treatment in 17 females. 15 females were in CP CML, 2 in accelerated phase (AP) at diagnosis. Two of them had subsequent pregnancies: the 1st pregnancy ended with medical abortion at the beginning of imatinib treatment and at the 2nd one was planned during complete molecular response (CMR) and resulted in healthy infants delivery in both cases. Imatinib dose was from 400 to 600 mg daily. One female had a treatment interruption at conception. The general approach was to stop imatinib after pregnancy diagnostics. The females in CMR were monitored by Real-time polymerase chain reaction (PCR) for minimal residual disease (MRD) and did not require any therapy. The supportive treatment was used in case of cytogenetic or hematologic relapse (interferon alpha, hydroxyurea, one woman refused from therapy). One woman received imatinib during the whole pregnancy period. After delivery the women restarted imatinib immediately, 2 women continued the treatment interruption for 1 and 3 months correspondently for safe breast-feeding. We also report about 4 pregnancy cases in CML CP patients on TKI2 therapy who were switched to TKI2 due to hematologic relapse on imatinib. 1 pregnancy in the male's partner on nilotinib ended with premature delivery (the child had severe hyperbilirubinemia). 1 pregnancy (ongoing, 22nd week) in female was diagnosed on imatinib+hydroxyurea therapy, then the patient was switched to nilotinib since 10th week of therapy. 2 pregnancies occurred on dasatinib

Table 1. Pregnancy outcomes in CML patients on TKI.

Pregnancy outcome	Therapy				
	Imatinib		Nilotinib		Dasatinib
	Partners of males	Females	Partners of males	Females	Females
Delivery at term, healthy infants	12	9			1
Premature delivery		1 (death)	1 (hyperbilirubinemia)		
Ongoing pregnancy	1	3		1	
Spontaneous abortion		1			
Medical abortion		5			1
Total number of cases	13	19	1	1	2

therapy. For the 1st one a medical abortion was planned. The 2nd one was prolonged: dasatinib was stopped since 6th week and the patient continued on hydroxyurea since the 2nd trimester, a healthy infant was born at term. *Conclusions.* In most cases of pregnancy in CML males partners on imatinib the outcomes were favorable. For CML females on imatinib the disease status at conception was very significant. The lowest risks of safe pregnancy management was observed for patients in CMR. The data about pregnancy outcomes on TKI2 therapy are limited, the current recommendation should be to avoid pregnancy. A careful study of all cases is needed to develop further recommendations.

0700

DASATINIB EFFICACY AND TOLERANCE IN THE TREATMENT OF CHRONIC MYELOID LEUKEMIA (CML) PATIENTS RESISTANT/INTOLERANT TO IMATINIB IN THE CONTEXT OF REAL CLINICAL PRACTICE MANAGEMENT

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Background. Most of the data about dasatinib treatment of CML patients resistant or intolerant to imatinib come from clinical trials. The data from real clinical practice are still scarce. *Aims.* To evaluate efficacy and tolerance of dasatinib as the second line therapy for CML patients resistant/intolerant to imatinib managed in the context of every day clinical practice. *Methods.* We analyzed data about CML patients from the defined region stored in a detailed database INFINITY. Minority of the patients (N=18) were included in the clinical trials (CA180-034, CA180-035). We assessed rates and cumulative incidences of complete hematologic responses (CHR), major (MCgR) and complete cytogenetic responses (CCgR), major molecular responses (MMolR), and a comprehensive set of survivals: overall (OS), transformation-free (TFS), progression-free (PFS), where progression was defined as in the START-R,-A,-B,-L trials (Kantarjian, Blood, 2007; Cortes, Blood, 2007; Guilhot, Blood, 2007), failure-free (FFS), where also loss of CCgR, failure to the second line therapy as provisionally defined by ELN (Baccarani, J Clin Oncol, 2009), and dasatinib discontinuation for toxicity were included, total failure-free (TFFS), where also stop of the treatment for any reason was added; and alternative treatment-free survival (ATFS), reflecting the real proportion of patients remaining on dasatinib despite an event. Among others we evaluated dasatinib toxicity according to CTCAEv.3. *Results*. A total of 98 patients (median age at diagnosis 53.5 years, 19-75; at dasatinib start 57 years, 19-79; 43 males and 55 females) underwent the analysis; 68% were in CP and 32% in AP or BC at the start of dasatinib treatment. Ninety (91.8%) patients were pretreated with imatinib in a median of 24 months (range: 0.2-75). Dasatinib was administered after a median of 39 months (range: 1-175) from the time of CML diagnosis and the median follow-up on dasatinib was 12.9 months (0.2 - 50.8). Reasons for the second line dasatinib therapy were: resistance (77%), intolerance (14%), and others (9%). Estimated cumulative incidences of CHR, MCgR, CCgR and MMolR at 24 months were 92.1%, 76.2%, 66.7% and 58.5% for patients in CP, and 51.6%, 30.8%, 17.4% and 9.7% for patients in AP/BC, respectively. The best response rates for CP and AP/BC patients were as follows: 95.5% and 51.6% of CHR, 52.2% and 12.9% of CCgR, 59.7% and 19.4% of MMolR. Estimated OS, TFS, PFS, FFS, TFFS, and ATFS at 24 months were 90.6%, 92.9%, 90.4%, 66.8%, 63.7% and 66.9% for CP patients and 37.2%, 46.8%, 34.2%, 15.1%, 14.6% and 25.1% for AP/BC patients, respectively. Non-hematological and hematological toxicities of all grades occurred in 63.3% and 85.7% of patients, with 26.4% and 48.4% of cases with grade 3/4 toxicity, respectively. In total, 44 patients permanently discontinued dasatinib therapy for various reasons. Twenty one patients died during the follow-up. *Conclusions*. Dasatinib in second line setting was confirmed as a safe and effective therapy as well as with patients treated generally outside clinical trials at well-managed specialized centers. As for advanced phases of the disease, the results are still not satisfactory.

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0701

DELAYING THE INITIATION OF DASATINIB AFTER IMATINIB FAILURE HAS A NEGATIVE IMPACT ON OUTCOME FOR PATIENTS WITH CP-CML: RESULTS FROM A EUROPEAN OBSERVATIONAL STUDY (FORTE; CA180-211)

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Background. After 6-8 years' follow-up, 45-56% of patients with chronic-phase chronic myeloid leukemia (CP-CML) discontinue first-line imatinib (Hochhaus *et al.* Blood 2009), primarily due to inadequate response and/or intolerance. Interventional studies suggest that earlier and deeper responses to dasatinib correlate with better outcomes. Also, in resistant patients, time from first detection of imatinib failure to initiation of second-line BCR-ABL inhibitor therapy is a significant predictor of response (Milojkovic *et al.* Haematologica 2010). However, very limited data have been gathered, to date, from real-life observation. *Aims.* FORTE, a large, real-life, observational, European study, aimed to estimate the relationship between time elapsed from first detection of imatinib failure until initiation of dasatinib and best response to dasatinib, adjusted for other potentially explanatory factors. *Methods.* Adult patients with CP-CML who had failed imatinib and were treated with dasatinib for ≥2 months were enrolled at 124 sites across 12 European countries. Disease history, response to imatinib, criteria defining imatinib failure and response to dasatinib were collected from patient charts retrospectively and prospectively for up to 6 months. A predefined selection of covariates (gender; age at dasatinib initiation; time from diagnosis to dasatinib initiation; best response to imatinib and last imatinib dose) were entered/removed in a Proportional Odds model to identify factors potentially influencing dasatinib best response over the entire observation period. *Results.* Of 457 eligible patients, 176 (38.5%) were imatinib intolerant and 352 (77%) imatinib resistant, including 71 patients who were both imatinib resistant and intolerant. Approximately half the patients (51.6%) were male. Median age at dasatinib initiation was 57.2 years; median times from diagnosis to imatinib and dasatinib initiation were 2.2 and 45.1 months, respectively, and Sokal and Hasford scores were intermediate/high in 219/306 (71.6%) and 188/268 (70.1%) patients, respectively. Overall, 51.6% of patients evaluated had achieved complete cytogenetic response (CCyR) or major molecular response (MMR) on prior imatinib. Median time from imatinib failure to dasatinib initiation was 8.8 months and 67.6 % of patients received a starting dasatinib dose of 100 mg/day. During the entire observation period, 336/454 patients (74%) achieved CCyR or MMR on dasatinib. An analysis of 443 patients showed a statistically significant effect of time, in months, from imatinib failure to dasatinib initiation on the achievement of a better response to dasatinib ($p<0.023$), with an estimated odds ratio [95% CI] of 0.987 [0.976-0.998] after adjusting for the effect of time from diagnosis to dasatinib initiation and best imatinib response. The odds ratio suggests that with a 6-month delay in starting dasatinib, there would be a 7.5% decrease in the odds of achieving a better dasatinib response (including CCyR/MMR). The corresponding decrease in the odds with a 12-month delay is 14.4%. *Conclusions.* Delaying time to dasatinib initiation has a negative impact on response to dasatinib in CP-CML patients with previous imatinib failure. Results from this observational study are consistent with data from interventional trials in resistant patients and underscore the importance of earlier intervention in patients with CP-CML who have failed imatinib.

Clinical bleeding disorders

0702

QUALITY OF LIFE IN CHILDREN WITH HEMOPHILIA A UNDERGOING PROPHYLAXIS OR EPISODIC THERAPY:RESULTS FROM THE ESPRIT STUDY

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Introduction. Bleeding events lead to joint impairments, arthropathy and chronic pain. Prophylaxis was shown to be the most effective method to prevent bleeding events. The ESPRIT study has investigated whether Health-Related Quality of Life (HR-QoL) was improved in patients treated in prophylaxis compared to those on episodic treatment. To compare HR-QoL in children on prophylaxis VS episodic treatment. *Methods.* ESPRIT study was a multicenter, randomized, comparative, open, trial aimed to evaluate the efficacy of prophylaxis. Forty-five severe hemophilia A patients aged 1-7 years, with no clinical and radiologic signs of joint damage were enrolled and randomized to prophylaxis or to episodic treatment and followed-up for 10 years. Self-reported and proxy-reported HR-QoL was assessed by Haemo-QoL questionnaires at the end of the study. *Results.* The Haemo-QoL score for both treatment groups was 29.9 (SD=9.3), similar to that given by parents (mean 28.90; SD=11.2). Patients and parents reported mainly problems in the dimension friends (mean 56.3; SD=22.2), perceived support (mean 52.92; SD=30.2) and dealing (mean 51.15; SD=33.1). A significant difference was found between episodic treatment and prophylaxis for the dimension family ($p<0.029$), which was more impaired in the episodic treatment group (mean 44.0; SD=22.6) than in prophylaxis group (mean 11.27;SD=8.7). In fact, children on episodic treatment felt often/always more overprotected by their mother (80%) and their father (80%) than those was on prophylaxis (11% by their mother; 20% by their father). Eventualy 20% of patient on episodic treatments perceived that their parents had often or always to limit their time at work or leisure, 10% felt their parents had to limit their work or leisure sometimes, compared to none of the patients on prophylaxis. *Discussion.* HR-QoL was found to be significantly worse in children on episodic treatment in the dimension family compared to those on prophylaxis. Indeed children complained that parents had to limit their work/leisure time to take care of them: probably prophylaxis gives parents definite reassurance, so that they do not have the need to look after them obsessively.

0703

PROPHYLAXIS TREATMENT IN YOUNG SEVERE HEMOPHILIA A PATIENTS: EFFICACY, FVIII CONSUMPTION, TROUGH FVIII LEVELS AND THERAPY COMPLIANCE

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Background. Long-term prophylaxis is the gold standard treatment for severe hemophilia A patients (pts); it is effective in the prevention of hemophilic arthropathy in children and in young pts. The standard prophylaxis regimen consists of the administration of FVIII ~30 IU kg-1, every other day or three times a week, with the aim of maintaining a level of FVIII >1%. Venous access, especially in children, can be a barrier to prophylaxis; thus, different regimens which involve a lower number of venipunctures are under evaluation. *Aims.* To evaluate different prophylaxis regimens in young severe hemophilia A pts, and to compare efficacy, FVIII consumption, trough FVIII levels and patient/family's compliance. *Methods.* Twenty severe hemophilia A pts (≤18 years) started prophylaxis because of either increasing hemorrhages or presence of a target joint. Three prophylaxis regimens were planned: FVIII, 50 IU kg-1 once a week in 2 pts, 50 IU kg-1 twice a week in 12, 30 IU kg-1 thrice a week in 6. The median age of pts at the start of prophylaxis was 6.9 years (1.1-13.5). *Results.* All pts were treated with rFVIII. Actual rFVIII doses were: once a week, 50 IU kg-1; twice a week, mean dose 46.5 IU kg-1; thrice a week, mean dose 37 IU kg-1. The median annual number of hemarthroses/other bleedings pre-prophylaxis was 4 (1-12) and 5 (1-20), respectively. During the last 12 months of prophylaxis, we recorded: hemarthroses, median 0 (0-2), other bleedings, median 1 (0-2). Mean/median values of trough FVIII levels were 1.1% and 0.7% (0.22%-8%), respectively. Values of trough FVIII >1% were recorded in 5 pts (4 under twice, 1 under thrice a week regimen). No significant differences in concentrate consumption were recorded between twice and thrice a week schedules. There was no difference in the orthopedic score before (median 0; 0-2) and during prophylaxis (median 0.5; 0-2). Median follow-up was 12.7 years (2.6-17.3). During prophylaxis, no inhibitor development was recorded; moreover, 4 low-responding inhibitors (titer <5 BU mL-1) which were present before the start of prophylaxis start disappeared. *Conclusions.* Twice a week prophylaxis can be an alternative regimen to the standard one in young severe hemophilia A pts. Indeed, we found no significant differences both in trough FVIII levels and efficacy between twice/week and thrice/week regimens. Moreover, reduction of venipunctures, especially in small children, improves the compliance of pts and their families.

0704

SONOGRAPHY FOR THE ASSESSMENT OF HAEMOPHILIC ARTHROPATHY OF ANKLES AND KNEES - A NEW TOOL IN HAEMOPHILIA CARE

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Introduction. Imaging such as plain film radiography imaging has always been considered an important tool for the evaluation of complications and for therapeutic follow-up of haemophilic arthropathy. Recently, magnetic resonance imaging has been used for its capability of identifying early joint damages. Unfortunately it is expensive, requires time and sedation in small children, limiting its routine and repeated use. Sonography has been increasingly used for evaluation of joint status for its greater feasibility and better sensitivity for synovial changes. *Aims.* To evaluate the clinical utility of sonography in hemophilic patients *Methods.* In order to evaluate the clinical utility of sonography in hemophilic patients, the authors we have retrospectively analyzed medical records of subjects who underwent sonography of at knees and ankles at annual check-up from January 1, 2009 to June 30, 2010. The findings were compared to orthopedic evaluation. *Result.* Overall 325 joints (115 ankles, 210 knees) in 131 patients with haemophilia (aged 6-79 years, median 32 years) were examined: sonography showed abnormalities in 49% of ankles and in 34% of knees. Synovial hypertrophy and cartilage alterations were the most frequently features (96% of abnormal ankles and 86% of abnormal knees, respectively). Prevalence of abnormalities increased with age and defect severity. Only joints of patients on early prophylaxis showed a lower prevalence of changes of patients on delayed prophylaxis or on demand treatment (2/22, 9% vs. 126/303, 42%). Comparison of sonographic and orthopedic findings showed the latter had a lower sensitivity, especially in the evaluation of ankles (18%). *Discussion.* In this series of cases, sonography was able to identify soft tissues changes of ankles and knees unidentified by the clinical evaluation. For its properties of low running costs and practicability represents a very good tool for a better assessment and monitoring. Therefore, its use should be intensified in the routinary management care of patients with haemophilia

0705

REDUCED BONE MINERAL DENSITY IN PATIENTS WITH HEMOPHILIA A AND B IN NORTHERN GREECE

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Background. Hemophilia A and B has been associated with increased prevalence of osteopenia or osteoporosis (67-86%) in a few studies. *Aims.* The aim of this study is to estimate (i) the prevalence of bone disease in hemophilics followed-up in the hemophilia centre of Northern Greece and (ii) its association with hemophilic arthropathy, physical activity as well as with the presence of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections. *Methods.* 104 male patients and 44 age-matched controls were screened. Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry

(DXA) in lumbar spine (LS), femoral neck (FN) and total hip (TH). Severity of arthropathy was estimated with Pettersson scores and Arnold-Hilgartner classification system. For patients >50 years of age, T-score values between -1 and -2.5 standard deviations (SD) were defined as "osteopenia", while values below -2.5 SD were defined as "osteoporosis". For patients <50 years, Z-score of <-2 SD was defined as "below the expected range for age", according to the criteria for the definition of osteoporosis in males by the World Health Organization. *Results.* Ninety-nine patients aged 45.4±15.2 years (85 with hemophilia A, 14 with hemophilia B) were included. Five patients with diseases related to secondary osteoporosis were excluded. Eighteen patients (18%) were suffering from severe hemophilia, 21 (21%) from moderate and 60 (60%) from mild disease. HCV infection was diagnosed in 38 (38%) and HIV in 7 (7%) patients. Low BMD was diagnosed in 26 of 99 patients (26%) being significantly higher than its incidence in controls (20%) (P=0.0001). Seven patients manifested decreased BMD only in LS, 8 only in TH and 11 in both LS and TH. With respect to the severity of haemophilia, decreased BMD was observed in 5 (28%) of those with severe, in 9 (43%) of those with moderate and in 12 (20%) of those with mild disease. With respect to virus infections, 11 (29%) of the HCV and 3 (43%) of the HIV patients manifested decreased BMD. Low BMD was significantly associated with the severity of hemophilia (P=0.0001), the presence of HCV (P=0.026) or HIV (P=0.0001) and the degree of physical activity (P=0.0001), in unadjusted analysis. An inverse association between the degree of arthropathy assessed by Pettersson or Arnold-Hilgartner score was evident only for the knees (P=0.032 and P=0.019, respectively). In multiple regression analysis, after adjusting for age, BMI, HCV, HIV, number of affected joints and risk factors for developing osteoporosis, such as smoking, alcohol, previous fracture, family history of osteoporosis or hip fracture, only the degree of physical activity and the severity of arthropathy in ankles assessed by Pettersson score were associated with low BMD. *Conclusions.* Our study showed a high incidence of decreased BMD in patients with hemophilia, lower than usually reported. The degree of physical activity and the severity or arthropathy are independent risk factors associated with low BMD.

0706

OSTEOPOROSIS IN EGYPTIAN PATIENTS WITH HEMOPHILIC ARTHROPATHY AND ITS CORRELATION WITH SERUM COPPER, MAGNESIUM AND ZINC LEVELS

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Background. Hemophilia is a coagulation disorder characterized by acute hemorrhages into the musculoskeletal system leading eventually to arthropathy and disability. Patients with severe hemophilia are at risk for developing reduced bone density in childhood and adolescence for number of reasons as arthropathy and joint deformities result in prolonged immobilization, reduced physical activity and predispose them for osteoporosis. This can lead to an increasing tendency of bone fragility and fractures in patients after trivial trauma. Osteoporosis is a multifactorial disease with particular considerations to calcium, magnesium and other trace elements as copper and zinc. *Objective.* To find out the presence of osteoporosis in patients with hemophilic arthropathy and its relation to serum levels of trace minerals as zinc, copper and Magnesium. *Methodology.* Twenty male patients with hemophilia A and twenty healthy age and sex matched controls were enrolled in the study. Evaluation was carried out clinically, functionally and radiologically. The lower limb joint score of ankles and knees was done according to world Federation of Hemophilia. Juvenile arthritis functional assessment report scale was used. Plain x ray on both knees with bone densitometry (DEXA) were done at femoral neck and lumbar spine (L1 - L4) for all patients and controls. Laboratory investigations included Hb, complete liver and kidney function tests, serological screening for Hbs Ag and HCV. Serum Calcium, phosphorus and alkaline phosphatase to rule out metabolic bone disorders. Determination of magnesium, copper and zinc levels in serum were done by using the flawless atomic absorption spectrometry. *Results.* Severity of osteoporosis assessed by DEXA revealed highly significant lower T and Z scores of lumbar spine and neck of femur in hemophlic arthropathy patients versus controls ($p < 0.001$). T score of neck of femur correlated negatively with total joint score ($r = - 0.46$, $p = 0.03$), functional assessment score ($r = - 0,45$, $p = 0.04$) and total xray score ($r = - 0.46$, $p = 0.03$). There was no significant difference in either T or Z score of lumbar spine and neck of femur between patients with or without hepatitis C virus ($p > 0.05$). In hemophilic arthropathy patients, a highly significant decrease was found in serum levels of Mg, Cu and Zn compared to controls ($p < 0.001$) while there was no statistically significant difference as regards serum calcium levels ($p > 0.05$). Also, serum levels of Cu and Zn correlated positively with Z score of neck of femur ($r = 0.61$, $p = 0.004$ and $r = 0.83$, $p = 0.001$ respectively). On the other hand, there was no significant correlation between serum levels of either calcium or magnesium and the severity of osteoposis as measured by T and Z scores ($p > 0.05$). *Conclusion.* Osteoposis represents a frequent concomitant observation in patients with hemophilia which can complicate management of these patients. Screening of young haemophiliacs for reduced bone density is recommended with measuring the serum levels of trace elements as Zn, Cu and Mg for better assessment of the disease.

0707

A REVIEW OF PCC PROPHYLAXIS IN SEVERE FACTOR X DEFICIENCY IN THE REPUBLIC OF IRELAND

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Severe Factor X (FX) deficiency is a rare autosomal recessive bleeding disorder. Patients with FX:C level of <0.01-0.03 iu/ml have a severe bleeding phenotype with haemarthrosis occurring in 69% of patients and intracranial haemorrhage accounting for 15% of all bleeding events. Prophylactic FX replacement is challenging because there is no purified FX concentrate available. Fresh frozen plasma, solvent detergent plasma and prothrombin complex concentrate (PCC) contain FX but there are concerns regarding possible thrombogenicity. Seven patients aged 7-303 months old with severe FX deficiency are treated with PCC prophylaxis. Six are born to consanguineous parents. One patient was diagnosed by cord blood FX level while five presented with bleeding episodes; 2/5 with gastrointestinal bleeding at day 1 and day 3 of life, 1/5 presented with umbilical stump bleeding at day 4 of life and 2/5 with intracranial haemorrhage at day 1 of life. The seventh patient is from a non-consanguineous relationship and presented with epistaxis at day 3 of life. All seven patients commenced prophylactic FX replacement therapy with PCC in the first week of life. Dosing regimens range from 25 - 60 IU per kilogram once to twice weekly. 5/7 patients have had central venous access devices to facilitate the administration of PCC. Two patients have had line related thrombosis. 1/7 has Spastic Diplegia secondary to the intracranial bleed at day 1 of life and 2/7 have required extra PCC to treat bleeding episodes secondary to traumatic events. There have been no spontaneous life or function threatening bleeding episodes while on prophylaxis. We conclude that prophylaxis with PCC reduces the number and severity of bleeding episodes a population with severe FX deficiency.

0708

MANAGEMENT OF MENARCHE AND JUVENILE MENORRHAGIA IN GIRLS WITH VON WILLEBRAND DISEASE AND CONGENITAL SEVERE FACTOR VII DEFICIENCY

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Background. Women with hereditary bleeding disorders are compromised in their lives with many situations increasing demands on hemostasis. Menarche may be the first occasion for the manifestation of the mild bleeding disorder, however, in girls with severe coagulopathy it may lead even to a life-threatening bleeding. Menorrhagia is a well-known complication of childbearing age in patients with von Willebrand disease (vWD), however, in other bleeding disorders this problem is often underestimated. *Aim.* We report on the management of menarche and juvenile menorrhagia in patients with severe congenital bleeding disorders. *Methods*. Girls with bleeding dosorders and menorrhagia are managed by hematologist and pediatric gynecologist in the comprehensive hemophilia care centres. Pictorial blood assessement chart is used for assessing the intensity of menstrual bleeding. *Results.* Eight girls with severe congenital bleeding disorders (age 11-13 years) were referred to our haemophilia centre with heavy menstrual bleeding despite factor replacement therapy started in regional hospitals: four girls suffered from severe FVII deficiency (FVII<

1%), three had type 3 von Willebrand disease (vWD) and one severe vWD type 2A. Abnormal bleeding occured in 6 and 2 patients at menarche and during the second menstrual cycle, respectively. The median duration of the first abnormal menstruation was 12 days (7-30 days). In four patients severe bleeding resulted in severe posthemorrhagic anemia (Hbg 5,7-9,0 g/dL), requiring prolonged hospitalization and administration of 2-30 RBC transfusions. Mean treatment duration of the first menorrhagia with Haemate P and Factor VII concentrate or r-FVIIa was 15 days (range 2-25) and 8.5 days (range 2-22), respectively. Subsequent abnormal menstrual cycles despite the use of prophylactic replacement led to the start of hormonal treatment with gestagens in all the girls after a median of the first 3 (2-6) cycles. Prophylaxis with factor concentrates and antifibrinolytics during menstruation were used for additional 4-8 cycles, then the stabilization of menses with hormons was achieved. Later on monophasic combined oral contraceptives were used with no further need for replacement therapy. The median of PBAC score is 68; range 56-108 (normal <80), hemoglobin 12.3; range 10.6-13.2 g/dL (normal limit 12.0-13.5g/dL) and serum ferritin 25, range 10-40 µg/L (normal limit 15-150µg/L), respectively. *Conclusion.* The course of the first menses is similar in patients with severe forms of vWD and FVII deficieny. Despite availability of high effective factor concentrates the juvenile menorrhagia still represent a high risk situation in these girls. The preventive approach and close cooperation of hematologist with patient family and pediatrician-gynecologist are of paramout importance for proper managent of this situation. The control of menstrual bleeding can be succesfuly achieved with hormonal therapy and factor replacement can be avoided.

0709

IMMUNE TOLERANCE WITH PLASMA DERIVED FVIII/VWD CONCENTRATE IN BOYS WITH SEVERE HAEMOPHILIA A AND RESISTANT INHIBITORS

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Background. High titre alloreactive FVIII antibodies remain the most challenging complication of severe haemophilia A in countries with access to FVIII concentrate. There is some evidence that using plasma derived FVIII containing von Willebrand factor (pdFVIII/VWF) to induce immune tolerance (IT) increases the likelihood of success. Most data available includes patients exposed to a variety of different pdFVIII/VWF concentrates. *Aims.* We present 5 boys (aged 7-15 years) with high titre inhibitors who have failed at least one attempt at IT with recombinant FVIII (rFVIII) and were then switched to pdFVIII/VWF with a single concentrate (Fanhdi, Grifols). *Methods.* We carried out a retrospective case note review on our cohort as part of a national data collection exercise which is ongoing. The parents and where appropriate the patients gave informed consent. Data collected included, IT regimen, ethnicity, FVIII mutation, Bethesda titres, number of bleeds pre and post switching to Fanhdi and concurrent use of any immune suppression. *Results.* Three of the boys are Black African and 2 are Caucasian. They all had historical inhibitor titres of >10BU but at the time of switching to Fanhdi 3 of them had inhibitor titres of <5BU. Two boys had had 25-46 months of high dose rFVIII IT prior to switching, 2 boys had failed a previous IT attempt at another centre and were on bypassing agents and the fifth boy had had a relapsed inhibitor. Four boys were given 100iu/kg bd or 200iu/kg od of FVIII and 1 had 80iu/kg 3 times a week. Four of the boys received a total of 6 courses of rituximab (375mg/m2 x 4) and 3 of the boys had mycophenolate mofatil (10mg/kg bd) for 5-12 months (both off-label uses). The mean inhibitor titre pre Fanhdi IT was 18.2BU and the mean most recent inhibitor titre was 2.4BU. The mean follow-up is 39 months. Four of the boys continue on daily or alternate daily treatment with Fanhdi with measurable FVIII levels at 24 or 48 hours and a gradually decreasing FVIII dose in 3 boys, but only 2 have a inhibitor level of <0.5BU currently (achieved at 31 and 32 months after switching). One boy has stopped IT and is now on daily or alternate daily prophylaxis with an activated prothrombin complex concentrate. None of the patients had any complications from immune suppression. *Summary.* In this small cohort attempting IT with a single PDFVIII/VWF concentrate we found that all the subjects had a reduction in their inhibitor titre after switching from rFVIII IT and that they had few bleeding problems and were able to attend school and partake in most normal activities. The most rapid reductions in inhibitor titre were observed in the 2 boys who started IT with Fanhdi at the same time as having a course of rituximab.

0710

SAFETY AND EFFICACY OF RALTEGRAVIR IN PATIENTS WITH HAEMOPHILIA AND ANTI-HIV MULTIDRUG RESISTANCE

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Background. The treatment of patients with HIV infection and congenital bleeding disorders poses specific questions on its safety and efficacy because of presence of long duration of HIV infection, antiretroviral drug multi-resistance due to previous sequential mono-/dual-therapy before the advent of highly active antiretroviral therapy (HAART), and concomitant HCV-/HBV- related chronic liver disease. Raltegravir (Isentress, Merck, NJ, USA), the first approved inhibitor of HIV integrase, is a recently approved drug licensed initially for patients with multidrug resistance. *Aim.* We retrospectively assessed safety and efficacy of raltegravir in patients with hemophilia A or B, HIV infection, multidrug resistance, on treatment with raltegravir for at least three months. *Methods.* For safety evaluation, we checked for clinical adverse events, clinical course, blood tests, CD4 lymphocyte subsets, plasma viral load and overall consumption of coagulation factors, in order to recognize a higher bleeding tendency and/or resistance to replacement therapy. Effectiveness criteria were disease progression, viral load decrease <5,000 HIV-RNA copies/ml, increased or stable CD4 lymphocyte count above 200 cells/µl. *Results.* Six patients, all with HCV coinfection (3 also with HBV infection) had been treated with raltegravir for overall 111 months (median 20 months, min-max: 5-29 months). One patient had suffered from an AIDS-defining disease (Pneumocystis jiroveci pneumonia), 2 patients had CD4 count <200 cells/µl. The median viral load was 8,785.5 copies/ml (min-max <40-37,807 copies/ml). No new opportunistic infections or AIDS-related cancers occurred. All patients showed suppression of viral replication (<40 copies/ml). The CD4 cell count showed a median increase of 137.5 cells/ml (min-max: 27-458/µl). No adverse events occurred. There was no evidence of increased bleeding frequency, modification of bleeding sites, lack of response to replacement therapy. *Conclusions.* HAART-containing raltegravir appeared to be effective and well tolerated in patients with haemophilia with multidrug resistance and it might represent an useful option in these patients

0711

VENOUS THROMBOEMBOLIC COMPLICATIONS IN PATIENTS WITH SEVERE COAGULOPATHY UNDERGOING MAJOR SURGERY

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The risk management for venous thromboembolism (VTE) is still unsatisfactory in patients with severe coagulopathy undergoing major surgery that requires peri-operative replacement of lacking coagulation factor. In fact, information about the incidence of proven VTE is lacking in this setting. Some patients receive pharmacological antithrombotic prophylaxis but the efficacy and safety of such approach is doubtful; moreover, when applied, the dosage and duration of anticoagulant prophylaxis is controversial. The first step to evaluate whether patients with severe coagulopathy may need antithrombotic prophylaxis is to evaluate the incidence of post-operative VTE. For this purpose, we conducted, among four centres in Italy (Palermo, Milano, Castelfranco Veneto and Parma), a prospective investigation to objectively evaluate the occurrence of proximal deep vein thrombosis (DVT) and or pulmonary embolism (PE) in consecutive patients with severe coagulopathy planned for major surgery. During the period 2003-2007, 47 patients with congenital coagulopathy who underwent major surgery were evaluated in the post-operative period for symptomatic DVT or PE and asymtpomatic DVT, detected by compression ultrasonography (C-US) of the lower limbs. C-US was performed 10 _ 3 days after surgery (short-term follow-up); the clinical surveillance was conducted for the entire period of 3 months after surgery (long-term follow-up). None of the patients received pharmacological antithrombotic prophylaxis pre or post-operatively; mechanical prophylaxis (with compression graduated stocking) was applied in most of them

Table 1. Events.

Intervention	C-CUS basal	C-CUS post	DVT
Major surgery (n=43)	42/43	41/43	none
Minor surgery (n=4)	4/4	4/4	none
Invasive procedures (n=2)	2/2	2/2	none
Total (n=49)	48/49	47/49	0

(41/47, 87.2%) in the post-operative period. During the shortterm followup (10 _ 3 days) or during the long term clinical surveillance (3 months) no symptomatic or C-US detected thromboembolic events were registered (0/47, 0% 95 CI-0.7-0.7). In conclusion, in patients with severe coagulopathy undergoing major surgery requiring replacement therapy, the incidence of proven DVT in the immediate or delayed followup is low, < 1%; in these patients, mechanical antithrombotic prophylaxis seems to be an effective and safe approach.

0712

COMPARISON OF ATTITUDES OF EUROPEAN HAEMOPHILIA CLINICIANS TOWARDS SPORTS ACTIVITIES IN PATIENTS WITH HAEMOPHILIA

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Background. Until the '70s sport activities were not recommended for patients with haemophilia (PWH) due to risks of muscle and joint bleeds. Although sport activities are considered beneficial for physical health, motoric coordination and psychological equilibrium, there are still several obstacles towards sport activities such as worries of the family and limited knowledge of potential benefits. Therefore three surveys into the assessment of attitudes of haemophilia specialists towards sports activities in PWHs were conducted in Italy, Germany and the UK. *Methods.* All haemophilia centres/treaters in Italy (n=49), Germany (n=70) and he UK (n=73) received a questionnaire via email/mail containing the following information: physician's sociodemographic characteristics, information about the haemophilia centre (number of patients, experience in the field, and cooperation with other disciplines) attitudes towards sports for haemophilic children (recommendation of sports, frequency, sport competition and specific circumstances) and own sports activities. *Results.* The questionnaire was compiled by 47 centres in Italy (96%), 35 centres in Germany (50%) and 26 centres in the UK (36%). Most of the British haemophilia centres collaborated with a physiotherapist (83%), followed by German (75%) and Italian centres (62%). In Germany one third collaborated with a sports physician, but only one fifth in Italy, while in the UK only one centre did so. In general physicians recommended sports activities for PWH twice a week. Different recommendations were found among countries: a 100% consensus on recommended and not recommend sport activities was achieved only for few sport categories such as swimming, gymnastics and rugby. *Conclusions.* Attitudes of haemophilia specialists towards sports activities are quite important, since physicians are influential on the decisions of parents. Physicians should support parents to let their children develop normally.

0713

EFFICACY AND SAFETY OF ANTITHROMBOTIC PROPHYLACTIC THERAPY WITH LOW-MOLECULAR WEIGHT HEPARIN (LMWH) AFTER ORTHOPEDIC SURGERY IN HEMOPHILIA PATIENTS

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Background. Deep venous thrombosis (DVT) is a common post-operative complication in normal subjects undergoing orthopedic surgery of lower limbs. Therefore, thromboprophylaxis with LMWH or other antithrombotic agents is strongly recommended. On the contrary, LMWH thromboprophylaxis after orthopedic surgery is still controversial in hemophilia patients (pts). *Aims.* To evaluate the efficacy and safety of post-operative anti-thrombotic therapy with LMWH in severe or moderate hemophilia A and B pts, treated with factor concentrates, undergoing orthopedic surgery of lower limbs. *Methods.* Twelve pts [severe/moderate hemophilia A: 9/1; severe/moderate hemophilia B: 1/1; median age 36 years (30-60)], underwent orthopedic surgery because of a significant hemophilic arthropathy. The following surgical interventions were performed: 3 knee replacements, 3 ankle replacements, 3 knee synovectomies, 2 ankle synovectomies, 1 femur fracture reduction. Recombinant FVIII or FIX were administered as bolus infusions at the following dosages: 1) joint replacement: 1 hour before surgery (t0): 100 IU Kg-1; from the 12th to 60th hour (t12hàt60h) after surgery: 50 IU Kg-1 every 12 hrs; from the 3rd to 7th post-operative day: 40 IU Kg-1, 2 bolus day-1; 2) synovectomy: t0: 80 IU Kg-1; t12hàt60h: 40 IU Kg-1, 1 bolus every 12 hrs; from the 3rd to 7th post-operative day: 25 IU Kg-1, 2 bolus day-1; 3) femur fracture reduction: t0: 100 IU Kg-1; from the 12th hour to the 7th post-operative day: 50 IU Kg-1, 1 bolus every 12 hrs, followed by tapering. All patients received post-operatively prophylactic antithrombotic therapy with enoxaparin at a dosage of 50 IU kg-1 day-1; the first administration of LMWH was performed at the 12th hour after surgery and was continued until complete mobilization of the pts (5-7 post-operative days); in case of femur fracture reduction, enoxaparin was given until plaster cast removal, 4 weeks after surgery. *Results.* Twelve severe/moderate hemophilia A/B patients underwent orthopedic surgery. They were prophylactically treated either with factor concentrates or with LMWH, and no bleeding or thrombotic complications were recorded. *Conclusions.* Adequate pre- and post-operative replacement therapy, enables the use of LMWH given at lower doses than the standard ones. Hence, thromboprophylaxis with low doses of LMWH is effective and safe in hemophilia pts.

0714

MANAGEMENT OF DENTAL EXTRACTIONS IN EGYPTIAN CHILDREN WITH BLEEDING DISORDERS OPTIMIZING THE USE OF TRANEXAMIC ACID: A SINGLE CENTER STUDY

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Background. Most of the reports in the literature deal with management of dental extractions usually of adults and rarely of children with haemophilia A and B of variable severity whilst few studies discussed patients with von Willebrand Disease (VWD), rare coagulation disorders and platelet disorders.Replacement therapy remains the mainstay of management inspite of its hazards. The use of tranexamic acid, systemically and locally, as a single not adjunctive therapy to reduce the incidence of postoperative bleeding in these disorders is a subject of debate and large studies are not available till now evaluating its use as opposed to replacement and local measures with none conducted in Egypt. *Aim.* To optimize the use of tranexamic acid pre and post dental extraction in Egyptian children with bleeding disorders and so minimizing the use of replacement therapy. *Methods.* One hundred patients with bleeding disorders who required dental extractions were recruited from the paediatric haematology and dental outpatient clinics, Cairo University Paediatric Hospital over a 3-year period after a consent was obtained. This included 50 children with platelet disorders [45with Idiopathic thrombocytopenic purpura (ITP), 3 children with Bernard Soulier and 2 with Glanzmann's thrombasthenia]. In patients with ITP, intervention was done with platelet count ≥ 30,000. The study also included 50 children with inherited coagulation disorders [37 children with haemophilia A, 3 children with haemophilia B, 4 with type 1 vWD and one with type 3vWD, one with FX deficiency and 4 with FV deficiency]. The management protocol was individualised according to the severity of the bleeding disorder and dental history. Patients were divided into two groups according to the treatment regimen, group 1(n=83) including patients with mild and some with moderate bleeding tendency receiving only tranexamic acid systemically and then tranexamic acid mouth wash (TAMW) 5% post-extraction and group 2 (n=20)including few with moderate and all severe bleeding disorders receiving replacement therapy to increase the deficient factor to 20 % and then TAMW 5% post-extraction. Follow up was done on day one, three and seven post-extraction Results 103 extractions were done in children with a mean age of 11.2 years. Post-extraction complications were reported in 11/103 (10.7%) [6 children with haemophilia A, 1

child with vWD, 3 patients with ITP and 1 patient with thrombasthenia) but none required hospitalization or extra replacement. The patient group on tranexamic acid showed less frequent bleeding complications as compared to those receiving replacement but the latter had a more severe bleeding tendency. Though the protocol was to use TAMW for a week yet bleeding usually stopped after 2 days and all children found the mouthwash easy to use, palatable and very effective. *Conclusion*. The protocol proposed in this study is feasible allowing haemophiliac children to be treated on an outpatient basis with containment of cost and relatively low number of haemorrhagic complications especially for mild and some moderate bleeding disorders. Further multicenter controlled studies are recommended on a larger scale exclusively on children to standardize guidelines for this unique group of patients.

0715

SUCCESSFUL PREGNANCY AND DELIVERY IN PATIENT WITH CONGENITAL COMBINED DEFICIENCY OF FACTORS V AND VIII ASSOCIATED WITH LUPUS ANTICOAGULANT

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Combined deficiency of coagulation factors V (FV) and VIII (FVIII), known as F5F8D, is an autosomal recessive bleeding disorder characterized by the concomitant reduction in both factors and bleeding symptoms. F5F8D is extremely rare (1:1.000.000) and developed due to mutations in genes lecitin mannose-binding 1 (LMAN1) and multiple coagulation factor deficiency 2 (MCFD2) which encoded proteins involved in the FV and FVIII intracellular transport. Mutation of LMAN1 gene accounts 70% of F5F8D families, whereas mutations in MCFD2 account 30%. In MCFD2 mutations, FV and FVIII are significantly lower than in LMAN1 mutations. F5F8D is characterized by mild bleeding, as the observed FV and FVIII levels are sufficient to prevent major spontaneous bleeding. However, bleeding commonly follow surgery, dental extraction and trauma. Treatment of bleeding requires substitution with FV (FFP) and FVIII (desmopressin or FVIII concentrates). Recently, recombinant activated factor VII (rFVIIa) has been used for the treatment of bleeding in F5F8D. The aim of our study is to present clinical, molecular features and treatment of F5F8D associated with strong lupus anticoagulant (LA) during pregnancy and delivery. A 28-year-old pregnant woman had history of abnormal bleeding after tooth extraction. The family bleeding history was unremarkable for abnormal bleeding. Results of prothombin time (33%) and APTT (123 s) were prolonged. Laboratory assays showed decrease of FV:C 5%, FV:Ag 2%, FVIII:C 4%, FIX:C 28%, FXI:C 41%, and FXII:C 30%. Strong LA (1.8-2.1), anticardiolipin (IgM 35.5) but no beta 2 glikoprotein I antibodies were detected. Molecular diagnosis indentified homozygous mutation c.149+5 G/A in MCDF2 gene. Her mother (FV:C 105%, FV:Ag 100%, FVIII:C 109%) and father (FV:C 75%, FV:Ag 97%, FVIII:C 50%) were heterozygous for the same mutation. A diagnosis of F5F8D with LA were established 5 years before. During the pregnancy levels of FV:C and FVIII:C were low but she did not have a bleeding, and LA was elevated but results of D-dimer were normal. Patient underwent delivery after administration of FFP (10 ml/kg) and FVIII concentrates (30 U/kg) with increment of FV:C to 14%, FVIII:C to 17% and LA 1.55. However, when FV, FVIII and LA were measured in platelet reach plasma (PRP) before and after platelets lysis, by frozen and heating, level of FV:C increased from 36% to 74% and FVIII:C from 46% to 66%. We supposed that phospholipids released from platelets neutralised LA (from 1.39 to 0.68) and factors increased on real levels. Patient was successfuly delivered (female, 2750 g, Apgar score 9) without bleeding complications and thrombotic events. She has been substituted on same maner two days after delivery and then doses were reduced for 50% next 7 days. Prothrombin time was prolonged at newborn, and after the treatment with vitamin K it has been normalised. To our knowledge this is the first patient with F5F8D associated with LA who was successfuly delivered without bleeding and thrombotic complications. LA is a risk factor of thrombosis and it was main reasons why she has been substitute with FV and FVIII on lower levels and rFVIIa was not used.

0716

GASTROINTESTINAL HAEMORRHAGE IN PATIENTS WITH BLEEDING DISORDER

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Introduction. In recent years improvements in the treatment for individuals with bleeding disorders in the United Kingdom (UK) means the life span of haemophiliacs and people with severe Von Willebrands disease is similar to that of the UK general population. The emergence of a population of healthy adults with inherited bleeding disorders presents a new challenge for their care. They develop medical and surgical diseases not previously seen in this group. In the UK practice is based on guidance from the UK Haemophilia Doctors Organisation and the World Federation of Haemophilia. Registered hospital centres delivery care; however there is ongoing debate regarding the appropriate clinical setting for the treatment of this patient group. The RCH Haemophilia Centre delivers care to 180 registered patients and based on national guidelines has a policy covering attendances in such patients. *Aims.* To review the presentation, management, investigation and outcome of patients with bleeding disorders that have presented to the RCH with significant gastrointestinal (GI) bleeding between January 2007 and January 2011. *Methods.* Patient's details were obtained from the Haemophilia Centre records. Relevant clinical details were extracted from hospital notes. Details of factor concentrate used were retrieved from the laboratory records. *Results.* There were seven events of GI haemorrhage in six patients with bleeding disorders; 3 moderate haemophiliacs, 1 mild haemophiliac, 1 severe Von Willebrands disease and 1 2A Von Willebrands disease. Duration of symptoms ranged from 24hours to ten days. Three presented with dark blood loss and four with fresh blood loss. Four patients presented out of hours directly to the admissions unit and 2 contacted the haemophilia nurse in working hours. In 6 cases there was documented discussion with on call Haematologist before factor concentrate was administered however in 3 patients there was a delay in administration of factor concentrate. Four patients required blood transfusion. All patients had Factor concentrate; two had continuous infusions and five had bolus therapy. Investigations and management for the GI haemorrhage was in accordance with the RCH guidelines in all but 1. This patient had a barium meal rather than endoscopy. This may have been in part due to a possible risk of vCJD infection and the need to quarantine an endoscope. The diagnoses were varied; 1 haemorrhoids, 2 diverticular disease, 2 colonic polyps, 1 a small bowel bleed and 1had no diagnosis. All patients were discharged when symptoms settled. *Conclusions.* Patients with bleeding disorders do present to other specialities with bleeding unrelated to their original diagnosis. These are often emergencies and there is therefore a need for local management. They should be managed according to established protocols for these conditions along with specialist haematology input. It is essential to clearly communicate the practicalities of factor 8 issue, prescribing and administration to ward staff. Some patients presented late with significant bleeding. This may be because they have not needed recent treatment for their bleeding disorders and/or did not think their bleeding disorder was relevant. Continued patient education is important.

0717

DIRECT COSTS OF CHILDREN WITH HAEMOPHILIA A UNDERGOING PROPHYLAXIS OR EPISODIC TREATMENT: RESULTS FROM THE ESPRIT STUDY.

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Introduction. Bleeding events lead to progressive arthropathy and invalidity. Prophylaxis was shown to be the most effective method to prevent bleeding events and slow down joint destruction. The ESPRIT study has assessed the direct costs of prophylaxis and episodic treatment in children with hemophilia A from the third party payer perspective. *Methods.* ESPRIT study was a multicenter, randomized, comparative, open, trial aimed to evaluate the efficacy of prophylaxis. Forty-five severe hemophilia A patients aged 1-7 years, with no clinical and radiologic signs of joint damage were enrolled and randomized to prophylaxis or to episodic treatment and followed-up for 10 years. Cost analysis was based on the annual FVIII concentrate use. The third party payer perspective was adopted (Italian National Health System). After having quantified the resources absorbed,

costs were expressed in Euro as at 2010. *Results.* Monthly FVIII usage per patient was 8'852 IU (patients on prophylaxis) vs. 3'981 (patients on episodic treatment). Assuming an average price per IU of recombinant FVIII concentrates of 0.75€, yearly cost for prophylaxis was 79'668€ vs. 35'829€ for patients on episodic treatment. The Incremental Cost-Efficacy Ratio (ICER) for bleeding event avoided in patients on prophylaxis was 7'537€ (10'048.6 IU x 0.75€). We calculated the ICER for maintaining all joints pristine over the whole treatment period (mean study time: 82 months): it was 201'601.12€ (28'800.16€ a year). *Discussion.* The cost of prophylaxis was more than the double compared to episodic treatment. ICER per bleed avoided showed the need for a high investment of resource (7'537€). On the other hand, the additional cost for maintaining pristine joints in a child with hemophilia was lower than 30,000 € a year.

0718

BLEEDING PROPHYLAXIS WITH AN ANTI-INHIBITOR COAGULANT COMPLEX (AICC) IN PATIENTS WITH HEMOPHILIA A AND INHIBITORS CAN IMPROVE QUALITY OF LIFE: RESULTS OF THE PRO-FEIBA STUDY

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Background. Patients with hemophilia A and inhibitors are at high risk for severe bleeding and progression of joint disease with consequent deterioration of their health-related quality of life (HRQoL). Prophylaxis with bypassing agents has been suggested as a potential therapeutic option in these patients. *Method.* A prospective, randomized, crossover study (Pro-FEIBA Study) was designed to evaluate safety and efficacy of an anti-inhibitor coagulant complex (AICC) for bleeding prophylaxis in hemophilia A patients >2 years with high-responding inhibitors. The study compared 6 months of AICC prophylactically dosed at 85 U/kg ±15% on 3 nonconsecutive days per week with 6 months of on-demand therapy (target dose: 85 U/kg ±15%). The 2 study periods were separated by a 3-month washout, during which time patients used on-demand therapy. Quality of life in patients >14 years was assessed at the beginning and end of each study period with 2 generic instruments: the Short-Form 36 (SF-36) and the Euro-QoL 5-Dimensions (EQ-5D). *Results.* Twenty-five patients with median age of 30.2 years (range: 16.1-67.9 years) completed at least one QoL questionnaire. Sixteen patients completed both the SF-36 and the EQ-5D at the beginning and end of each study period, 1 patient completed only the SF-36, and 1 patient completed only the EQ-5D. A comparison of the 2 study periods showed a trend towards HRQoL improvement favoring prophylactic therapy. The results reached statistical significance (for conventional levels) for the SF-36 physical component summary score. Specifically, the mean difference in the summary scores before and after episodic treatment was -1.7 (SD 7.9) versus +4.4 (SD 8.4) before and after prophylaxis (p=0.025). *Conclusion.* AICC prophylaxis significantly improved HRQoL as compared with episodic treatment. Larger cohorts and longer follow-up are necessary to confirm these data.

0719

COST OF IMMUNE TOLERANCE INDUCTION IN HEMOPHILIA A PATIENTS: RESULTS FROM THE ITER STUDY

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Background. Although immune tolerance induction (ITI) is generally accepted as first choice treatment to eradicate inhibitors (Inh) in hemophilia A patients (pts), little is known about determinants of outcomes and cost consequences. *Aim and Methods.* The Immune Tolerance and Economics Retrospective (ITER) study is an observational, retrospective, multicentre, multinational study aiming to estimate cost of treatment in hemophilia A pts, undergoing ITI after 1995 with any type of FVIII. Data on hemostatic treatment given in the following time periods were collected: up to 12 months before the diagnosis of Inh, between Inh diagnosis and ITI start, during ITI, and 12 months after the end of ITI. Costs of treatment were calculated in the perspective of the third party payer and expressed as mean €/patient-month. *Results.* 71 valid pts, with median age at ITI start=3.8 (0.4-41) years, were enrolled. Before ITI the median Inh peak titre was 18.5 (0.80-704) BU. ITI was applied for a median of 1.22 (0.1-14.0) years and was successful in 84.5% pts. Before Inh diagnosis, pts cost 670€/patient-month for on-demand or prophylaxis treatment. Cost was 3,188€/patient-month between the Inh diagnosis and ITI start (92.1% for bypassing agents), and 60,078€ during ITI (76.8% for ITI, 19.4% for extra FVIII treatment, 3.8% for extra treatment with bypassing agents). The mean cost after ITI was 13,211€/patient-month. No significant relationship was found between cost during and after ITI and the success rate. Discussion: ITI applied on pts with the characteristics of those involved in the ITER study is successful in 84% of them at a mean cost of 60,000€/patient-month during ITI, plus 13,000€/patient-month through 1 year later. Further research is encouraged to value long term benefits and costs attributable to ITI versus non-ITI, in order to identify the most efficient treatment option for the pts' and for the health care system.

0720

THE IMPACT OF TRANSITION FROM ADOLESCENCE TO ADULTHOOD ON THE HEALTH-RELATED QUALITY OF LIFE OF HAEMOPHILIA PATIENTS (THE HYQOL-EUROPE STUDY)

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Introduction. Despite considerable evidence demonstrating chronic pain, disability and reduced health status in haemophilia patients, little is known about the impact of transitional life events in terms of educational, vocational and relational changes on the disease and its treatment, and patients' health-related quality of life (HRQoL). For adolescents and young adults with haemophilia independence, self-management of treatment and integration in job life are main concerns. The HyQoL-Europe Study aims to evaluate the impact of transitional events on HRQoL. Additionally, key transitional life events like partnership, living and professional situation will be identified. *Methods.* In this prospective, longitudinal, multicenter, non-interventional study ca. 100 patients aged 14-35 years with moderate or severe haemophilia A using Helixate from 7 countries are enrolled. The study is divided into two phases: a recruitment phase of up to 18 months, followed by a data-collection phase of 36 months for each individual patient. The following variables are assessed: socio-demographic characteristics, HRQoL (generic: SF-36, EQ-5D; disease-specific: Haemo-QoL, Haem-A-QoL), sports and physical functioning (HEP-Test-Q, short EPIC Norfolk index), living situation, sexuality, spirituality/religious beliefs, treatment adherence and transitional life events. Clinical data on bleeding history, treatment, orthopaedic status, etc. are collected by physicians. All evaluations will be carried out at baseline and yearly over 3 years. *Results.* In Europe 3 regions were identified capturing different socio-economic, cultural and transitional situations (Regions: I: Germany, Austria, Switzerland, II: France, Belgium, III: Italy, Spain). 44 patients are enrolled up to now in Italy (n=12), Austria (n=10), Germany (n=9), France (n=8), Belgium (n=1), Switzerland (n=3), Spain (n=1). Mean age of patients is 24.2 years (SD=6.2), 68% are living with their parents and 63% are working. 81.8% of the patients are severely affected, 25% receive on-demand treatment, 75% prophylaxis (twice/week). In average they report 6 days off at school/work (range 0-30) due to haemophilia in the past 12 months. Discussion: The HyQoL-Europe Study together with a similar project in Canada is the first study investigating the crucial period of transition in the life of young haemophilia patients. Recognition of specific transitional life events and their impact on HRQoL in adolescents and young adults may allow treaters to provide and/or tailor better quality of medical care, improve support of patients' self management and facilitate a more efficient allocation of resources.

Clinical hemoglobinopathies 2

0721

ASSOCIATION BETWEEN HAEMOLYSIS AND MICROALBUMINURIA IN ADULTS WITH SICKLE CELL ANAEMIA

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Background. Sickle cell nephropathy (SCN) is highly prevalent and potentially life-threatening in sickle cell anaemia (SCA). End stage renal failure is present in around 11% of SCA patients, increasing with age. While significant associations have been found between microalbuminuria and markers of haemolysis in the paediatric population, similar studies in adults have not consistently replicated these findings. *Aims.* To investigate the prevalence of microalbuminuria amongst adults with SCA, and to examine their association with haemolysis. *Method.* This is a retrospective study based on adult patients (> 16 years of age) attending King's College Hospital over a four year period (1st of January 2006 to 31st of December 2010). Steady-state data linked to clinic visits were collected, including haemoglobin (Hb), reticulocyte, bilirubin and lactate dehydrogenase (LDH) levels, serum cystatin C, estimated glomerular filtration rate [eGFR] calculated using modified diet in renal disease [MDRD] and Hoek formulae [using cystatin C]) and urine albumin/creatinine ratio [ACR]. Bilirubin levels were adjusted for the UGTA1 genotype. Data was also collected on sex, age at clinic visit and alpha-thalassaemia status. Patients were excluded from the study group if they were regularly transfused, or had other causes of albuminuria or chronic renal failure. Analysis was limited to patients with Hb SS and Hb Sβ0 who had at least one ACR value. All laboratory test results were treated as continuous variables and were log transformed if appropriate. Random effects regression was used to allow for multiple observations from each individual. Variables were analysed using both simple correlation and controlling for co-variates (sex, age, genotype- including alpha thalassaemia trait, hydroxyurea use and white blood cell count). *Results.* The study group consisted of 207 patients (59% female), mean age 31 years (SD+/- 11, range 16-60). Microalbuminuria (ACR ≥4.5mg/mmol) was present in 97 (47%) of the group. Using simple correlation, ACR correlated significantly with LDH (p<0.0001 95% CI 0.002-0.003), bilirubin (p<0.0001 95%CI 0.500-1.09), reticulocyte count (p=0.024 95%CI 0.00 -0.001), and negatively with Hb (p=0.001 95%CI -0.229 to 0.062). Alpha-globin genotypes were available in 39% patients. Presence of alpha-thalassaemia correlated negatively with ACR (p>0.008 95%CI-0.824"'0.124). After controlling for co-variates the correlation with reticulocytes became non-significant, however all other correlations remained significant; LDH p<0.0001 (95%CI 0.002-0.003), bilirubin p<0.0001 (95%CI 0.431-1.05) and Hb p=0.0006 (95%CI -0.246 to -0.061). eGFR (both MDRD and Hoek methods) correlated positively with reticulocyte count using simple analysis (p<0.0001 95%CI 0.027-0.065 and p<0.018 95%CI 0.003-0.028, respectively) and when controlling for co-variates (p<0.0001 95%CI 0.044-0.084 and p<0.0001 95%CI 0.012-0.040, respectively). Bilirubuin and LDH levels correlate positively with eGFR (p<0.001). *Summary.* Microalbuminuria is a common complication of SCD and correlates strongly with all markers of haemolysis, the first time that this has been shown in an adult population with SCD. Correlation of bilirubin and LDH with eGFR suggests an association of haemolysis with hyperfiltration. Alpha-thalassaemia, known to reduce haemolysis in SCA, has a protective effect against microalbuminuria adding further support to the theory that sickle cell nephropathy is linked to the haemolytic sub-phenotype of SCD.

0722

EFFECT OF (6R)-5,6,7,8-TETRAHYDRO-L-BIOPTERIN ON REAL TIME NITRIC OXIDE AND REACTIVE OXYGEN SPECIES GENERATION IN NEUTROPHILS OF SICKLE CELL DISEASE PATIENTS WITH AND WITHOUT HYDROXYUREA THERAPY

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Background. Sickle cell disease (SCD) is associated with decreased bioavailability of nitric oxide (NO) via the reactive oxygen species (ROS)-mediated consumption of this vasoactive molecule. Decreased bioavailability of NO is mainly due to reduced availability of substrate (L-arginine) and cofactor (6R)-5,6,7,8-tetrahydro-L-biopterin (BH4) of enzyme NO synthase (NOS). The NO donor property of hydroxyurea (HU) effectively participates in treatment of SCD. Administration of hydroxyurea and L-arginine increases NO to greater level than either hydroxyurea or L-arginine treatment alone. Augmenting BH4 levels also has greater efficacy for NO production than L-arginine supplementation alone. However, the effect of BH4 on NO/ROS generation in presence of hydroxyurea remains unexplored. *Aims.* We therefore investigated modulation of real time NO/ROS generation by BH4 in neutrophils of steady state SCD patients and SCD patients on hydroxyurea therapy (SCD-HU), as compared to controls. *Methods.* Neutrophils from controls, SCD and SCD-HU patients were included in study with informed consent. Real time NO/ROS generation was monitored utilizing specific probes (4,5-diaminofluorescein-2-diacetate (DAF-2DA;10 μM) for NO; 2,7-dichlorofluorescein (H2DCF-DA;10 μM) for hydrogen peroxide (H2O2) and dihydroethidium (DHE;10 μM) for superoxide anion (O2-), respectively, using confocal microscopy. To examine effect of BH4 (10 μM), cells were preincubated with BH4 before adding probes and alterations in fluorescence was quantitated by flow cytometry as mean fluorescence intensity (MFI). Preincubations with NOS inhibitor, Nω-nitro-L-arginine-methyl-ester (L-NAME;1mM), ROS scavenger, superoxide dismutase (SOD;100U/ml) were also done and their effect on NO/ROS generation was assessed. *Results.* Flow cytometric studies revealed that NO production was significantly lower in SCD neutrophils as compared to controls (1.54±0.42; 5.31±0.59 MFI, respectively, n=6, p<0.005) but hydroxyurea treatment augmented NO production (3.97±0.17 MFI, n=6, p<0.005) in SCD-HU neutrophils. ROS generation was higher in the neutrophils of SCD patients as compared to neutrophils of SCD-HU patients [84.75±6.71; 62.32±5.85 (H2O2) and 30.17±2.12; 12.53±0.26 (O2-) MFI, respectively, p<0.005]. On pretreatment with BH4, there was significant increase in NO production (6.26 ±0.18; 5.31±0.59 MFI, respectively, p<0.005) and decreased generation of ROS [36.82±3.54; 40.46±6.11 (H2O2) and 13.66±0.45; 16.09±0.82 (O2-) MFI, respectively, p<0.005] in control neutrophils. NO production increased (1.69±0.08; 1.54±0.42 MFI, respectively, p<0.005) with decreased ROS generation [50.85±4.12; 84.75±6.71 (H2O2) and 15.80±0.61; 30.17±2.12 (O2-) MFI, respectively, p<0.005] in presence of exogenous BH4 in neutrophils of SCD patients. Interestingly, exogenous BH4 did not significantly increased NO levels in SCD-HU patients (3.97±0.17; 4.02±0.32 MFI, respectively) but instead increased ROS generation [79.15±6.21; 62.32±5.85 (H2O2) and 14.58±0.75; 12.53±0.26 (O2-) MFI, respectively, p<0.005], possibly suggesting autooxidation of BH4 in these cells. Results were corroborated by confocal microscopy where real time NO/ROS generation was monitored. Addition of NOS inhibitor L-NAME and ROS scavenger, SOD abolished fluorescence of DAF-2DA and DHE respectively, confirming the specificity of the probes. *Conclusions.* Hydroxyurea treatment inceases NO production in SCD patients and might further deplete arginine stores in SCD-HU patients. Exogenous BH4, instead of augmenting NO further, increases ROS generation and possibily suggests BH4 autooxidation in presence of increased superoxide radicals in neutophils of SCD-HU patients. dr.rashmisaini@gmail.com

Table 1. NO/ROS generation in neutrophils of SCD patients.

		Control MFI (n= 6)	SCD PATIENT MFI (n= 6)	SCD-HU PATIENT MFI (n=6)
DAF-2DA Fluorescence (NO)	Basal	5.31 ± 0.59	1.54 ± 0.42	3.97 ± 0.17
	BH4	6.26 ± 0.18	1.69 ± 0.08	4.02 ± 0.32
H2DCF Fluorescence (H2O2)	Basal	40.46 ± 6.11	84.75 ± 6.71	62.32 ± 5.85
	BH4	36.82 ± 3.54	50.85 ± 4.12	79.15 ± 6.21
DHE Fluorescence (02-)	Basal	16.09 ± 0.82	30.17 ± 2.12	12.53 ± 0.26
	BH4	13.66 ± 0.45	15.80 ± 0.61	14.58 ± 0.75

0723

SICKLE CELL DISEASE DATA BASE: NEONATAL SCREENING OR NOT? A BELGIAN EXPERIENCE

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The objective of this study was to determine the efficiency of the neonatal screening (NS) program for Sickle Cell Disease (SCD) which was implemented in 1994 in few maternities and extended in 2003 to all maternities in Brussels. It is a systematic screening performed on liquid cord blood. Affected children are referred to a specialized center. We reviewed 146 medical records of patients with SCD born in Belgium and prospectively followed from the time of their diagnosis in three Brussels' Academic Centers and focused on the subgroup of patients older than 3 years of age at December 31, 2007. They were divided into two groups: the NS and the no NS groups. The incidence of major events (first septicemia, first stroke, first severe anemia, number of hospitalization days from 0 to 3 years, and death) was compared. 55 patients (median age 6.7 year, range: 3-16) were in the NS group and 49 (median age 11.2 year, range: 4-27) were in the no NS group. The median age at diagnosis for the no NS cohort was 1 year (range: 1-6). The follow-up of the NS and no NS cohorts accounted for 301.5 and 473.8 patient-years, respectively. Most of the patients were homozygous for Hb S. Incidence of a first episode of septicemia was similar in both groups (9.1% versus 12.3%). The median age at the time of sepsis was 26 months and 13 months in the NS and no NS groups, respectively. All the patients from the NS group were on penicillin prophylaxis versus 50 % in the no NS group. The main pathogen remained Streptococcus pneumoniae and there were no resistant strain despite regular prophylaxis. Incidence of stroke was 1.8% (1/55) in the NS cohort compared to 8.2% (4/49) in the no NS cohort. There was no difference in the incidence of the first severe anemia or the number of hospitalization days for both groups. Two deaths occurred in the NS cohort in the very early childhood (septicemia in one and acute severe anemia for the other). These deaths were attributable to no compliance to antibio-prophylaxis and poor follow-up. Furthermore these deaths occurred in the very early period after NS has been initiated. No death occurred in the NS group since 12 years probably due to better parents' education and comprehensive care. One death was observed in the no NS group (sudden rupture of cerebral aneurysm). In conclusion, NS program is feasible, safe and appropriate to detect SCD. Although the relative small size of our study and the bias due to unreported early deaths by infection or severe anemia in the no NS group before the diagnosis of SCD has been done, our results are very encouraging: NS delays the age of the first severe infection and might reduce the incidence of early neurological complications. It also underlines the better outcome with improvement of parental education and comprehensive care. These data emphasize the need to continue NS for SCD in Brussels and to extend it to all Belgian maternities.

0724

CO-INHERITED A-THALASSEMIA BLUNTS THE CLINICAL RESPONSE TO HYDROXYCARBAMIDE IN SICKLE CELL DISEASE

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Background. Hydroxycarbamide (HC), a key therapy in the treatment of sickle cell disease (SCD) reduces transfusion requirement and the frequency of acute pain episodes, mainly through fetal hemoglobin (HbF) induction. Although HbF levels are markedly increased in many sickle patients receiving HC therapy, up to 40% of sickle patients do not respond clinically. Various genetic variants have been implicated, including co-existing α-thalassemia (data from our previous retrospective study). As α-thalassemia is present in approximately 30% of SCD patients, any effects on HC response would have clinical implications. In a separate study we showed that plasma cell-free DNA (cfDNA), a marker of tissue damage, is elevated during sickle acute pain and reduced with HC therapy. *Aims.* We performed a larger, prospective study to further investigate the effects of co-inherited α-thalassemia on HC response. We wished to expand on our preliminary findings by collecting data on acute hospital admission and combining this with a comparison of cfDNA levels between patients with SCD alone (SCD) or with co-existing α-thalassemia (α-SCD). *Methods.* Participants: 62 participants were recruited from the specialist hematology clinics at King's College (n=45) and St Thomas' (n=17) hospitals, London. 18 patients had α-thalassemia. All participants gave informed, written consent. 33 patients were already receiving HC therapy, 22 never had HC therapy, 4 commenced HC during the study and 3 were excluded due to intermittent HC therapy or frequent transfusions. Clinical Data: For patients treated with HC, clinical data (hematological indices, hospital admissions, HC dosing) was collected retrospectively for the year prior to starting HC therapy and prospectively throughout the study from 6 months after commencement of HC therapy. "Non HC"patient data was collected prospectively. Blood samples: Blood samples were collected prospectively for cfDNA measurement. Samples were processed using published methods and cfDNA was measured using real-time PCR. *Results.* We compared data for the SCD and α-SCD groups, on and off HC therapy. The α-SCD group showed: significantly higher cfDNA levels, both for patients on HC (p=0.04) and off HC (p=0.04); significantly smaller increases in MCV (p=0.02) and MCH (p=0.01) in response to HC therapy; smaller reduction in the number of days spent in hospital due to SCD pain (p=0.02). *Conclusion.* Clinical response to HC (as measured using MCV, MCH and days spent in hospital due to sickle pain) was significantly attenuated in the SCD patients with co-existing α-thalassemia. We also showed that cfDNA levels (a known marker of trauma and organ damage) were higher in the α-SCD group, both on or off HC therapy. This may be related to reduced hemolysis in α-SCD patients. Co-inherited α-thalassemia, present in a third of all SCD patients, should be taken into consideration both in the clinical and laboratory setting when interpreting HC response.

0725

CLINICAL MANIFESTATION OF SICKLE CELL DISEASE IN THE HBS/HBA CHILD: CAN CRITERIA TO IDENTIFY 'NON HEALTHY' CARRIERS BE ESTABLISHED?

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Background. Sickle-cell trait (SCT) affects more than 300 million people worldwide (100.000 in Italy) and has been considered as a benign carrier state. Recently, evidence of severe clinical manifestations (renal medullary cancer, exercise related deaths, exertional rhabdomyolysis, venous thromboembolic events) associated with the carrier state has emerged, mainly in the adult population. Moreover, it has been reported that adults with SCT can develop conditions that are typical of sickle disease (SCD), such as acute chest syndrome (ACS) and vaso-occlusive pain crises (VOC). Less information is available for SCT children, even if an increase in invasive pneumococcal disease and single case observations of VOC have been reported. *Aims.* To evaluate if clinical features of SCD can develop in SCT children; to determine social, clinical or laboratory predictive factors for precocious development of such complications in SCT children in order to identify a subgroup of SCT carriers that might benefit from close clinical observation or therapeutic options. *Methods.* All SCT children at the Sickle Cell Clinic of the Pediatric Hematology Oncology Unit receive a full blood test and clinical examination once a year. We performed a retrospective analysis of the charts of SCT children evaluated at our Center from January 2000 to December 2010 to identify admissions or reports of clinical events related to SCD. Social, clinical and laboratory variables were evaluated to identify possible predictors of a more severe phenotype of the SCT child. Informed consent was obtained. *Results.* 50/163 patients were HbA/HbS, therefore SCT. 23 M and 27 F. 86% were African, 10% European and 4% South-American. Mean age at diagnosis was 37 months. 7/50 children had been admitted at least once for some cause; 4 of them (4/50, 8%) were admitted for SCD related complications: ACS (1), VOC (2) and haemolytic crisis (1) (Table 1). Moreover, patient n3 (see Table 1) displays frequent VOC that require minor opioid treatment at home. At steady state, children admitted for SCD related complications (Group 1) had higher mean

Table 1.

Diagnosis and treatment for children with SCT admitted due to SCD related complications

	Age	Clinical Manifestation	Treatment
Patient 1	2 years	VOC crisis with Hemolysis	i.v. pain killers, i.v. hydration Top-up Transfusion
Patient 2	6 years	ACS- Necrotizing Pneumonia Pneumococcal Sepsis	O2, Salbutamol, i.v. Antibiotics, Red Cell Exchange Transfusion
Patient 3	6 years	Recurrent VOC requiring hospitalization	i.v. pain killers-morphine, i.v. hydration
Patient 4	2 years	Hemolytic crisis with fever	i.v. hydration, i.v. Antibiotics

WBC (9827/m3 vs 7019; p=0.002), Hb (12 g/dl vs 11; p=0.014), MCV (77 fL vs 70; p=0.002) compared to children never admitted for SCD related complications (Group 2), while percentage of HbS and HbF were similar in the two groups (32.9% vs 32.3%, p=0.73 and 2% vs 5%, p=0.38, respectively). Interestingly, Group 1 children also showed higher FVIII activity (183.6% vs 124.7%, p=0.001) and VWF antigen (171.8% vs 113%, p=0.001), with normal inflammatory markers in both groups. *Conclusions.* A subgroup of SCT children displays clinical manifestations of SCD, even at young age. Several laboratory values seem to be higher in symptomatic SCT children. The expression of a hypercoagulable pattern could represent a risk factor for disease severity in children with SCT. Some coagulation tests might be a non invasive and useful tool to identify a subset of carriers with an increased risk of SCD related complications. These children could deserve a clinical approach similar to that recommended for SCD patients. Prospective and larger studies are needed to confirm our findings. rcolombatti@gmail.com

0726

NEWBORN COHORT WITH SICKLE CELL ANEMIA: ASTHMA INCREASES THE RISK FOR ACUTE CHEST SYNDROME BUT NOT THE RISK FOR THOSE OCCURRING FOLLOWING VASO-OCCLUSIVE CRISES

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Background. Acute chest syndrome (ACS) is a frequent cause of morbidity and a leading cause of death among individuals with sickle-cell-anemia (SCA). ACS is characterized by new pulmonary infiltrates on the chest X-ray with varying degrees of respiratory signs and fever. ACS can have infectious (pneumococcus, mycoplasma…) or noninfectious (vascular thrombosis, fat embolism) causes. Asthma is associated with a risk of frequent ACS. *Aims.* to evaluate if asthma also increases the risk of ACS occurring following vaso-occlusive crises (VOC/ACS). *Methods.* SS/Sb0 patients of the Creteil Newborn Cohort, seen before 3 months of age and followed at the SCA-Center in Créteil (CHIC) from 1986 to 2010, were included in this study (n=286). Alpha/beta genotypes and G6PD activity were determined. Blood parameters were recorded at steady state during the second year of life. VOC requiring hospitalizations, ACS and ACS occurring following VOC (VOC/ACS) were recorded. The latter was ascribed to patients who were not febrile and had a normal chest X-ray at admission for VOC and who subsequently developed ACS with presence of a new pulmonary infiltrate on the chest X-ray. The yearly rates of VOC, ACS and VOC/ACS were calculated for the period before any intensification (transfusion program, hydroxyurea or stem-cell transplantation). *Results.* Out of the 286 SCA-patients (140M, 146F), 29 (10.1%) had a physician-assessed diagnosis of asthma. Alpha-Thal was present in 102/232 (44%) patients; beta-haplotypes, available in 207 patients, were Car/Car in 91 (44%), Ben/Ben in 45 (22%), Sen/Sen in 15 (7%), and others in 56 (27%). G6PD-deficiency was present in 32/249 (12.8%). At baseline, mean (SD) blood parameters were: Hb: 8g/dL (1.2); Ht: 24% (3.9); MCV: 79.1 fL (8.9); HbF: 14.8% (8.4); LDH: 947 UI/L (380) and reticulocytes: 294 (121); WBC: 14.1 (5.1), neutrophils 5.8 (3.2) x 109/L. Median follow-up was 8.2 yr (range 0.2-22), providing an overall of 2638 patient-years during which 1661 VOC, 455 ACS including 158 VOC/ACS, were observed. Intensification (n=165/286) consisted of hydroxyurea (n=87), transfusion program (n=129), geno-identical stem-cell transplantation (n=47), with several patients receiving more than one intensive therapy. During the1765 patients-years before any intensification, the mean rates (events/year of follow-up without intensive therapy) of VOC, ACS and VOC/ACS were 0.87, 0.35 and 0.15, respectively. The KM-estimated cumulative risk of at least 2 ACS episodes by age 8 was 36.2% (95%CI: 32.4-40%) in patients without asthma but 62.3% (95%CI: 52.5-72.1%) in those with asthma (Log-Rank p<0.001). In contrast, the risk of VOC/ACS was not significantly different in patients with or without asthma [39.9% (95%CI: 29.7-50.1%) vs 35.1% (95%CI: 31.4-38.8%)] and (Log-Rank p=0.1). Cox-regression analysis showed that gender, G6PD deficiency, beta-haplotypes and alpha-thal were not risk factors for ACS and VOC/ACS whereas asthma significantly increased the risk of ACS occurrence (HR: 3.6, 95% CI:2.1-6.2,p<0.001) but not that of VOC/ACS (HR: 1.6, 95% CI:0.9-3,p=0.11). *Conclusion.* Results from this newborn cohort confirm that a diagnosis of asthma increases the risk of ACS but show that it does not associate with VOC/ACS, suggesting that only ACS due to infections are implicated.

0727

SERUM FERRITIN AND TOTAL UNITS TRANSFUSED FOR ASSESSING IRON OVERLOAD IN SICKLE CELL DISEASE

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Background. Blood transfusion therapy in sickle cell disease (SCD) is increasing and with this comes the morbidity and mortality associated with the consequent iron overload. Whilst patients on regular blood transfusions have their iron indices monitored regularly, patients receiving "as required" blood transfusions can accrue a substantial iron load unnoticed. Serum ferritin (SF) is widely available but can be unreliable in SCD due to the inflammatory nature of the condition. R2 magnetic resonance imaging (R2MRI) is a recognised non-invasive method of estimating LIC but has limited availability. A simple method of assessing iron load in patients having regular and intermittent transfusions is required. *Aims.* To assess the prevalence of iron overload in patients with SCD with a view to highlighting those for further investigation or chelation. *Method.* This is a retrospective review of patients in our sickle cell database (King's College Hospital [KCH], London) over a 20 year period from 1st January 1990 to 31st January 2011. Adult patients (≥16 years of age) were identified who had steady-state SF levels and accurate transfusion history. Patients of all genotypes and chelation histories were included. LIC was assessed non-invasively by R2MRI (Ferriscan®) in 52 patients and cardiac T2* was performed concurrently in 18 cases. Clinical characteristics obtained were age, frequency of transfusion (regular vs as-required), total top-up units transfused (TUT) and LIC. *Results.* 317/660 patients had received at least one unit of blood. 52/317 patients (mean age 35 years, range 20-62) had SF ≥1000 ng/ml, mean 2550ng/ml (range 1003-16000). R2MRI data was available in 31/52 patients; 30 (97%) of which had LIC ≥2mg/g dry-weight [gDW] (NR 0.7-1.8), mean 21 mg/gDW (range 2.1 - >43). 27/30 (87%) of the patients with increased LIC had received ≥20 TUT (mean 152, range 26-342), transfusions were planned in 13 and "as required" in 14. The mean LIC in this group of 27 patients was 23.4mg/gDW (range 4.4->43), 22 patients had LIC≥7mg/gDW (mean 27.2, range 7.2->43). Transfusions were planned in 18/52 (35%) patients, their mean SF was 3985ng/ml (range 1244-9093) and mean TUT, 181 (range 22-342). 13/18 patients had R2MRI data and all 13 had increased LIC (mean 20.1, range 4.7->43 mg/gDW). Transfusions were sporadic in 34/52 (65%) patients; their mean SF was 2826 ng/ml (range 1003-16000) and mean TUT, 76 (range 12-335). 18/34 patients had R2MRI data of which 17 had LIC ≥2 mg/gDW (mean 21.6, range 2.1 - >43) and 11 had LIC ≥7mg/gdw (mean 31.5 range 8.5->43), none of whom were chelated. Only 6 patients with SF ≥1000ng/ml had received <20 TUT, these were all sporadically transfused; 3 had LIC data (2.1, 2.1 and 2.3mg/gDW, respectively). All cardiac T2*MRIs were within normal limits (mean 34 ms, range 27-43.1). *Summary.* Sporadically transfused patients can become heavily iron overloaded with mean SF and LIC on par with those on transfusion programmes; a combination of ≥20 TUT with SF≥1000ng/ml is suggested as a useful screening test. This audit highlights the need for addressing iron chelation therapy in both sporadically and regularly transfused SCD patients.

0728

REVISITING RISK FACTORS FOR SILENT CEREBRAL INFARCTION IN A COHORT OF LEBANESE PATIENTS WITH SICKLE CELL DISEASE

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Background. Studies from the US and Europe continue to confirm a high rate of silent cerebral infarction (SCI) in children and adults with sickle cell disease (SCD), and highlight several potential risk factors. Data from Middle Eastern countries is scarce. *Aims.* to prospectively evaluate the prevalence and risk factors for SCI in Lebanese patients with SCD. *Methods.* This was a prospective study on a sample of SCD patients attending two comprehensive SCD centers in Lebanon. Patients with history of overt stroke were excluded. All patients underwent brain magnetic resonance imaging (MRI) as part of their annual surveillance. Complete blood counts, hemoglobin electrophoresis, steady-state serum ferritin, bilirubin, and lactate dehydrogenase levels were determined. Patient charts were reviewed for demographics, transfusion and spleen status. Written informed consent was provided by all patients. *Results.* A total of 123 patients (87 SS and 36 SThal) were included in the analysis. The mean age was 14.6 ± 10.3 years (range, 2-51 years), with 73 (59.3%) patients being males. Twenty (16.3%) patients had evidence of silent cerebral infarction (SCI) on brain MRI. Patients with SThal had a lower yet statistically similar frequency of SCI as patients SS (11.1 vs. 19.3%, P=0.274). Patients with SCI were significantly older than patients without SCI (mean age 20.2 ± 10.2 vs. 13.5 ± 10.0 years, P=0.007), but had a similar proportion of males (65% vs. 58.3%, P=0.574). A statistically similar proportion of patients were splenectomized (SCI: 10% vs. no SCI: 26.3%, P=0.118) or had persistent splenomegaly (SCI: 40% vs. no SCI: 43.4%, P=0.777) in both groups. Patients with SCI had significantly lower mean HbF% (11.9 ± 8.3% vs. 17.3 ± 11.8%, P=0.019) and higher mean HbS% (79.9 ± 13.3% vs. 69.3 ± 17.4%, P=0.012) than patients without SCI; but had similar mean total hemoglobin levels (9 ± 1.1 mg/dl vs. 9 ± 1.2 mg/dl, P=0.956). There were no statistically significant differences in mean white blood cell counts, reticulocyte count, total or direct bilirubin, or lactate dehydrogenase levels between the two groups. The median number of total life time transfusions and the median steady-state serum ferritin levels were comparable in both groups (SCI: 17 units vs. no SCI: 10 units, P=0.0311) and (SCI: 258.5 ng/ml vs. no SCI: 245.5 ng/ml, P=0.531). On multivariate logistic regression, a 1-% increase in HbS% was associated with a 1.06 increased odds of having a SCI (95% CI 1.01-1.11) and a 1-year increase is age was associated with a 1.06 increased odds of having a SI (95% CI: 1.01-1.10). *Conclusions.* Silent cerebral infarction in patients with SCD is associated with advancing age and increasing HbS%, but not with anemia, hemolysis, transfusion status or iron overload. SThal patients can have SCI as frequent as patients with SS.

0729

MATERNAL AND FETAL COMPLICATIONS IN SICKLE CELL DISEASE: OUTCOMES FROM A 10 YEAR STUDY AT MOUNT SINAI HOSPITAL IN TORONTO, CANADA

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Background. Sickle cell disease is an inherited recessive hemoglobinopathy affecting nearly 1 in 600 African Americans. The disease is caused either by homozygous substitution of valine for glutamic acid (HbSS), or less commonly compound heterozygous with lysine (HbSC), in the number 6 position of the β globin polypeptide chain causing haemoglobin polymerization and subsequently sickling of the red blood cells in deoxygenated states. This leads to hemolysis-related nitric oxide depletion, anemia and painful vaso-occlusive crises with resultant chronic ischemia-reperfusion injury. While pregnancy was discouraged in the past, recent improvements in patient management, transfusion medicine and neonatal care have led to pregnancy and delivery becoming increasingly safe. As one of the largest hemoglobinopathy centres in North America, we have undertaken the first known study in Canada to examine these outcomes in our population. *Aims.* To identify maternal and fetal complications in pregnant women with Sickle Cell Disease, with reference to the Ontario population. *Methods.* A retrospective study of consecutive deliveries of sickle cell patients (SS, SC, HbS/BetaThal) at Mount Sinai hospital from September 2000 through December 2010 was conducted. Maternal demographics, pregnancy complications including transfusions, mode of delivery, apgar scores and fetal complications were recorded. Patients were identified from the Special Pregnancy Database and the Delivery Databases of Mt. Sinai Hospital. Electronic patient records from the Toronto General Hospital, and paper charts from Mt.Sinai Hospital were reviewed. *Results.* A total of 83 pregnant patients: 71 (86%) HbSS, 10 (12%) HbSC and 2 (2%) HbS/BetaThal was reviewed. All had singleton live birth deliveries with no neonatal mortality. Median maternal age was 27 years (range 17-38) with a median gestational age of 38 weeks (range 26-42). Twenty two women (27%) delivered before the 37th week and 32 women (39%) delivered by Caesarean Section. Pregnancy complications: preterm premature rupture of membranes 4 (5%), pregnancy induced hypertension 5 (6%), gestational diabetes 5 (6%), hospitalizations for pain crisis 22 in 15 patients (18%) (range 1-4), transfusions 15 (18% of pregnancies) acute chest syndrome 2 (2%), pneumonia 3 (4%), urinary tract infection 4 (3 upper and 1 lower; 5%).The mean birth weight was 2838±734 g. Eighteen neonates (22%) had a birth weight< 2.5 kg. Mean apgar scores at 1 and 5 minutes were 7.9±1.9 and 8.8±0.8 respectively. Fetal complications were few: IUGR 3(3.6%), oligohydramnios 2(2%), meconium stained amniotic fluid 23%. There were no fetal malformations. *Summary/Conclusions.* The C-section rate was higher than the 2006/2007 Ontario rate for the general population of 28%, but similar to other sickle cell studies reported in the literature with reported rates of 29-36%. Preterm labour and neonates with a low birth weight(<2.5 kg) are similar to the 25% and 21%, respectively found in the Cooperative Study of Sickle Cell disease but larger than the 2005/ 2006 Ontario rates of 8.6% and 7% respectively. Other pregnancy and fetal complication rates compare favourably to previous reports. Pregnancy in women with Sickle Cell Disease can be managed effectively by an experienced multidisciplinary team including hematologists and high risk pregnancy obstetricians.

0730

CARDIAC, RENAL, AND THROMBOEMBOLIC COMPLICATIONS OF SICKLE CELL TRAIT AND DISEASE

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Background. Many complications of Sickle Cell Trait (SCT) and Sickle Cell Disease (SCD) have been well established, but other potential comorbidities remain controversial. Few large cohort studies have addressed these questions. *Aims.* To examine the frequency of select cardiac, renal, and thromboembolic complications which may be associated with SCT (hemoglobin AS) or SCD (hemoglobin SS) by examining a large cohort of African-American patients with SCT, SCD, and normal hemoglobin, in the Kaiser Permanente Northern California patient databases. *Methods.* Institutional review board approval was obtained according to institutional standards. A retrospective cohort study was conducted of African Americans in the Kaiser Northern California patient databases. Inclusion criteria were all African Americans at least age 18 on December 31, 1996 who were known to have normal hemoglobin, SCT, or SCD. Cohorts were defined through a combination of laboratory data and charted ICD-9 codes. The following outcomes were investigated: renal disease (acute, chronic), coronary artery disease, congestive heart failure, ischemic stroke, and thromboembolism. Outcomes were defined using both chronic conditions registries and charted ICD-9 codes. Preliminary analysis compared 2794 patients with SCT to 282 patients with SCD and 11,609 patients with normal hemoglobin. Relative risks were calculated and adjusted for sex, age-group, hyperlipidemia, obesity, and months of membership during 1997 through 2008. *Results.* The following adjusted relative risks compared to patients with normal hemoglobin were calculated. The relative risk of chronic kidney disease in SCT patients was 1.14 (95% confidence interval [CI] 1.04-1.25) and the relative risk of pulmonary embolism was 1.31 (95% CI 1.03-1.67). There were no other significant differences in the outcomes for SCT patients compared to the AA cohort. The relative risk of acute kidney injury (2.24, 95% CI 1.75-2.86), chronic kidney disease (1.38, 95% CI 1.12-1.69), congestive heart failure (1.46, 95% CI 1.03-1.65), and pulmonary embolism (3.50, 95% CI 2.31-5.31) were significantly higher in patients with SCD compared to controls with normal hemoglobin. Other venous thrombosis/embolic events were not significantly increased in the SCD group. *Summary/Conclusions.* We report a slightly elevated risk of chronic kidney disease and an elevated risk of pulmonary embolism in SCT patients relative to those with normal hemoglobin, however we did not demon-

strate any relative increase in any of the interrogated cardiac outcomes. Patients with SCD demonstrated an increased risk of acute and chronic kidney disease, CHF, and pulmonary embolism.

0731

ELEVATED TRICUSPID REGURGITANT JET VELOCITY IN LEBANESE PATIENTS WITH SICKLE CELL DISEASE IS ASSOCIATED WITH MORE SEVERE DISEASE, FAMILIALY CLUSTERED AND NOT RESPONSIVE TO HYDROXYUREA

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Background. Elevated tricuspid regurgitant jet velocity (TRV), a measure of pulmonary hypertension, is associated with increased morbidity and mortality in adult patients with sickle cell disease (SCD). Its significance in a younger population is unclear. *Aim.* The objective of our study was to find an association between elevated TRV and parameters of disease severity, hemolysis and hydroxyurea (HU). *Methods.* We conducted a review of all patients with SCD referred to our comprehensive care center. We assessed disease severity by the frequency of vaso-occlusive crises, acute chest syndrome, findings on transcranial doppler screening (TCD), in addition to the presence of retinopathy, stroke, splenic sequestration, proteinuria, and avascular necrosis. Elevated TRV was defined as ≥2.5m/s. *Results.* We studied 220 patients with SCD at our institution, 92 (41.8%) patients had a TRV ≥2.5 m/s. Four patients underwent cardiac catheterization, and all of them were found to have an elevated pulmonary artery pressure. The median age was 15 years (range: 3 to 43 years) with a M:F ratio of 1.04. Of the 92 patients, 78 (84.7%) had HbSS, 10 (10.8%) HbSβ+, 3 (3.2%) Sβ0, and one has HbSD. Patients with an elevated TRV were significantly more likely to have at least 3 vaso-occlusive crises per year (42.4%) and recurrent episodes of acute chest syndrome (45.6%) as compared to patients with a TRV<2.5m/s. Seventeen patients (18.5%) had avascular necrosis of at least one joint, four patients (4.3%) had proteinuria, three (3.3%) had retinopathy, and 2 patients (2.1%) had a stroke. Nineteen patients with elevated TRV underwent transcranial Doppler (TCD) screening and all were within normal limits. Fifty-seven patients (62%) had been on hydroxyurea upon presentation with a mean dose of 18.5mg/kg/day. Currently, sixty-four (69.6%) patients are on HU with a mean dose of 21.5 mg /kg/day. Increasing the HU dose by a mean of 3mg/kg/day, did not have any effect on lowering the TRV, but was associated with a decrease in LDH by a mean of 82 IU/L, and a modest mean decrease in total bilirubin (0.3mg/dL), WBC count (2285/cu.mm) and reticulocyte count (1.5%). Hemoglobin and MCV increased by a mean of 0.5g/dL and 8fL respectively. Similar results were seen when using 2.6m/s as a cutpoint. In addition to that, we found that 17 families had 2 or more members with elevated TRV, and patients with a positive family history of high TRV had a higher index of severity for the disease. *Conclusions.* The prevalence of elevated TRV ≥ 2.5 m/s was 42% in our population of patients with SCD. While increasing the hydroxyurea doses did result in a decrease in the hemolysis parameters, it did not seem to lower the TRV. Patients with elevated TRV had severe disease as compared to those with normal TRVs. Familial clustering of elevated TRVs was seen in our highly consanguineous population, which might point to an underlying genetic predisposition for developing pulmonary hypertension.

0732

RENAL IRON LOAD IN SICKLE CELL DISEASE CORRELATES WITH HAEMOLYSIS BUT NOT WITH HEPATIC IRON

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Background. Sickle cell disease (SCD) is characterised by chronic hemolysis and frequent vaso-occlusive episodes eventually leading to end organ damage. Renal impairment affects 11% of patients with sickle cell anaemia, increasing further with age. Both the vaso-occlusive and hemolytic aspects of SCD have been implicated in renal failure, as assessed by glomerular filtration rate (GFR) and microalbuminurea. Additionally, the increasing use of blood transfusion therapy SCD has resulted in a sub-population with secondary iron overload. Studies have shown that, for similar liver iron concentration (LIC), the prevalence of organ damage differs between patients with β-thalassemia and SCD. *Aims.* To investigate the significance and etiology of renal iron accumulation in SCD patients. *Methods.* 41 patients from the specialist hematology clinic at King's College Hospital, London had already had an assessment of hepatic iron loading by spin density projection assisted R2-MRI (FerriScan®) as part of their clinical care programme and/or as part of another study approved by the NHS Research Ethics Committee (REC 05/Q0703/21). Due to the retrospective nature of the study, a waiver was obtained from the REC and informed consent was not required of patients for this study. Renal R2 (R-R2) values were derived from monoexponential fits to kidney image data obtained during LIC assessment in order to assess kidney iron concentration. Clinical data were collected retrospectively for the 2 year period prior to the MRI scan. Thresholds for renal hyperfiltration and microalbuminurea were MDRD eGFR ≥140 ml/min/1.73m^2 and albumin:creatinine ratio ≥4.5 mg/mmol, respectively. *Results.* R-R2 values showed no correlation with LIC. Highly significant correlations were seen between R-R2 and bilirubin ($r=0.62$, $p<0.0001$) and with lactate dehydrogenase levels ($r=0.61$, $p<0.0001$). α-globin genotypes were available in 37 patients; those with co-inherited α-thalassaemia had a lower mean R2 (22.86 ± 1.727 N=12) than those without (28.22 ± 1.924 N=25), but this did not reach significance (p=0.086). R-R2 was significantly higher (p=0.014) in patients with renal hyperfiltration (mean 30.48 ± 2.655) than those without (23.11 ± 1.468). A weak but significant negative correlation was observed between renal R2 and age ($r=-0.35$, $p=0.027$). We note that the renal hyperfiltration group were also significantly younger (p=0.003) than those with normal filtration rates (29.8 ± 2.9 vs. 42.3 ± 2.5 years). However, microalbuminurea showed no relationship with R-R2. *Conclusion.* We showed that renal R2, a surrogate for renal iron load, is not related to liver iron concentration, but correlates strongly with markers of hemolysis. Though not significant, mean renal R2 was noticeably lower in the α-thalassemia group, in keeping with studies showing that reduced hemolysis and delayed onset of renal impairment in SCD patients with α-thalassemia. The relationship between R-R2, hyperfiltration and age could be related to the increased total renal blood flow and increased GFR at a young age falling as nephropathy progresses with age. While hemolysis is a major determinant of renal vasculopathy and impaired renal function, the small sample size do not seem to show any correlation between microalbuminuria and renal R2.

0733

BONE DISEASE IN YOUNG EGYPTIAN PATIENTS WITH BETA-THALASSEMIA MAJOR AND INTERMEDIA: CORRELATION WITH BIOCHEMICAL, HORMONAL AND GENETIC PROFILES

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Background. Bone disease is an important cause of morbidity in beta-thalassaemia major (TM) and thalassemia intermedia(TI) patients. Thalassemia osteopathy results from a variety of genetic and acquired factors. Objectives: to study the magnitude of bone disease in TM and TI patients and to assess the influence of biochemical, hormonal and genetic factors. *Methods.* In a cross sectional study ,95 patients (55 TM ,40 TI), aged 13-30 years (mean 17± 3.8 years) were evaluated for BMD by Dual x-ray absorptiometry for lumbar spine and femoral neck. Anthropometric measures and Tanner stage were assessed. The effects of sex, transfusion/chelation program as well as disease duration were evaluated. Laboratory evaluation included: CBC, serum calcium, phosphorus, serum ferritin level, bone alkaline phosphatase, parathyroid hormone and 25-OH-vitamin D. PCR - RFLP technique was used to analyze VDR gene FokI polymorphism. *Results.* Weight/Height SDS, Height/Age SDS, sitting height SDS were comparable in TM and TI; BMI SDS was lower in TM compared to TI (P=0.02). Hormonal replacement therapy was given to 65% of studied patients. Six TM patients (5%) reported pathological fracture, 61.7% gave history of bone pain (66% TM and 40% TI). BMD was reduced in 90 % of thalassemics: 60% had osteoporosis and 30% had osteopenia. In TM patients spine Z-score (-3.3 ± 1.4) was lower than femoral Z-score (-0.68 ± 1.3, p= 0.000). The spine Z score was lower

in TM compared to TI patients (P=0.039). Negative correlation was found between the age and femur Z-score [P= 0.000], femur T-score [P= 0.000] and spine Z-score [P= 0.03]. Sex was not correlated to BMD. Positive correlation was present between pretransfusion Hb and spine Z score (P<0.05); transfusion frequency and both spine Z score (p<0.000) and Femur T-score (P<0.04). Negative correlation was present between serum ferritin and femur T-score [P= 0.019], femur Z-score [P= 0.03], spine T-score [P= 0.03]. BMI SDS had positive correlation with femur T-score [P= 0.04], spine T-score [P= 0.03] and spine Z-score [P= 0.04]. Positive correlation was found between spine Z score and Height /Age SDS [P= 0.004], sitting Ht SDS [P= 0.03]. 75% had low serum calcium, none was symptomatic. 72.5% had low PTH level and 9% had hypoparathyroidism. None had low 25-OH-vitamin D. Bone alkaline phosphatase was elevated in 95% of patients. BMD was not correlated to serum calcium or PTH levels. Spine Z-score was negatively correlated with bone alkaline phosphatase [P<0.001]. The genotypic frequency of VDR FokI gene was: FF 52.5%, Ff 22.5%, ff 17.5%. BMD femur Z score was highest in FF genotype (p = .041). *Conclusion.* Thalassemia osteopathy is multifatorial but in our community inadequate transfusion and the resulting bone marrow expansion as well as poor chelation resulting in iron toxicity to the osteoblasts are important factors, possibly modified by the VDR gene polymorphism.

0734

RENAL DYSFUNCTION IN CHILDREN AND YOUNG ADULTS WITH B-THALASSEMIA MAJOR

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Backround. Patients suffering from β-thalassemia major have now improved life expectancy due to regular transfusions and chelation therapy, however, chronic complications still develop - some only recently identified. Both tubular and glomerular dysfunction has been reported, although in a relatively limited number of reports. Renal involvement has been attributed to iron overload, chronic anemia, transfusion rate and chelation therapy, mainly to the newer chelator deferasirox. The exact mechanism of renal injury still remains unclear to a large extent. *Aims.* Evaluation of renal involvement in children and young adults with β-thalassemia major and correlation of findings with iron overload and chelation therapy. *Methods.* Thirty three patients on regular transfusion and chelation therapy, aged 4-20 years (mean age 12±4.7 years), participated in the study. Mean pre-transfusional haemoglobin level was 9.4±0.37 g/dl. For analysis purposes, patients were divided into 2 groups based on chelation therapy: group A consisting of 18 patients receiving deferasirox and group B of 15 patients receiving other chelation (3/15 patients receiving deferioxamine, 2/15 patients deferiprone and 10/15 patients combined deferioxamine and deferiprone therapy). The two groups did not differ with regards to age, sex and haemoglobin, but group A presented with lower ferritin values compared to group B (mean 1003±505ng/dl and 1744±592ng/dl, respectively, p=0.001). None of the patients presented additional risk factors for renal involvement. In addition to conventional renal biochemistries, acid-base balance, glomerular filtration based on the Schwartz formula, fractional excretion of sodium (FENa), tubular phosphorus re-absorption and urine calcium and protein were measured. *Results.* All participating patients had normal blood markers of renal function and electrolyte values, as well as normal acid-base balance parameters. Mean glomerular filtration rate was 125±19.9ml/min/1.73m2, mean tubular phosphorus re-absorption 93±1.9% and mean FENa 0.54±0.2%. 75.7% of patients (25/33) presented with increased urine calcium, with a mean value of 6.4±3mg/kgr/24h. 45% of patients (15/33) had an increased urine protein level, with a mean of 146±102 mg/24h. Protein and calcium urine levels did not differ statistically between the two chelation groups (p=0.85 and p=0.77, respectively). With regards to iron overload, there was a negative correlation between ferritin value and urine protein (r=0.454, p=0.09). Finally, a positive correlation was found between protein and calcium urine levels (r=0.304, p=0.085). *Conclusions.* Study results confirm the presence of renal involvement in thalassemia patients, developing at an early age. In addition, the study demonstrates a high risk of proteinuria in patients with lower ferritin values, possibly due to chelation nephrotoxicity developing at lower iron burdens. Although the study does not demonstrate correlation between tubular or glomerular dysfunction with any of the iron chelation methods, further studies are needed in order to arrive at safer conclusions.

0735

EVALUATION OF RENAL INVOLVEMENT IN CHILDREN AND ADOLESCENTS WITH SICKLE/BETA THALASSEMIA OF GREEK ORIGIN

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Background. Sickle cell nephropathy encompasses a large spectrum of abnormalities. However, few studies have assessed parameters of renal involvement in patients of young age with sickle cell disease (SCD), while relative literature in sickle/beta-thalassemia (S/b-thal) patients is even more limited. *Aim.* The aim of the present study was to evaluate the renal function in children and adolescents with sickle/beta-thalassemia of greek origin. *Patients and Methods.* For the purpose of the study 17 S/b-thal patients, aged 3.5 to 18 years (mean 10.97 ± 4.87 years) were evaluated. Mean haemoglobin (Hb) level was 9 ± 0.7 g/dl. None of the patients was recently hospitalized, was on hydroxyurea or a chronic transfusion program, nor presented additional risk factors for renal disease. In addition to conventional renal biochemistries, estimated glomerular filtration rate, serum cystatin C (Cys C), fractional excretion of sodium (FENa), tubular phosphorus re-absorption and urine calcium, protein, microalbumin and β2 microglobulin (β2 MG) were measured. Moreover, renal ultrasound was performed. *Results.* Mean eGFR was 142.2 ± 22.5 ml/min/1.73m2, with approximately half of the patients (8/17) presenting with an eGFR of > 150 ml/min/1.73m2. Mean urine specific gravity was 1011.3 ± 3.9. No patient presented with microscopic hematuria or with hypercalciuria. Biochemical urine analysis revealed normal sodium excretion and phosphate re-absorption. In all patients serum Cys C and urine β2 MG levels were within normal range. However, 29.4% of patients (5/17) demonstrated impaired glomerular function with proteinuria or microalbuminuria (11.8% and 17.6%, respectively). Regression analysis revealed no correlation between age, annual number of vaso-occlusive crisis, Hb, HbF, eGFR, LDH, Cys C or β2 MG levels with the presence of proteinuria or microalbuminuria. Renal ultrasound was normal in all cases. *Conclusions.* Our study revealed a considerably high rate of proteinuria and microalbuminuria in the young S/b-thal group studied. To the best of our knowledge, this is the first study to specifically assess renal involvement in young patients with sickle/beta-thalassemia and of the same ethnic origin. Of interest is the finding of proteinuria and microalbuminuria in patients during their first decade of life. Given that glomerular damage seems to develop early and irrespective of pain rate in S/b-thal patients, it is recommended that regular testing of relative markers is performed in this patient group.

0736

PERCUTANEOUS ENDOSCOPIC GASTROSTOMY FEEDS IMPROVE WEIGHT AND BODY MASS INDEX IN CHILDREN WITH SICKLE CELL DISEASE AND FALTERING GROWTH

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Background. Children with Sickle Cell Disease (SCD) frequently experience malnutrition, faltering growth and delayed puberty. Growth failure in children with SCD is linked to increased nutritional requirements, endocrine dysfunction, metabolic derangement and deficiencies in specific nutrients. It is postulated that haemolysis and increased erythropoietic activity lead to increased protein and nutrient turnover and utilisation. Faltering growth is unusual in children with SCD below 2 years old, becomes more apparent by 8-9 years of age, and is seen most frequently in the pubertal years. Nutritional supplementation, hydroxyurea and long term blood transfusions have been shown to improve growth in children with SCD. *Aims.* This study aims assess the impact of instigating Percutaneous Endoscopic Gastrostomy (PEG) feeds on growth parameters in children with SCD and faltering growth. *Methods.* Children attending the Paediatric Haematology clinic at the Royal London Hospital aged 5-16 with SCD and faltering growth (Body Mass Index (BMI) <9th centile, and/or weight static for 6 months), in whom dietary fortification and nutritional supplementation had failed, had a PEG inserted. An overnight feed regime of 500-1000 mls Fresubin Energy was then instigated over 8-10 hours. Weight and height data was collected at 3 monthly intervals pre- and post- PEG insertion. Weight, height and BMI Z-scores were calculated and change

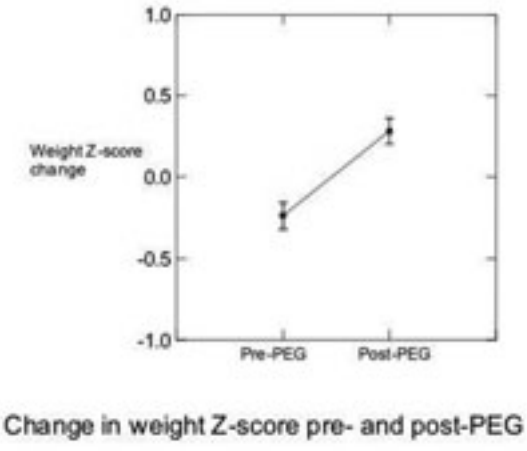

Figure 1.

in Z-scores analysed using SYSTAT 10.2. *Results.* Gastrostomies were inserted in 12 children between November 2008 and February 2011 (9 boys and 3 girls). The median age at PEG insertion was 14 years (range 11-15 years). There was one death prior to completion of the study unrelated to the PEG. Change in weight, height and BMI Z-scores were calculated and compared pre- and post-PEG insertion. Using the ANOVA Estimate Model there was a statistically significant difference in weight Z-score change (P=0.001, see fig. 1) and BMI Z-score change (P=0.01) after commencing PEG feeds. There was a non-significant positive change in height Z-score (P=0.329). *Summary/Conclusion.* Faltering growth and maturational delay are common in children with SCD. This study provides evidence that PEG feeding improves weight and BMI Z-scores in children with SCD and faltering growth. Although height Z-scores did not change significantly this may reflect the lag in linear growth versus weight gain, or the small sample size. Pubertal delay was frequently seen and the majority of subjects demonstrated improved pubertal development. Patient and carer surveys demonstrated high a level of patient and carer satisfaction with PEG feeding. We plan to commence a randomized controlled trial of PEG feeding versus standard nutritional supplementation in children with SCD and faltering growth

0737

LONG-TERM EFFICACY AND SAFETY OF DEFERASIROX IRON CHELATION IN 104 PEDIATRIC PATIENTS WITH TRANSFUSION-DEPENDENT ANEMIAS IN TWO UK INSTITUTIONS

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Background. Children with transfusion-dependent anemias require lifelong iron chelation. Deferasirox, a once-daily oral iron chelator, provides an attractive alternative to subcutaneous chelation. Published sub-analyses demonstrate that, compared with adult patients, children achieve slower dose escalations and have differing pharmacokinetics. This may impact efficacy. These concerns, coupled with the requirement for lifelong usage in pediatric patients, provide compelling reasons to monitor the long-term safety and efficacy of deferasirox in children. *Aims.* To examine the efficacy and safety of deferasirox in 104 pediatric patients with transfusion-dependent anemias in two UK institutions. *Methods.* Data on 104 patients pediatric patients (<16 years) with transfusion-dependent β-thalassemia and sickle cell disease were retrospectively collected in two UK institutions. Data were collected from initiation of deferasirox therapy (up to 54 months). Efficacy was monitored via serum ferritin (SF) and MRI T2*/R2 data. Iron burden was calculated from annual transfusional requirement. Dosage, toxicity and safety were assessed by examination of contemporaneously recorded patient records and measurement of laboratory parameters, including serum creatinine and liver enzyme levels. *Results.* The median age of patients was 9y 1m (range 26m -16y). Disease characteristics were: Sickle cell disease (n=45) and thalassemia (n=59). Annual blood transfusion was 188 ml/kg for β-thalassemia and 121 ml/kg for sickle cell disease. Median duration of evaluation for sickle cell patients was 18 months (range 3-54m). Thalassemic patients were followed for a median duration of 24 months (range 12-39m). The mean starting dose of deferasirox for thalassemic patients was 15mg/kg, with a mean dose of 27mg/kg at final follow-up. In sickle cell disease the mean doses were 19mg/kg and 21 mg/kg respectively. Mean (SD) SF at baseline for thalassemic patients was 2009 ng/ml (770 ng/ml), 2773 ng/ml (967 ng/ml) for sickle cell disease. Mean SF was significantly reduced over time (p=0.003). The change in SF over time was inversely correlated with the dosage of deferasirox for the thalassemia cohort (Fig. 1).

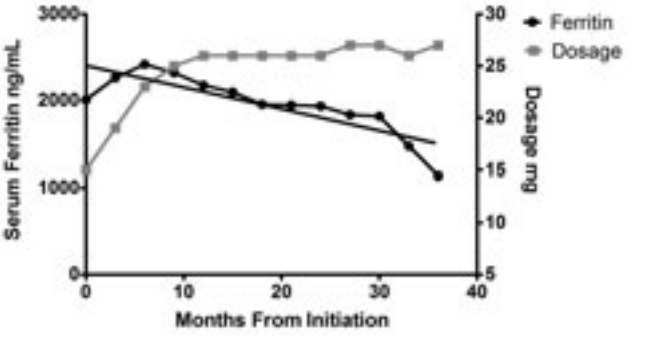

Figure 1.

This association was not apparent in sickle cell disease. Sequential T2* MRI Data was available for 36 patients over at least 2 consecutive years. A reduction in the mean cardiac T2* time of 6.04ms (95% CI -9.907 to -2.175) was seen (p=0.007). Liver T2* showed no significant change. The most commonly recorded adverse events were transient gastrointestinal symptoms. 23 patients (22%) experienced abdominal pain, nausea, vomiting and diarrhoea. These symptoms rarely required dose adjustment (n=4) or discontinuation (n=3). Skin rash occurred in 6 patients with 1 discontinuation. Increases in serum creatinine were observed in 19% of patients receiving deferasirox. Sustained increases of greater than 33% outside of the age appropriate normal range occurred in 7 patients. 4 of 7 responded to dose reduction with 3 patients being discontinued. Discontinuations for other reasons included one patient with an increased alanine aminotransferase and two with unsatisfactory efficacy. *Summary/Conclusions.* Our data confirm deferasirox effectively reduces the SF and cardiac iron burden and highlights the importance of dose titration. Deferasirox was generally well tolerated in pediatric patients with a safety profile consistent with data from previous clinical trials.

Genomics

0738

ROLE OF ETO2 IN THE EPIGENETIC REGULATION OF ERYTHROID GENES

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Background. Developmental control mechanisms often utilize multimeric complexes containing transcription factors, coregulators, and additional non-DNA binding components. It is challenging to ascertain how such components contribute to complex function at endogenous loci. We recently analyzed the function of components of a complex containing master regulators of hematopoiesis (GATA-1 and Scl/TAL1) and the non-DNA binding components ETO2, the LIM domain protein LMO2, and the chromatin looping factor LDB1. We revealed that ETO2 and LMO2 regulate distinct target gene ensembles in erythroid cells. Furthermore, it was found that ETO2 commonly represses GATA-1 function via suppressing histone H3 acetylation, and also regulates methylation of histone H3 at lysine 27 (H3-trimeK27) at select loci, which suggested that ETO2 might be an important determinant of the erythroblast epigenome (Fujiwara *et al.* PNAS. 2010). *Aims.* We investigated the role of ETO2 in the epigenetic regulation of erythroid genes. *Methods.* CBFA2T3 mRNA (which encodes ETO2 protein) was cloned into pcDNA3.1 (Clontech) and Flexi HaloTag vector (Promega), and ETO2 was transiently overexpressed in K562 cells using Amaxa nucleofection technology® (Amaxa Inc.). Quantitative ChIP analysis was performed using anti-acetylated H3K9 (abcam), anti-trimethyl H3 (Lys 9 and 27) (Millipore) and anti-Myc (Santa Cruz). To induce erythroid differentiation of K562 cells, hemin was treated at a concentration of 30 uM for 24h. For transcription profiling, human whole expression array was used (Agilent). Gene Ontology analysis was based on DAVID software (http://david.abcc.ncifcrf.gov/home.jsp). *Results.* Overexpression of ETO2 in hemin-treated K562 cells resulted in decreased ratio of benzidine-staining positive cells, suggesting ETO2 retards the erythroid differentiation of K562 cells. Next, we conducted microarray analysis to characterize ETO2 target gene ensemble in K562 cells. The analysis demonstrated that 598 genes were downregulated in the ETO2-overexpressed cells (>2 fold). To test what percentages of ETO2-repressed genes could be direct target genes of GATA-1 or GATA-2 in K562 cells, we merged the microarray results with ChIP-seq profile (n= 5,749 and n=21,167 for GATA-1 and GATA-2 ChIP-seq peaks, respectively) (Fujiwara *et al.* Mol Cell. 2009), and demonstrated 23.1% and 40.5% of ETO2-repressed genes contained significant GATA-1 and GATA-2 peaks in their loci, respectively. Gene Ontology analysis among ETO2-repressed genes revealed significant enrichment of genes related to "oxygen transport/ hemoglobin complex" (p=0.00128). Overexpression of ETO2 in K562 cells resulted in the significant decrease in the expression of globin genes such as HBG, HBB, HBE1, HBA, HBM and HBZ by quantitative RT-PCR. Quantitative ChIP analysis revealed ETO2 occupancy at globin HS2. Furthermore, the overexpression significantly increased H3-trimeK27 and reduced acetylated H3K9 at γ-globin promoter. Co-immunoprecipitation analysis revealed the interaction between ETO2 and EZH2, a member of polycomb repressor complex responsible for H3-trimeK27-mediated transcriptional repression. We are currently analyzing the mechanism of ETO2-dependent transcriptional repression and how ETO2-dependent histone marks are established in erythroid cells. *Conclusion.* In conjunction with the evidence that ETO2 binds histone deacetylases and associates with GATA-Scl/TAL1 complex that binds epigenetic modifiers, ETO2 appears to have important roles in establishing the erythroblast epigenome. We consider this is important from the perspective of elucidating mechanisms of hematopoiesis and leukemogenesis.

0739

INTEGRATION OF GLOBAL SNP-BASED MAPPING AND EXPRESSION ARRAYS WITH MICRORNA PATTERNS REVEALS DEREGULATION OF MIR-370 AND PERMITS THE IDENTIFICATION OF ITS TARGET GENE NF1 IN ACUTE MYELOID LEUKEMIA

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Deregulated microRNA (miRNA) expression has been largely reported to play a crucial role in tumorigenesis. Recent studies have shown different mechanisms leading to miRNA deregulation in cancer: mutations, chromosomal translocations, epigenetic alterations, and defective miRNA biogenesis; however, alterations affecting miRNAs by DNA copy number variations (CNV) remain poorly studied. Our aim was to identify if CNVs affect the expression levels of miRNAs in acute myeloid leukemia (AML), modulating the expression levels of their target genes. We analyzed 16 myeloid cell lines by an integrative approach including high resolution SNPs arrays, mRNA expression arrays, and quantification of 250 miRNAs by real-time PCR. We found correlation between the expression levels of 19 miRNAs and CNVs affecting the genomic regions in which these miRNAs are located: 16 miRNAs were upregulated and located in two genomic regions of amplification (11q24 and 14q32), and 3 miRNAs were downregulated and located in regions with genomic deletions (13q14 and 9p21). These results prompted us to analyze the expression levels of their predicted target genes. After performing a whole genome expression analysis in the cell lines, we obtained a set of candidate genes whose altered expression (B>0) may result from deregulation of 9 out of these 19 miRNAs. The differential expression of these selected 9 miRNAs and their predicted targets were validated by QRT-PCR. Three miRNAs had NF1 as a potential target gene; therefore, we analyzed whether miR-370, miR-379, or miR-494, all located on 14q32.31, could regulate NF1. The AML cell line HL-60, with low expression of the miRNAs was chosen as a cellular model for miRNA overexpression experiments. Analysis by QRT-PCR confirmed overexpression of these microRNAs after transfection with the corresponding pre-miRNAs. Western blot analysis showed that NF1 levels decreased after miR-370 overexpression. No changes in NF1 levels were observed after ectopic expression of miR-379 and miR-494. Furthermore, transfection with pRL-NF1(3'UTR) in cells ectopically expressing miR-370 showed decreased luciferase reporter activity, indicating that miR-370 binds to 3'UTR of NF1 regulating its expression. These results highlight that presence of copy number alterations affecting miRNAs represent an alternative mechanism to deregulate the expression of genes with importance in myeloid leukemia development. Further studies are in progress to identify other genes with importance in AML and deregulated by CNVs affecting miRNA expression. e-mail address: laura_garcia_orti@hotmail.com

0740

ANTIBODY ARRAY-BASED PROTEOMIC PROFILING OF CHILDHOOD ACUTE LEUKEMIA

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Background. Mapping of signaling pathways and searching for new prognostic molecular markers is a promising approach to design specific treatment of leukemia while minimizing the adverse effects. Large-scale proteomic profiling is expected to yield more direct answers to functional and pharmacological questions than does transcriptional profiling on mRNA level. Performing large-scale proteomic profiling on primary leukemia samples and understanding the effect and functional consequences of targeted anti-cancer agents requires high-throughput technologies as well as novel analytical strategies. Here, we employ bead-based antibody arrays, which can follow changes in protein expression levels that may associate with clinical presentation and therapy efficacy of acute leukemias. *Methods.* Cellular proteins are separated by a differential detergent treatment to cytoplasmic, membrane and nuclear proteins, labeled with biotin and subjected to size-exclusion chromatography to obtain 24 fractions. Fractions are incubated with a mixture of 1700 color-coded beads each carrying antibodies against single protein target. Mixture of all beads binding their specific proteins is analyzed by flow cytometer that resolves beads' color-code and records the amount of captured protein.[1] This novel concept has several advantages: 1) High level of multiplexing by color-coded beads (1700 bead types) 2) Additional information based on protein size profiles (e.g. protein-protein interactions) 3) Flexibility in array composition (custom selected e.g. phospho-specific antibodies) 4) Semi-automated analysis (gating, quality control, normalizations) Algorithms for semi-automated analysis are implemented in R-project environment. It allows for color-code interpretation, captured protein signal read-out and downstream batch analysis including protein size recognition and clustering of results. Array performance was tested on ten representative B-cell precursor (BCP-ALL), T-cell acute lymphoblastic leukemia (T-ALL) and acute myeloid leukemia (AML) cell lines. Next, 30 bone marrow specimens of childhood leukemia at diagnosis were analyzed.

Results. Antibody arrays can accurately determine lineage assignment of leukemia cells (BCP-ALL, T-ALL, AML) in cell lines as well as in patient samples. In parallel to surface proteins, intracellular proteins are quantified (transcription factor Ikaros, E2F, Pax-5; cell cycle machinery CDKN1A, CDKN2D; phosphorylated signaling molecules BLNK, CBL, FYN, IRS1). In total, over 300 protein entities were found. The array allowed us to obtain dose dependent and reproducible results. *Conclusions.* High-throughput quantitative analysis of cellular proteome opens new possibilities for understanding cellular signaling and biology of acute leukemias.

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Reference

1. Wu et *al*., Mol Cell Proteomics, 2008.

0741

MICRO-ARRAY AND 2D-PAGE ANALYSIS REVEAL DIFFERENTIAL REGULATION PATTERNS OF ANTI-CD20 ANTIBODIES GA101 AND RITUXIMAB IN MCL

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Mantle cell lymphoma (MCL), an aggressive B-cell NHL is characterized by a poor long-term prognosis with a median survival of 3-5 years. Obinutuzumab (GA101), a Fc-engineered type II CD20 humanized IgG1 antibody has been shown to result in higher direct cell death induction, increased ADCC and a significant higher cytotoxicity when compared to type I anti-CD20 antibody Rituximab. We conducted our study to investigate possible differences in downstream signal pathways of the antibodies. Using sensitive MCL cell lines Rec-1 and Granta-519 we evaluated the effect of GA101, rituximab and the combination of both antibodies on cell viability and proliferation. Granta 519 and Rec-1 were treated at a cell density of 5x105 cells/ml with GA101 or rituximab at a dose of 10 µg/ml. Samples of 3x106 cells were harvested and processed for 2D-PAGE analysis after a 4h exposure. Distinct protein spots with altered expression after antibody treatment from untreated controls were identified and analyzed by mass spectrometry. Affymetrix micro-array of MCL cell lines (Granta-519, HBL-2, Jeko-1, Rec-1, Z-138) was performed after 4h exposure of either GA101, rituximab or combination of both antibodies. Ingenuity Pathway Analysis of the identified genes was performed to elucidate downstream pathways. Mono-treatment with GA101 achieved 70% cell reduction in Granta-519 and 40% in Rec-1. In contrast, rituximab led to 25% and 5% cell reduction in Granta-519 and Rec-1. Combination treatment of both antibodies led to a cytotoxicity comparable to rituximab mono-exposure. Analysis of 2D-PAGE protein maps revealed 40 and 39 distinct differently expressed protein spots after GA101 and rituximab treatment. Analysis revealed that 23 of these protein spots were commonly altered after both antibodies (CCDC158, MACF1, RAB39, RAD23B). 17 (ENO1, MKI67, NPM1, HSPA5) and 16 proteins were uniquely altered after GA101 and rituximab treatment only (e.g. DST, G3BP2, LMO7, PSMD13). Micro-array-analysis showed 2-3 (Granta 519) to 14-78 (HBL) modulated genes after antibody exposure in all five MCL cell lines. Distinct sets of candidate genes after rituximab (BCL2A1, CHL1, LILRA4, LPL, LY9, RHEBL1, SOX11, WNT3) and GA101 (EGR2, EGR3, NFATC1, SPRY2, ZBTB24) were affected in multiple cell lines. Proteome and transcriptome-based analysis depicted different sets of candidate molecules, which were mapped to common cellular functions including e.g. "cell death", "cellular growth and proliferation" and "cell cycle". Interestingly, combination of GA101 and rituximab resulted in a rituximab-like expression pattern, both on RNA and protein level. Proteome and transcriptome-based experiments showed antibody-specific downstream expression patterns of GA101 and rituximab. These results might represent the molecular basis of the superior effect of GA101 in comparison to rituximab. Combination treatment with both antibodies revealed a rituximab-like expression pattern that confirmed our previous experiments on cell viability and proliferation. Proteomic screening identified a group of proteins relating cellular stress response mechanisms and cellular energy metabolism to the treatment of the therapeutic antibody GA101. These results indicate a possibly involved autophagy mechanism after GA101-treatment and a stress response mechanism effecting regulation of cellular metabolism. These results will provide a further step into identifying molecular-based rationales for new combined therapeutic approaches to treat MCL.

0742

ULTRA DEEP AMPLICON SEQUENCING OF TET2 IN PEDIATRIC MDS/JMML SPECIMENS DETECTS SEQUENCE VARIANTS AND A NOVEL MISSENSE MUTATION

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Background. MDS is a clonal bone marrow disorder characterized by cytopenia due to ineffective hematopoiesis and a high risk of evolution to AML. MDS is mainly a disease of the elderly and is rare in pediatric patients. The WHO classifies MDS in several subgroups in the first place based on morphologic assessment of bone marrow cells. Even if similar morphological diagnosis is used to identify MDS it is understood that pediatric MDS is quite different from adult MDS. Since some years genomic aberrations in MDS are reported and aid in improving molecular-risk classification. Mutations in the *TET2* gene are described in MDS and MPN with percentages varying from 12-22% and in AML *TET2* mutations found in 23% of patients have prognostic impact and are significantly association with older age. In fact, several studies have reported that *TET2* mutations were not identified in pediatric MDS specimens. NGS (NextGenerationSequencing) 454 amplicon sequencing offers a fast, reliable and highly sensitive method of screening for sequence variants and is currently investigated for reproducibility across laboratories. *Aims.* To set up a platform for NGS sequencing for 454 amplicon sequencing of pediatric leukemia and MDS/JMML specimens and to verify in the first place the occurrence of *TET2* mutations in pediatric MDS/JMML. *Methods.* In the context of the Interlaboratory Robustness of Next-Generation Sequencing study (IRON) nine pediatric patients, 6 with diagnosis of JMML and 3 with MDS were sequenced for 27 amplicons covering all coding exons of *TET2*. Primers pairs included a 10-base molecular identifier barcode sequence (MID) to recognize each specific patient. We used Genome Sequencer Junior instruments (Roche Applied Science). All data was generated using the GS Junior Sequencer Instruments Software and sequence alignment and variant detection was performed using the GS Amplicon Variant Analyzer (AVA) software version 2.5 (Roche Applied Science). Sequencing analysis by Sanger sequencing was used to confirm mutation detection in 454 ultra deep screening. *Results.* We generated amplicon ultra deep sequencing of 27 amplicons of *TET2* gene for 9 MDS/JMML patients with a median coverage per amplicon of 650-fold. In total we identified 68 variants in *TET2* gene occurring in more than 1% of bidirectional reads. Of these variants, 6 were known SNPs in MDS and JMML patients with a coverage of > 40% (40-100%) of reads per patient (rs61744960, rs12498609, rs17253672, rs2454206 and rs 34402524). In one MDS patient, a 16 years old boy we found a novel missense mutation C/T in exon 11 of *TET2* at aminoacid T1980I (ENST 00000380013) (RMA = 43.91% of reads). The mutation was confirmed by Sanger sequencing. *Summary/Conclusions.* Ultra-deep amplicon sequencing offers a fast and reliable approach for mutation detection and can be basically implemented in mutation screening of candidate genes in leukemia diagnostics laboratories. We have counter currently detected a novel *TET2* mutation in an adolescent boy with MDS seemingly indicating that *TET2* mutations are rare in pediatric MDS/JMML but may be found in the upper age group of pediatric patients.

0743

CD209 GENE POLYMORPHISMS FOR PREDICTION OF SUSCEPTIBILITY TO INVASIVE PULMONARY ASPERGILLOSIS INFECTION

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Invasive Pulmonary Aspergillosis (IPA) is the most common cause of infection-related deaths in patients with haematological cancers. Despite

the availability of new antifungal drugs, the incidence of IPA is rising as a result of a wider use of broad-spectrum antibiotics, novel immunomodulatory therapies and an increasing proportion of susceptible patients such as solid organ transplantation recipients or critical care patients. Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Nonintegrin (DC-SIGN), Dectin-1 and Dectin-2 are transmembrane C-type lectins that are involved in the recognition of fungal pathogens. The recognition of Aspergillus conidia by dendritic cells or macrophages as well as the chemotactic activity mediated by CCL2/CCR2 ligand-receptor axis, are critical steps in the defence response against Aspergillus. It has been suggested that both processes might be, at least in part, genetically determined. Thus, the aim of this study was to assess whether the presence of single nucleotide polymorphisms (SNPs) within DC-SIGN, Dectin-1, Dectin-2, CCL2 and CCR2 genes influence on the risk of Invasive Pulmonary Aspergillosis. A tag SNP approach resulted in a selection of twenty-seven tagging polymorphisms that were genotyped in 217 high-risk patients with haematological malignancies. Out of 217 haematological patients, seventy patients were diagnosed with proven or probable IPA (following the EORT/MSG criteria, update 2008) and the remaining 147 patients were classified as not infected. Our results revealed that patients bearing the DC-SIGN_rs4804800_G, DC-SIGN_rs11465384_T and DC-SIGN_rs7248637_A alleles had an increased risk of IPA infection (per-allele OR=2.73 95%CI 1.53-4.87; PTrend=0.0004; OR=2.73 95%CI 1.39-5.40; PTrend=0.0035 and OR=2.08 95%CI 1.16-3.72; PTrend=0.013, respectively). In addition, carriers of the DC-SIGN_rs7252229_C showed a slightly increased risk of IPA infection (per-allele OR=1.68 95%CI 0.94-3.00; PTrend=0.078). Likewise, patients harbouring the DC-SIGN_rs4804800_G or DC-SIGN_rs7252229_C alleles showed an increased frequency of galactomannan positivity that those carrying the A or G allele, respectively (p=0.06 and p=0.08). These results strong suggest that DC-SIGN polymorphisms might modulate the risk of IPA infection and might be useful as biomarkers for patient stratification and to develop personalized treatment strategies. Nonetheless, these results need to be confirmed in larger cohorts of haematological patients.

0744

LEUKEMIA GENE ATLAS - A PUBLIC PLATFORM TO SUPPORT RESEARCH AND ANALYSIS OF MOLECULAR DATA OF LEUKEMIAS

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Background. The number of published studies regarding leukemias has been increasing enormously over the last few years. A vast amount of molecular data is collected, analyzed and published every year. Nevertheless, meta-analyses and comparisons of published studies are performed rarely. For researchers it is time consuming to get an overview of the range of published data and results. To obtain an insight into published data or to re-analyze data, e.g. to compare it with ones own data and results, is demanding for researchers who may lack the statistical background to analyze high-dimensional data. Conventional repositories, such as GEO, ArrayExpress and Oncomine, do not feature leukemia specific biological and medical annotations and do thus not cope with this particular kind of research. *Aims.* The Leukemia Gene Atlas was designed to support research and analysis of molecular data of leukemias and hence to fill this gap. *Methods.* The central part of the Leukemia Gene Atlas is a database storing the molecular data of published studies with the corresponding laboratory and clinical data, as well as results, e.g. gene signatures. Currently, the data spans from gene expression to methylation to next-generation sequencing data. The samples associated with the molecular data were classified and annotated leukemia specifically according to their biological and clinical characteristics. The database is publicly available via a website which supports the search and selection of studies and samples with comprehensive search functions. The website also provides a wide range of visualization and analysis tools to process the stored data. An exceptional implement is the search in published result tables which supports, for example, the search for studies and groups of samples whose gene expression patterns significantly differ for certain genes of interest. The website also offers the down- and upload of data. *Results.* Currently, the database stores 290 result tables and the data of 5,000 samples from nine studies and four data types (gene expression, methylation, genotypes and NGS). We intend to integrate many further data sets and thus to increase its versatility and usability. *Conclusion.* The Leukemia Gene Atlas supports the interpretation of newly measured data and is now online: www.leukemia-gene-atlas.org

0745

IDENTIFICATION OF CHARACTERISTIC IGF2BP EXPRESSION PATTERNS IN B-LINEAGE NEOPLASMS

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Insulin-like growth factor 2 mRNA-binding proteins IGF2BP1, IGF2BP2, and IGF2BP3 have been shown to have diagnostic and prognostic utility in a number of epithelial and soft tissue tumors but the expression of these molecules in different types of leukemia is largely unknown. By using an RT-qPCR approach we have systemically analyzed the expression of the three *IGF2BP* coding genes in normal hematopoietic tissues and diverse hematopoietic malignancies. We show that low *IGF2BP1* and *IGF2BP3*, and high *IGF2BP2* expression are characteristic to donor bone marrow (BM) and peripheral blood (PB). Myeloid malignancies - acute myeloid leukemia (AML) and chronic myeloproliferative neoplasms (MPN) - essentially retain the "normal" *IGF2BP* expression profile. In contrast, lymphoid lineage neoplasms - acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM) - are associated with characteristic perturbations of *IGF2BP* expression pattern (Figure 1a). Namely, all lymphoid malignancies tend to underexpress *IGF2BP2* when compared to the normal corresponding tissue and myeloid lineage malignancies though it was statistically highly significant only in the case of CLL and MM ($p<0.001$) as revealed by 1-way ANOVA. In addition, CLL also shows a remarkable variation in *IGF2BP3* expression levels while MM appears to be virtually negative for this transcript (Figure 1a). The most prominent perturbations were identified in ALL where *IGF2BP1* and *IGF2BP3* varied over five and four orders of magnitude, respectively. As ALL is comprised of biologically and clinically different disease entities *IGF2BP* profiles were further reanalyzed with respect to this (Figure 1b). We have identified significant associations of overexpressed *IGF2BP1* with *ETV6/RUNX1*-positive ($p<0.001$), underexpressed *IGF2BP2* with *E2A/PBX1*-positive ($p<0.01$), and overexpressed *IGF2BP3* with *MLL/AF4*-positive ($p<0.001$) leukemias. In contrast to T-ALLs, B-ALLs negative for recurrent fusion genes underexpress *IGF2BP2* ($p<0.01$) and overexpress *IGF2BP3* ($p<0.001$) when compared to donor BM. Altogether, our results show that deregulation of normal *IGF2BP* expression pattern is associated with malignant B-lymphopoiesis. The potential utility of *IGF2BP* profiling in B-lymphoid neoplasms will emerge as the functions of *IGF2BP*s are further delineated.

This study was supported by the European Economic Area and Norwegian Financial Mechanism Grant No. 2004-LT0040-IP-1EEE.

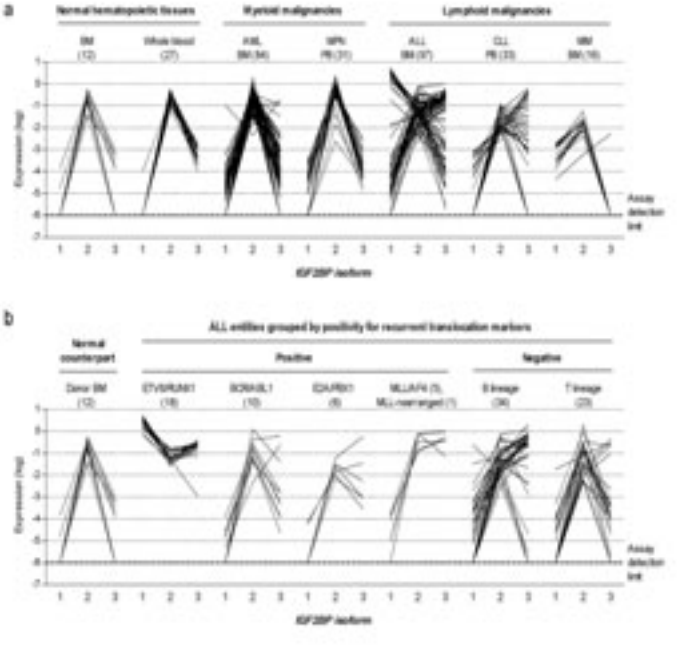

Figure 1. Expression patterns of IGF2BPs.

Hematopoiesis

0746

C-CBL MEDIATES CYTOSKELETAL SIGNALS THROUGH RAC AND REGULATES INTERACTION OF IMMATURE HEMATOPOIETIC CELLS WITH THE BONE MARROW MICROENVIRONMENT

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c-Cbl is a ubiquitin E3 ligase and functions as a negative regulator for signals induced by various activated tyrosine kinases, by promoting ubiquitination and proteasomal degradation of these kinases. Recently, mutations of the c-cbl gene have been reported in hematopoietic malignancies, and regulation of hematopoietic stem/progenitor cells (HSPCs) by c-Cbl is attracting attention. c-Cbl has a C-terminal end with putative tyrosine residues that can interact with several signal transduction molecules, e.g. p85 subunit of PI3-kinase and adaptor molecule Crk, and several reports have denoted that this interaction may be important for cytoskeletal regulation. Since it is known that cytoskeletal dysregulation and impaired interaction of hematopoietic cells with the bone marrow microenvironment might be associated with malignant transformation, we investigated cytoskeletal regulatory mechanisms mediated by c-Cbl in immature hematopoietic cells. To examine the migratory capacity of the HSPCs, we performed migration chamber assay for SDF-1 was performed. We found that c-Cbl deficient HSPCs showed significantly decreased migration to SDF-1; the migration capacity of the Cbl deficient cells was one eighth compared with the wild-type (Wt) counterparts. Then, to evaluate *in vivo* homing ability to the bone marrow microenvironment, transplantation assays were performed. We transplanted 2.5 x 10e6 of HSPCs from c-Cbl deficient or Wt Ly5.2 mice into lethally irradiated Wt Ly5.1 mice. Three hours after transplantation, chimerism of the transplanted Ly5.2 cells in the bone marrow was examined, and we found that the proportion of transplanted cells was smaller in c-Cbl deficient cells, showing that homing capacity of c-Cbl deficient HSPCs was impaired. In c-Cbl deficient HPSCs, activity of Rac, a member of small G-protein GTPases, was significantly decreased. To confirm the role of Rac in the c-Cbl-mediated cytoskeletal signaling pathways, we transduced lentiviral vectors expressing Rac1 G12V, a constitutively active form of Rac1, into the Wt or c-Cbl-deficient HSPCs. G12V-trancduced c-Cbl-deficient HSPCs regained migration capacity to SDF-1 and bone marrow homing ability, suggesting that Rac1 is a key mediator for the cytoskeletal regulation by c-Cbl. Reduced Rac activity, impaired migration and homing abilities of c-Cbl-deficient HSPCs were rescued by re-expression of the full-length c-Cbl. But transduction of the c-Cbl Y700F/Y774F mutant could not restore these defects. Interestingly, transduction of the Y731F mutant, which abrogates the putative biding site for PI-3K, could recover these cytoskeletal functions, indicating that either Y700 or Y774, not Y731 is essential for the cytoskeletal regulation in HSPCs. In summary, we found that c-Cbl-deficient HSPCs showed impaired migration activities to chemoattractants and altered homing ability to the bone marrow microenvironment, with decreased activation of Rac. This cytoskeletal regulation is mediated by Y700 or Y774 residues of c-Cbl. Since phosphorylation of Y774 and Y700 creates binding sites for Crk, signaling cascades via c-Cbl/Crk/Rac might have a role in the engraftment of HSPCs. Our data propose a novel biological function of c-Cbl on the regulation of HSPC-microenvironment interaction.

0747

CANONICAL WNT SIGNALLING MODULATES THE BEHAVIOUR OF STEM CELLS TRANSFORMED BY THE ACTIVE TYROSINE KINASE FUSION PROTEIN TEL/PDGFR BETA

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Background. Leukaemia is initiated by the transformation of a haemopoietic stem cell (HSC). Aberrant activation of the Wnt pathway has been linked to leukaemia through various mechanisms; autocrine up-regulation of Wnt morphogens, epigenetic silencing of negative regulatory molecules and activation of down-stream signalling molecules. Although evidence supports a role for the canonical Wnt pathway in normal HSC self-renewal and haemopoiesis, how vital intrinsic and extrinsic Wnt signals are in sustaining leukaemic stem cells (LSC) and the leukaemic phenotype is still unclear. *Aims.* To determine the importance of intrinsic and extrinsic Wnt signalling in self-renewal and differentiation decisions using a LSC model. *Methods.* To address this we utilised a previously established and characterized tetracycline regulated embryonic stem (ES) cell model to express the oncogene Tel/PDGFRbeta. Activation of the pathway was achieved via pharmacological GSK3beta inhibitors, Wnt3a stimulation or by over-expressing a dominant positive form of beta-catenin. Self renewal was assessed by alkaline phosphatase staining and TaqMan Stem Cell Pluripotency Array. Haemopoietic commitment was assessed by flow cytometry and haemopoietic colony assays. *Results.* Expression of Tel/PDGFRbeta down-regulated the Wnt/GSK3beta/beta-catenin pathway and reduced ES cell self-renewal. Activation of the Wnt pathway substantially suppressed Tel/PDGFRbeta mediated differentiation, with Wnt 3a stimulation, over-expression of beta-catenin and the GSK3beta inhibitor BIO all having this capacity. Analysis via the pluripotency array identified the gene signature involved in Tel/PDGFRbeta mediated early differentiation. As expected key self-renewal genes were down-regulated and key differentiation genes up-regulated by Tel/PDGFRbeta. Several of which are implicated in other malignancies the most interesting being; Flt1 an active tyrosine kinase involved in angiogenesis, HBZ and Cdx2 which are also linked to leukaemic transformation. Activation of the canonical Wnt pathway either through expression of active beta-catenin or BIO treatment enhanced expression of self renewal/pluripotency genes down-regulated by Tel/PDGFRbeta (Nanog, Rex1, GDF3, sFRP2 & c-Kit), whilst suppressing expression of differentiation genes (Dnmt3b, Flt1, Podx1, Cd9, GCM1 & GFAP) and haemopoietic genes (GATA family, Mafb, Egr1 & 2, Nab2). These findings indicate Wnt signalling reduces the transcriptional flux induced by this oncoprotein, leading to suppressed differentiation and enhanced self-renewal of Tel/PDGFRbeta expressing stem cells. During haemopoietic differentiation Tel/PDGFRbeta biased ES cells to form myelomonocytic colonies. Flow cytometry analysis indicates that the oncogene drives haemopoiesis, by up-regulating the HSC specific markers; Sca1 and c-Kit and multipotent progenitor marker Flt3, resulting in more CD45+ haemopoietic cells present by day 8 of differentiation and more myelomonocytic colonies by day 15. Over expression of active beta-catenin was able to suppress Tel/PDGFRbeta ability to up regulate c-Kit and Flt3, and myelomonocytic differentiation. *Summary/conclusions.* Overall our data indicates that ES cells are a valuable tool for modelling leukaemia, especially to delineate the complex interactions occurring within the BM microenvironment. We clearly demonstrate that activation of the canonical Wnt pathway modulates the behaviour of LSC in our model, leading to enhanced self-renewal and suppressed differentiation. This has important implications as it demonstrates that the Wnt morphogens potentially play a greater role in sustaining LSC within the BM niche than previously perceived.

0748

NOTCH REGULATES THE EXPRESSION OF HOXB4 AND GATA3 IN HEMATOPOIETIC STEM CELLS

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Background. The study of transcription factors (TF) and signaling pathways controlling processes involved in the biology of hematopoietic stem cells (HSCs), has conceptual and practical importance in the fields of stem cell expansion and differentiation for therapy purposes. In a previous study, we showed that the transcript levels of the NF-kB subunits RELB and NFKB2, NOTCH1, HOXB4 and GATA3 were at higher levels in the more primitive CD133+ enriched HSC subpopulation, as compared to total CD34+ HSC. Moreover, transcript levels of NOTCH1 were highly correlated to those of HOXB4 and GATA3, and of RELB and NFKB2. Additional promoter analyses, revealed the existence of binding sites (BS) for CSL (the DNA-binding partner of the active Notch intra cellular domain) in the promoters of all these transcripts; and also of NF-kB BS (except in NOTCH1 promoter). These results indicated the potential existence of a regulatory relationship among TF with important roles during T cell lymphopoiesis. *Aims*. To investigate, the potential regulation of GATA3 and HOXB4 by the Notch pathway, and its potential modulation by TNF-α, an activating-stimulus of the NF-κB pathway with known positive effects in T cell differentiation. *Methods*. Human CD34+ HSC were immunomagneti-

cally purified from umbilical cord blood (obtained after informed consent) and co-cultured for different periods (ranging from 12 to 132hs) with cells expressing the ligand Delta like 1 (OP9-DL1) agonist of Notch signaling, or not (OP9-GFP). Additional experiments were carried in the presence or absence of TNF-alpha (0.25ng/ml) and the Notch inhibitor DAPT (10μM), using HSC pre-treated (or not) with the protein synthesis inhibitor cycloheximidine (CHX) (10μg/ml). Real-time PCR was used to quantify transcriptional levels of the NOTCH targets, HES1 and HEY1, as well as GATA3 and HOXB4. GAPDH was used for normalization and relative expression was calculated using HSC co-cultured with OP9-GFP as reference samples. A nonparametric paired t-test was used to statistically access significance, using Prism 5 software. *Results*. As observed for HES1 and HEY1, the Notch pathway regulated the expression of GATA3 in the absence of de novo protein synthesis, inducing its expression after only 12h of coculture. The expression of HOXB4 was also induced by Notch signaling (peaking at 36h), probably indirectly, since, when protein synthesis was inhibited, its expression was observed even in the absence of Notch signaling (DAPT). Finally, Notch-induced expression of HEY1, GATA3 and HOXB4 were positively modulated by TNF-α at a physiologically relevant concentration (two orders of magnitude lower than commonly used in the literature). *Summary*. Our results demonstrate for the first time the transcriptional regulation of the HOXB4 and GATA3 by the Notch pathway in HSCs. This mechanism is very relevant in the context of early colonization of the thymic microenvironment, since, it establishes a direct link between the role of Notch in T cell commitment, with the role of HOXB4 in self-renewal and proliferation of early progenitors. Further dissection of these mechanisms may contribute for the development of new protocols for HSCs transplantation and in the study of leukemogenesis. Author email: josililian@gmail.com.

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0749

ACTIVATION OF DORMANT HEMATOPOIETIC STEM CELLS *IN VIVO* BY THE ENDOTOXIN LPS

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Maintenance of the blood system is dependent on dormant hematopoietic stem cells (HSCs). In order to both maintain a supply of mature blood cells and not exhaust HSCs throughout the lifespan of the organism, most adult HSCs remain quiescent and only a limited number are cycling at any given time. The balance between self-renewal and differentiation of HSCs is controlled by external factors such as cytokines, as well as interactions of HSCs with its niche environment. We have recently shown that the cytokine IFNa very efficiently activates dormant HSCs *in vivo*. Within hours after treatment of mice with IFNa HSCs exit G_0 and enter the active cell cycle. In general, IFNa is produced in response to viral infections by cells of the immune system, and plays an important role in the host defense against viral infections. We now questioned whether endogenous IFNa is also produced in response to other forms of bone marrow stress and whether this affects the proliferation rate of HSCs. To monitor IFNa production in the bone marrow *in vivo*, we have generated MxCre; ROSA-R25-EYFP mice and found that treatment with both the chemotherapeutic agent 5-FU as well as the endotoxin LPS leads to the production of IFNa in HSCs. In addition, LPS treatment *in vivo* induced a strong increase in proliferation of HSCs. To our surprise, mice lacking the IFNa receptor still respond to LPS, indicating that the induced proliferation of HSCs upon LPS treatment is independent of signaling via the IFNAR. In general, LPS will bind the TLR4-CD14 receptor complex on cells, leading to the cellular response to LPS. When mice lacking TLR4 are treated with LPS, HSCs are no longer activated in response to LPS, indicating that also the effect of LPS on HSCs is dependent on TLR4 signaling. Interestingly, LPS induced activation correlated with increased expression of Sca-1 on HSCs, similar to the increased Sca-1 expression upon IFNa treatment. As for IFNa, the up-regulation of Sca-1 is required for LPS induced proliferation, since Sca-1-/- mice do not respond to LPS stimulation. In summary, these data suggest that LPS induced bone marrow stress leads to the production of IFNa in the bone marrow and increased proliferation of the HSCs. Thus, in addition to viral infection also other forms of bone marrow stress, like LPS, result in activation of dormant HSCs in the bone marrow. Furthermore, both IFNa and LPS induced activation of HSCs are dependent on the up-regulation of Sca-1, suggesting a more general role for Sca-1 in the activation of stem cells.

0750

IGF2 DIFFERENTIALLY REGULATES HAEMATOPOIESIS IN FETAL AND ADULT HAEMATOPOIETIC STEM AND PROGENITOR CELLS

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Background. The insulin-like growth factor (IGF) signalling pathway is important for proliferation and differentiation of haematopoietic stem cells (HSC) in mice. Activating mutations and/or aberrant IGF signalling are implicated in human cancers, including childhood leukaemia and myeloma. The 2 principal ligands, IGF1 and IGF2, both signal through the same transmembrane receptor, IGF1R while IGF2R negatively regulates IGF signalling by binding and internalising IGF2. Previous studies in mice suggest that IGF2 regulates fetal haematopoiesis and blocking IGF2/IGF1R signalling in human ES cells reduces their survival and clonogenicity. However, whether IGF1 and 2 are produced by fetal liver (FL) and bone marrow (BM) haematopoietic niches and their role in human fetal haematopoiesis are not known. *Aims and Methods.* To investigate this we assessed: i. IGF1/2 production by human FL and fetal BM MSC and fetal hepatocytes (cBAL111) ii. FL HSC and progenitor numbers and IGFR expression and response compared to adult BM HSC/progenitors (MPP, LMPP, CMP, MEP, GMP and B-cell progenitors- BCP). *Results.* IGF2, as assessed by qRT-PCR, confirmed by Western blotting, was similarly highly expressed by FL, fetal BM and adult BM MSC (n=4 each) and human fetal hepatocytes. IGF1 was expressed by adult BM MSC but barely detectable in fetal MSC and hepatocytes. Flow cytometric analysis in conjunction with clonogenic and lymphoid differentiation assays showed that FL compared to adult BM CD34+ cells contained a 2-fold lower frequency of HSC and BCP but 2-4-fold higher frequency of MPP, LMPP, CMP, MEP and GMP. Consistent with a physiological role for IGF2 in fetal haematopoiesis we found distinctly different patterns of IGF1R/IGF2R on fetal and adult BM CD34+ cells. In FL >95% of HSC and progenitors highly expressed IGF1R. IGF1R and IGF2R were highly co-expressed on HSC while fewer MPP, LMPP, CMP, MEP, GMP and BCP co-expressed IGF2R with IGF1R and at considerably lower levels than HSC. In contrast to FL, fewer adult BM CD34+ cells expressed IGF1R and IGF2R and at much lower levels (MFI ~7-fold-3-fold less).The selective production of IGF2 by FL niche and differential expression pattern of IGF2R on FL CD34+ sub-populations is consistent with a modulatory role for IGF2-IGF2R interaction on these cells. Co-expression of high IGF2R levels with IGF1R on FL HSC may restrict HSC expansion hence the lower frequency of FL HSC while lower IGF2R in MPP and other progenitors would facilitate their expansion which may explain high MPP/HSC ratios in FL. In clonogenic assays IGF2, but not IGF1, increased clonogenicity of FL CD34+ cells. IGF2 caused marked stimulation of BFU-e and megakaryocyte (MK) colonies and some CFU-GEMM and EPO-independent BFU-e. IGF1 caused modest stimulation of EPO-independent BFU-e at the expense of CFU-GM, although did not stimulate MK or CFU-GEMM suggesting differential downstream effects of IGF1R signalling in response to IGF2 compared to IGF1 in FL CD34+ cells. *Conclusion.* Together these data suggest a unique role for IGF2, rather than IGF1, in regulating normal fetal haematopoiesis in humans which may provide insight into the role of aberrant IGF signalling in childhood leukaemia, particularly in Down syndrome.

0751

THE INFLUENCE OF MYCN GENE ON THE TRANSCRIPTIONAL REGULATION OF HEMATOPOIESIS

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Background. The MYCN oncogene is a well-established poor prognostic marker in hematopoietic malignancies (eg. AML), however the role of MYCN expression and the mechanisms by which it acts to promote an aggressive phenotype remain largely unknown. *Aims*. Induced murine MYCN gene overexpression in embryonic zebrafish

through heat-shock promoter, to establish stable germline transgenic zebrafish in order to study the influence of MYCN gene on the transcriptional regulation of hematopoiesis. *Methods*. Murine source MYCN cDNA was fused into the VEGF-tagged pSGH2 vector and was injected into zebrafish embryos at the one- or two-cell stage of development. These embryos were heat shocked in a 38°C incubator for 1 hour once between 14 to 19.5 hours post-fertilization(pf). MYCN-EGFP transgenic founder lines were identified on the basis of GFP expression at about 60 days pf. These founders were identified by fin clipping and genotyping, using PCR primers F:ATCACTGT-GCGTCCCAAGA, R:TTAGCAAGTCCGAGCGTGT. Then we established the stable germline transgenic zebrafish lines, to show its influence on hematopoietic regulatory factors through RT-PCR and the peripheral blood smear. *Results*. Eighteen of 256 (7.0%) mosaic F0 zebrafish embryos injected with the constructed vector were identified the germline transgenic zebrafish, including 8 male,10 female. We extracted blood cells at 60 dpf from wild-type and the F1, F2 generation of transgenic fish. Using cytology, we determined that the blood cells from wild-type fish are predominantly erythrocytes, with myeloid cells only occasionally observed. By contrast, the blood cells from the transgenic fish contain abundant blast-like cells, which are larger than the erythrocytes and have high nuclear to cytoplasmic ratios. We extracted RNA from embryos of 1 dpf, 3 dpf, 7 dpf, and adult fish of 2 months pf, using RT-PCR, we found that MYCN expression remarkablly results in scl (stem cells transcription factor, which is requirement in primitive hematopoiesis), mpo (myeloperoxidase; granulocyte specific gene) and gata1 (which is a master regulator in erythrocyte development) downregulation. Reversely, it upregulates c-mybwhich is predominantly expressed in immature hematopoietic cells, and its expression decreases as these cells differentiate. Correspondingly, MYCN expression downregulates NDRG1(N-Myc downstream regulated gene 1, which is defined as differentiation related gene 1). Therefore, the blood phenotype induced by MYCN expression results in an accumulation of immature hematopoietic blast cells, significantly inhibiting erythropoiesis, and the differentiation of myelopoiesis. *Summary.* Zebrafish offers the advantages of high-throughput scale in the study of gene function *in vivo*. We report here the generation of a highly tractable model of MYCN expression, showing that induced expression of MYCN in zebrafish embryos results in rapid manifestation of a robust phenotype that exhibits cytological and transcriptional hallmarks of human hematopoietic malignancies (eg. AML), suggesting that MYCN signaling pathways are likely to be conserved between human and zebrafish. Most importantly, using this model enabled us to track the molecular changes that take place well before morphological phenotypes can be detected, and to determine the roles of candidate MYCN target genes. We demonstrate that MYCN regulates scl and several lineage-specific transcription factors, reprogramming hematopoietic cell fate *in vivo*.

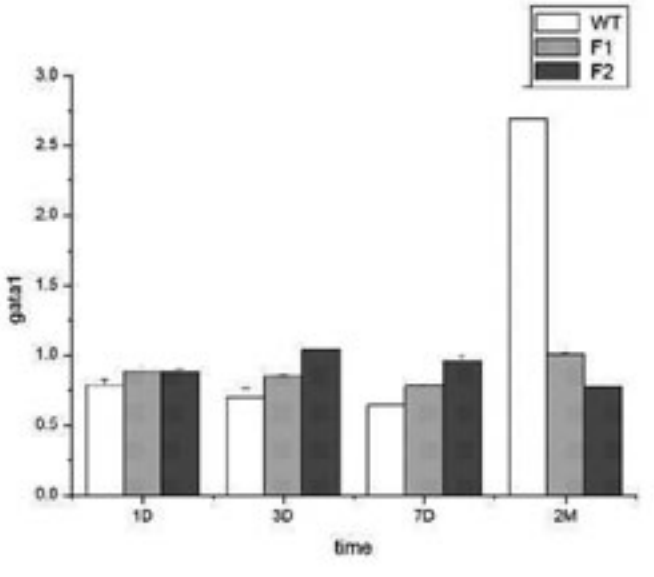

Figure 1. Comparation of gata1 expression between wild type.

0752

THE GENERATION OF MONKEY AND PIG INDUCED PLURIPOTENT STEM CELLS OF EASY HANDLING FOR HEMATOLOGICAL RESEARCHES

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Background. We have been studying hematopoietic reconstitution with stem cells *in vivo* in large animals such as monkeys and sheep. In this subject, induced pluripotent stem (iPS) cells have become very important as donor cells; however, human iPS as well as ES cells are not easy to handle. For instance, human iPS/ES cells grow slowly, their plating and subcloning efficiencies are very low, and transfection is quite difficult, as compared to mouse iPS/ES cells. iPS cells have also been generated from large animals including monkeys and pigs. These animal iPS cells have similar shortcomings to those of human iPS cells, and it hinders us to reproduce mouse results in large animal models. Recently, human iPS cells of easy handling with mouse-type characters have been generated by culturing the cells in the medium supplemented with LIF, signal transduction inhibitors (MEKi and GSKi), and an agonist for the protein kinase A pathway (forskolin) (Jaenisch *et al.* PNAS 2010;107:9222-9227). *Aims.* The aim of our study is to overcome the shortcomings (difficulties in handling) of human and large animal iPS cells previously generated, and to facilitate their use for hematological researches. In the present study, we have tried to generate monkey and pig iPS cells of easy handling (with characters similar to those of mouse iPS/ES cells). *Methods.* We prepared embryonic fibroblasts (PEFs) from cynomolgus monkeys and Clawn miniature pigs, and introduced Yamanaka's four genes (human Oct3/4, Sox2, Klf4, and c-Myc) with retroviral vectors into the cells. The cells were cultivated in the presence of forskolin. *Results.* After 14-20 days of the cultivation, emergent colonies were plucked and expanded for further analyses. Our newly generated monkey and pig iPS cells express pluripotency markers. They can be differentiated into three germ layer cells *in vivo* (<*ie.* teratomas in immunodeficient mice) and *in vitro*. Our monkey iPS cells resembled mouse iPS cells at first, but they became human iPS-like cells after several passages. On the other hand, our pig iPS cells have retained the mouse-type characters after extended passages unlike previously reported pig iPS cells. They show round-colony formation, rapid growth, LIF-dependency, capability of single-cell cultivation without a ROCK inhibitor, high viability after freeze-and-thaw, and easy transfectibility, like mouse iPS/ES cells. In addition, mouse iPS/ES cells do not express Xist (<*ie.* XaXa) or MHC class I, and that is also the case with our pig iPS cells. *Summary/Conclusions.* We have generated Clawn miniature pig iPS cells, which are easy to handle and similar to mouse iPS/ES cells in all the aspects tested. Their abilities to contribute to offspring chimeras remain to be investigated. In the Clawn miniature pigs, inbred animals are available and thus congenic transplant of our pig iPS cells is feasible. Currently, we are transplanting our pig iPS cells in the congenic setting.

0753

HETEROGENIETY OF HUMAN BONE MARROW DERIVED MESENCHYMAL STROMAL CELLS

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Background. In attempt to classify mesenchymal stem and progenitor cells basing on their proliferative potential (PP) the occurrence of hierarchy *in vitro* was revealed. Bone marrow (BM) mesenchymal cells were found to contain mesenchymal cells with high PP (HPP), with low PP (LPP), mesenchymal cluster-forming cells (MCFC), and mature mesenchymal cells (MMC). *Aims.* The aim of this study was to investigate the PP and clonal composition of BM derived multipotent mesenchymal stromal cells (MMSCs) during passages in culture. *Methods.* MMSCs from 6 donors (4 males and 2 females) 15-43 years-old were analyzed. $3x10^6$ BM nucleated cells were plated in 25 cm^2 flask in aMEM with 10% of fetal calf serum. The cells were passaged at the density 10^5 cells per flask after they reached confluent monolayer. MMSCs were infected with self-inactivating 4th-generation lentiviral vector bearing enhanced green fluorescent protein. At each passage the proportion of marked by lentivector (green) and viable cells was measured and MMSCs were cloned 1 cell per well in 96-well plate in standard medium supplemented with 5 ng/ml of basic fibroblast growth factor. Clones were transferred to 24-well plate, then to 6-well plate and finally to 25 cm^2 flask. The ability of MMSC clones to adipose and

osteogeneic differentiation was assessed at this stage. Clones were considered to have HPP if they reached confluent monolayer in 25 cm^2 flask, LPP if they grew to confluence in 6-well plate, were named MCFC if reached confluence in 24-well plate and MMC if they didn't manage to cover the bottom of 24-well plate. MMSC clones with HPP from 1 donor were analyzed by means of ligation-mediated polymerase chain reaction (LM-PCR). *Results.* It was shown that the proportion of MMSC clones with HPP was 3.7 ± 0.6% at 1st passage, reached maximum of 5.0 ± 1.4% at 3d passage and then declined. Clones with HPP were not detected at 7th passage. The proportion of MMSC clones with LPP reached maximum of 6 ± 2% by 2nd -3d passage and then also declined. Such clones were not detected at 7th passage as well. The maximum proportion of MCFC clones was 23 ± 12% at 3d passage and then declined to 7% at 7th passage. The majority of MMSC clones possessed very low PP (MMC) and their mean proportion through passages was 82 ± 4%. The vector integration sites were determined in 10 out of 14 MMSC clones with HPP obtained from one donor. Each analyzed clone contained unique integration sites. It means that every analyzed clone was a progeny of distinct parental MMSC. Clonal composition of MMSC was unique in each passage. The dominant clones were not revealed. Thus not a single true mesenchymal stem cell with the ability to self-renew was detected by LM-PCR among studied clones. *Conclusions.* MMSCs represent a heterogeneous population of cells with different but limited PP. The probability of the presence of genuine mesenchymal stem cells in the population of MMSCs seems to be very low.

0754

PERSISTENT MICROCHIMERISM IN BONE MARROW: INFLUENCE OF MESENCHYMAL STEM CELLS

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Background. Microchimerism in bone marrow (BM) can be detected even years after allogeneic hematopoietic stem cell transplantation (Allo-HSCT), in spite of complete donor chimerism (CDC) attainment in peripheral blood (PB). *Aims.* The aim of our study was to explore the source of the persisting autologous feature in BM with particular interest in mesenchymal stem cells (MSC), which are known to remain of recipient origin after Allo-HSCT. *Methods.* BM cells obtained from patients with CDC detected in PB (n=14; 8 after reduced intensity conditioning, 6 after myeloablative conditioning; the median day post Allo-HSCT= 1499) were cultured in cytokine-free medium, and MSC were expanded. After three or four passages, the origin of acquired cells was determined. All analyses were performed by real-time PCR exploiting insertion/deletion polymorphism with the detection limit of almost 0.01%. Authenticity of MSC was affirmed morphologically and by flow cytometry analysis (positivity for CD105, CD73, CD44, and CD90 and negativity for the hematopoietic markers - CD14, CD19, CD34, and CD45). *Results.* The proportion of autologous cells observed in the whole BM was below 0.1% in all samples (see table). On the other hand, analysis of cultivated MSC revealed their autologous origin, as only a minimum of donor cells was detected (below 0.1% in mean). The amount of donor cells decreased with the number of performed passages. Interestingly, the host origin of the MSC was also identified in two samples with no autologous cells detected in whole BM. *Summary/Conclusions***.** Our results confirm and extend previous observations of the autologous origin of MSC. For the first time, we clearly emphasize connection between MSC and persisting microchimerism in BM. The impact of MSC must be taken into account, especially when danger of incipient relapse relating to microchimerism analysis in BM is considered. Furthermore, undetected autologous cells in BM do not signify even partial donor origin of MSC. e-mail: ohorky@fnbrno.cz

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Table 1.

Patient No.	Dg.	Regimen	Graft	Day post Allo-HSCT	% of autologous cell detected	
					BM	MSC
60	AML	MAC	PBSC	2947	<<0.1%	>>99.9%
62	CML	MAC	PBSC	3418	<0.1%	100%
70	CML	MAC	PBSC	3339	<<0.1%	100%
102	CLL	RIC	PBSC	2879	<<0.1%	99.7-99.8%
166	ALL	MAC	PBSC	2199	<0.1%	>99.9%
173	CML	MAC	PBSC	2050	<0.1%	100%
200	AML	RIC	PBSC	1587	0%	>>99.9%
208	AML	RIC	BM	1411	0%	>>99.9%
230	AML	RIC	PBSC	1192	<<0.1%	99.7-99.8%
236	AML	RIC	PBSC	1140	<<0.1%	100%
239	AML	RIC	PBSC	924	<<0.1%	100%
253	FL	RIC	PBSC	863	<<0.1%	>99.9%
256	ALL	MAC	PBSC	1084	<<0.1%	>>99.9%
287	AML	RIC	PBSC	609	<<0.1%	>99.9%

Diagnoses: AML=acute myeloid leukemia (n=7); CML=chronic myeloid leukemia (n=3); ALL=acute lymphoblastic leukemia (n=2); CLL=chronic lymphocytic leukemia (n=1); FL=follicular lymphoma (n=1)
Regimen: MAC=myeloablative conditioning; RIC=reduced intensity conditioning
Graft: PBSC=peripheral blood stem cells (n=13); BM=bone marrow (n=1)

0755

THERAPEUTIC POTENTIALS OF MESENCHYMAL STEM CELLS IN BONE DEFECTS, THE CASE STUDY IN RABBIT TIBIA

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Background. Mesenchymal stem cells (MSC) have high proliferation and differentiation capacity into other cell lineages. MSC could be used in regenerative medicine because of these potentials. Distraction osteogenesis is the most prevalent therapy in bone defects. There are many restricted situations in bone defects therapy such as collapsing of callus, loss of lengthening and the long time of consolidation. *Aims.* The aim of this study is to examine therapeutic potentials of mesenchymal stem cells in bone defects. *Methods.* In this study, 21 New Zealand rabbit were used. These rabbits were separated into control, stem cell and osteoblast differentiated stem cells. Serum physiologic was applied to the first group of rabbit, adipose-derived mesenchymal stem cells were injected to callus site of the second group after distraction process to and osteoblasts differentiated from mesenchymal stem cells were injected to callus site of the third group of rabbits. Before injection, we characterized stem cells by flow cytometry and the cells were tagged with "green flourescent protein" (GFP). After four and eight weeks, the rabbits were sacrificed and evaluated radiologically, biomechanically and histopathologically. *Results.* Radiological analyses revealed that callus density and ossification rate increased in Group III as compared to Group I and Group II. In biomechanical tests, the highest rates were observed in Group III comparing to the others. As a result of histopathological studies, it was also observed that the quality of newly formed bone and the cells active in bone formation were significantly higher in Group III as compared to Group I and II rabbits. *Summary/Conclusions.* Taken together all these results revealed that osteoblasts differentiated from mesenchymal stem cells shortens the the consolidation period of distraction osteogenesis. Stem cells can be used effectively for the treatment of bone defects.

0756

MYOFIBROBLASTS ORIGINATING FROM MYELOGENOUS LEUKEMIA CASES FORM BLASTOMA IN SEVERE COMBINED IMMUNODEFICIENCY MICE

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Background and Aims. We recently reported that acute myelogenous leukemia (AML) blasts as well as chronic myelogenous leukemia (CML) cells convert to stromal myofibroblasts to create an environment for the proliferation of leukemic cells *in vitro* and *in vivo*. To ascertain the biological characteristics of the bone marrow-derived myofi-

broblasts, myelogenous leukemia-derived stromal myofibroblasts were transplanted to NOD/SCID mice *in vivo*. *Materials and Methods.* The institutional ethical committee approved our study, and after obtaining informed consent bone marrow cells were collected from AML and CML patients as well as from informed normal individuals. Mononuclear cells were separated with gravity sedimentation method, and adherent cells were isolated by the two hours-culture, and further cultured for a long term. During the culture, cells were splitted with trypsin/EDTA once a week. After two months the myofibroblasts were prepared,which characterized with flow cytometry to deter mine the expression of the specific proteins on their cell-surface. The prepared myofibroblasts (1 x 107 cells) were injected intravenously to two gray-irradiated NOD/SCID mice, and mice were bred in a pathogen free room with antibiotics-containing water. Anti-asialo GM1 antibody was injected every 11th day subcutaneously for the suppression of NK cells. *Results and Discussion.* Between at day 40 and 60 after transplantation mice were dead. Autopsy findings revealed tumor formation at the liver. The transplanted myofibroblasts expressed expressed STRO-1, and smooth muscle actin but not lineage-spcific molecules including CD56; however, blastoma-forming cells expressed CD56 strongly but not other lineage-specific cell-surface markers. CD31, CD73, CD140a, and CD326(EpCAM) were also negative. These myofibroblast-derived blastomas expressed vascular endothelial growth factor (VEGF)-A and its receptor type 1, and 2, and showed growth-promotion when cells were cultured with VEGF-A. These observation indicate that myeloid leukemia-derived myofibroblasts form blastoma in an autocrine-fashion of VEGF-A, and also that the CD56-positive specific fraction of myofibroblasts is selectively engrafted to form blastoma.CD56 positive myofibroblast sarcoma of the liver is very rare disease that called Nested stromal epithelial tumor of the liver. Here we can reproduce this tumor in NOD/SCID mouse system, and we think CD56 positive fraction of myofibroblasts are its origin. Further study will reveal its precise biological characteristics.

0757

ACCELERATED TELOMERE SHORTENING IS AN EARLY AND PERMANENT SIGNATURE IN CULTURED HUMAN MESENCHYMAL STEM CELLS EXPOSED TO CHEMOTHERAPY

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Background. Induction of cell senescence has been shown to be produced by various agents, including chemotherapeutic drugs. However, the mechanisms involved in the ageing pathway, in particular the distress on telomere produced by chemotherapy are still undefined, above all in human stem cells. *Aims.* In order to address these issues, human Mesenchymal Stem Cells (MSC) were assessed as target cells to investigate the initiation of the ageing process by chemotherapy. *Methods.* MSC were obtained from bone marrow (BM) cells from normal adults and grown in the presence of platelet lysates. Cultured MSC were identified for immunophenotype, and for growth and differentiation properties. MSC were exposed to sub-lethal doses of drugs able to induce double-stranded DNA breaks, i.e. doxorubicin (Doxo, 10 nM) and etoposide (Eto, 500 ng/ml). Telomere length (TL) was assessed by both flow-fish and southern blotting. *Results.* An initial TL shortening was detectable in MSC already at 5 days after exposure to Doxo and Eto, with a progressively marked reduction compared to untreated cells documented at 7, 14, 21 and 28 days in culture. Following a single drug exposure, MSC were unable to regain the lost telomere sequences, for up to 28 days in culture. ATM phosphorilation was documented early after Doxo and Eto exposure, while no telomerase activation was observed. Chemotherapy- Induced TL shortening was associated with reduced clonogenic activity *in vitro* and accelerated adipose differentiation. An analogous behavior in the differentiation pattern was observed in naturally aged MSC. *Conclusions.* the results indicate that: i. cultured MSC represent a useful cellular model to investigate novel drugs that may favor or, conversely, might prevent TL loss on human stem cells; ii. TL shortening is early detectable in cultured MSC following exposure to chemotherapy; iii. it remains as a permanent signature of previous chemotherapy-mediated DNA damage and predicts impaired proliferative and differentiation potential.

0758

CD146 AS A MARKER FOR CHARACTERISATION OF MSC POPULATIONS

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It is often assumed that expression of a certain broad set of markers defines various types of cell cultures as MSCs, but in reality, most of these markers are expressed by cultures of fibroblastic cells from any tissue. In addition, most of these markers are highly modulated in culture, which makes it difficult to characterize stromal cell cultures. In human BM, CD146 marks adventitial reticular cells a classically known stromal cell type residing in a subendothelial position over the abluminal surface of BM sinusoids. In other tissues, CD146 is expressed by pericytes, the cell that are considered as the MSCs found in different tissues. This study was designed to find out if CD146 could be used as a surrogate marker for characterization of MSC populations. *Patients and Methods.* 30 donors without bone marrow disorders were involved in this study. MSC cultures from BM (BM-MSC) were evaluated in successive passages (P)for immunophenotype (FACS analysis), frequency of colony-forming units (CFU-F) in MSC population, frequency of adipo- and osteo-progenitors (CFU-Ad, CFU-Ost) in the same population and for dynamic of changes in these properties with successive passage. CFU, CFU-Ad and CFU-Ost were studied by limiting dilution assay followed by induction of adipo- or osteo- differentiation: cell suspension was serially diluted two folds across the 8 columns of 96-well plates, resulting in columns containing from 50 to 0,39 cells per well. After 10 days of culture the number of positive and negative wells was determined for each cell concentration and CFU frequency was calculated. Then plates were induced to undergo adipogenesis and osteogenesis and CFU-Ad was determined by Oil Red staining and CFU-Ost by Alizarin Red staining after 14 and 21 days respectively. All calculations were performed as for CFU-F; β-galactosidase activity was used as biomarker for assessing replicative senescence; growth rate was estimated using on-line calculator www.doubling-time.com. *Results.* in present study we have found that, as expected, MSC at all passages were positive for stromal cell markers CD105/90/73 and negative for hematopoietic markers CD34/19/14/45. The level of expression of stromal markers remain stable at all passages. However, while the population of CD146+ cells was abundant in MSC at early passages, this population declined with successive passage (40+ 8% at P1 vs 14+ 6% at P5). This decline in CD146+ population correlated with decline in frequency of CFU-F (30,6%+4,9% at P1 vs 8,0%+1,5% at P4), CFU-Ost (17%+4,9% at P1 vs 5% + 2% at P4) and CFU-Ad (10% + 2% at P1 vs 3% + 1% at P4), in MSC sample. Additionally, while CD146+ population decreased in MSC sample, population doubling time increased dramatically and β-galactosidase activity increased. *Conclusion.* the population of CD146 positive MSCs declined with subsequent passages and correlated with the decrease of the ability to differentiate to Ost and Ad and increase of population senescence. The level of CD146 expression could be used to predict some of the properties of BM-MSC sample.

0759

NUCLEATED RED BLOOD CELLS, RETICULOCYTES AND IMMATURE RETICULOCYTE FRACTION IN TERM AND PRETERM INFANTS IN UMBILICAL CORD BLOOD

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Background. Nucleated red cell (NRBC) count varies widely at birth. Increased number of NRBCs seems to be related to fetal hypoxia. Furthermore the development of automatic flow cytometry techniques have led to improvement in the accuracy and precision of reticulocytes (RET). The immature reticulocyte fraction (IRF), which is the sum of the reticulocyte populations of high and medium immaturity, rather than just the reticulocyte count may reflect the intensity of erythropoietic stimulation. *Aim.* This study was aimed to establish normal range of reference values for percentage and absolute nucleated red cells (NRBC), absolute reticulocyte (RET) count and immature reticulocyte fraction(IRF) by using automated hematology analyzer, in term healthy newborns in umbilical cord blood (UCB), to compare these results with those corresponding to preterm fetuses and to define the state of erythropoiesis by using IRF versus RET count. *Methods.* UCB samples

from 329 (290 full term and 39 preterm) healthy newborns with gestational age between38-41 and 29-37 weeks respectively, delivered either vaginally or by cesarean section, were prospectively studied. Among preterm infants 4/39 were twins. Gestational age was determined from the last menstrual period and early ultrasonography. Blood samples were collected from the umbilical vein in tubes with EDTA K3, immediately after delivery and were analyzed within 3 hours. The hematological variables were determined in automated counter Sysmex XE2100 (Japan). Reference values were performed according to non-parametric percentile method (CLSI C28-A3). *Results.* Higher NRBC count was recorded in preterm newborn infants regardless from the type of delivery. Modest correlation was obtained between IRF and the RET count, but significantly different from zero. The data verified that the number of circulating RET corresponds to a greater fraction of young reticulocytes. Reference values in UCB in relation to gestational age for term infants (n=290) and preterm infants (n=39), respectively: NRBC% 0,00-20,508 (n=290) and 0,600-50,400 (n=39), NRBC 103 /uL 0,00-2,826 (n=287) and 0,080-5,550 (n=39), RET 106 u/L 0,093-0,244 (n=288) and 0,095-0,283 (n=37) and IRF% 18,0223-47,288 (n=288) and 20,70-44,00 (n=37). *Conclusion.* It is important to define reference values for the interpretation of blood count in the neonatal period. Our data showed decreased NRBC count with advancing gestation. We underline the possible value of high NRBCs in UCB suggesting an intrauterine hypoxic event several hours before birth. The IRF seems to have an independent role as an early and sensitive marker of erythropoietic activity and is becoming helpful in routine laboratory practice.

Hodgkin lymphoma - Biology & clinical

0760

CTLA4 POLYMORPHISMS AND MICROENVIRONMENT IN PEDIATRIC CLASSICAL HODGKIN LYMPHOMA. CLUES FOR THE UNDERSTANDING OF THE SUPPRESSOR PHENOTYPE

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Background. Classical Hodgkin lymphoma (cHL) is characterized by a low percentage of Hodgkin and Reed-Sternberg cells amidst a high number of infiltrating inflammatory cells, mostly CD4+ lymphocytes with a suppressor profile. The cytotoxic T-lymphocyte antigen-4 (CTLA4) is an immunoreceptor that inhibits T-cell proliferation and activation. Its polymorphisms appear to differentially influence its inhibitory activity. Particularly, the CTLA4 +49GG and CT60GG genotypes, and the CT60G/+49G haplotype were associated with a low inhibitory potential and with the susceptibility to autoimmune diseases. The inverse model was proposed for cancer, where genetic variants with high inhibitory potential (CT60A/+49A) would negatively influence anti-tumor immune surveillance. Thus, a better clinical response would be expected in the presence of genetic variants with increased anti-tumor capacity (low inhibitory potential). *Aims.* To investigate if CTLA4 polymorphisms influence the tumor microenvironment composition, clinical characteristics and outcome in cHL. *Methods.* 100 children (3 to 18y, median 15) diagnosed with cHL were included. DNA was extracted from peripheral blood or tumor diagnostic samples. Two CTLA4 polymorphisms (+49A/G and CT60A/G) were evaluated by TaqMan allele discrimination assays (ABI7000). CD4, T-bet (Th1), c-maf (Th2), CD8, Granzyme-B, Tia-1, FoxP3, CD20 and Ki67 expression were evaluated by immunohistochemistry in tissue microarray (TMA) slides. EBV was detected by EBER-ISH. *Results.* The presence of the CTLA4+49A allele was associated with high numbers of T lymphocytes (>451 /mm2, percentile 25) (p= 0.033). Genetic variants with high inhibitory potential (CT60A allele and +49A/CT60A haplotypes) were associated with high numbers of CD4+ lymphocytes (p= 0.023), which was secondary to an increase of Th2 lymphocytes(C-maf+), as compared to Th1 (T-bet+) lymphocytes (p= 0.046). CT60 low inhibitory potential variants (CT60G allele and GG genotype) were related with higher numbers of CD8+ (p= 0.014) and FoxP3+ lymphocytes (p= 0.007). These results suggested that while CTLA polymorphisms may influence the activation status and proliferation of Treg cells, the CD8+ lymphocytes sub-population appears to be a target for the inhibitory effect imparted by another cell population(s) expressing high CTLA4 levels. A better event-free survival (EFS) was associated with the CTLA4+49AA genotype (p= 0.045, Log-rank test), while a worst EFS was observed in cases with CTLA4 haplotype 49G/CT60G (p= 0.015). Differences in EFS were more marked in the EBV+ group, suggesting that FoxP3+ lymphocytes might be engaged in the immune response against EBV antigens. In the Cox regression adjusted by EBV status, haplotype 49G/CT60G maintained its independent prognostic impact (p= 0.043; HR CI95% 0.08-0.95). *Conclusions.* This is the first study to investigate CTLA4 polymorphisms in cHL and shows a potential effect of CTLA4 variants on cHL microenvironment, and outcome. In the pediatric cHL, high inhibitory potential CTLA4 variants were not associated with worse anti-tumor responses, but to favorable microenvironmental characteristics and better therapeutic responses. This reinforces the existence of a distinct anti-tumor model of immune response in hematopoietic malignancies as compared to solid cancers and highlight, through a genetic approach, the suppressor nature of the immune response against classical Hodgkin lymphoma.

0761

T(4;8)(Q27;Q24) IN HODGKIN LYMPHOMA CELLS TARGETS PHOSPHODIESTERASE PDE5A AND HOMEOBOX GENE ZHX2, ACTIVATING STAT1-SIGNALING

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Background. Hodgkin/Reed-Sternberg (HRS) cells represent the malignant fraction of infiltrated lymph nodes in Hodgkin lymphoma (HL). Although HRS cells display multiple chromosomal aberrations, few are

recurrent and the targeted genes unknown. *Aims.* Cell line L-1236 was used as a model to specify recurrent chromosomal breakpoints in HL. *Methods.* Fluorescence *in situ* hybridization (FISH), genomic copy number analysis by Agilent 4x180K-arrays, quantitativ real-time PCR (RQ-PCR), Western blot, Immuno-cytochemistry, RNAi-mediated knockdown, expression profiling by Affymetrix U133A arrays. *Results.* Here, we analyzed the karyotype of HL cell line L-1236 by multicolor FISH and identified multiple abnormalities, including t(4;8)(q27;q24). We focused on this alteration because both breakpoint regions (4q2 and 8q24) are recurrently involved in HL. Mapping these breakpoints was performed by FISH using tilepath BAC clones together with high density genomic arrays, while expression analysis of candidate target genes was tested by RQ-PCR and Western blot. The data revealed activation of phosophodiesterase PDE5A at 4q27 and activation of homeobox gene ZHX2 at 8q24. This breakpoint is located in the far upstream region of ZHX2, thereby removing potential binding sites for transcription factors XBP1 and MSX1. Expression analysis of ZHX2 and of promoter-constructs demonstrated ZHX2 activation by XBP1, and repression by MSX1 in combination with corepressor histone H1C. L-1236 cells showed silencing of XBP1 and elevated coexpression of MSX1 and H1C, associated with chromosomal alterations of the histone gene cluster at 6p22. Conspicuous expression of XBP1 in SUP-HD1 HL cells coincided with downstream deletions and enhanced ZHX2 levels, together confirming the activating role of XBP1 while highlighting an alternative mode of aberrant ZHX2 expression. To identify ZHX2 target genes we performed expression profiling of L-1236 cells following ZHX2 knockdown by siRNA treatment. Our data revealed suppression of STAT1 and several interferon-beta-targets, suggesting aberrant activation of STAT1-signaling by ZHX2. ZHX2-mediated STAT1-overexpression was confirmed in L-1236 cells by RQ-PCR and Western blot. Additionally, elevated expression of STAT1 protein was detected in all analyzed HL cell lines in contrast to control B-cell lines, highlighting a general feature of HL. Interestingly, treatment of L-1236 with PDE5A-inhibitor Sildenafil inhibited STAT1-phosphorylation and -signaling, indicating synergistic activity of the t(4;8)-target genes PDE5A and ZHX2. *Conclusion.* Taken together, we have identified a novel aberration in HL, t(4;8)(q27;q24), activating PDE5A and ZHX2. Both genes promote STAT1-signaling, which is generally elevated in HL cells and may contribute to the malignant phenotype. Furthermore, our data suggest that phosphodiesterase-inhibitors may represent a promising therapeutic avenue in HL.

0762

A LOW PERIPHERAL BLOOD CD4/CD8 T CELLS RATIO PREDICTS POOR PROGNOSIS IN CLASSICAL HODGKIN'S LYMPHOMA

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Background. Classical Hodgkin's lymphoma (cHL) is characterized by the presence of neoplastic cells in a rich background of T and B cells, macrophages, and other inflammatory cells. The contribution of these non tumoral cells to the pathogenesis of HL is still poorly understood; T cells constitute a significant component of the reactive infiltrate in cHL with an elevated T helper/T suppressor (CD4/CD8) ratio. T helper cells (Th) are central to the development of an immune response, activating antigen-specific effector cells and recruiting cells of the innate immune system. Until recently, the CD4/CD8 ratio was considered to be an index of immunosuppression in cancer patients. *Aim.* to evaluate the prognostic significance of a low peripheral blood (PB) CD4/CD8 cells ratio at diagnosis in cHL patients (pts). *Methods.* Flowcytometer immunophenotyping of PB samples was performed at diagnosis in 92 immunocompetent pts with cHL treated at our institution between January 2007 and December 2008 with first line ABVD chemotherapy. Median age at diagnosis was 34 years (range 15-82), 42 pts (45.6%) were male, 52 pts (56.5%) presented advanced stage disease (IIB-IV), 36 (39%) bulky disease, 40 (43.5%) had B symptoms, 24 (26%) spleen involvement, 25 (27.17%) extranodal disease. The histology was nodular sclerosis in 79 (85.9%), mixed cellularity in 13 (14.1%). *Results.* A PB CD4/CD8 T cells ratio <1.5 (Low CD4/CD8 ratio) was recorded in 40 pts (43,5%). The clinical features present with a higher rate in the low CD4/CD8 T cells ratio group were: nodular sclerosis histology (96% vs 77% p=0.07), high-stage disease (70.4% vs 45.72%; p<0.05), B symptoms (51.8% vs 37%; p<0.05), bulky disease (51.8% vs 28.57% p<0.05), spleen involvement (40.7% vs 14.3% p<0.05). In the low CD4/CD8 T cells ratio group a lower rate of complete remission was observed (77.7% vs 91.4% p<0.05) and a higher rate of disease progression

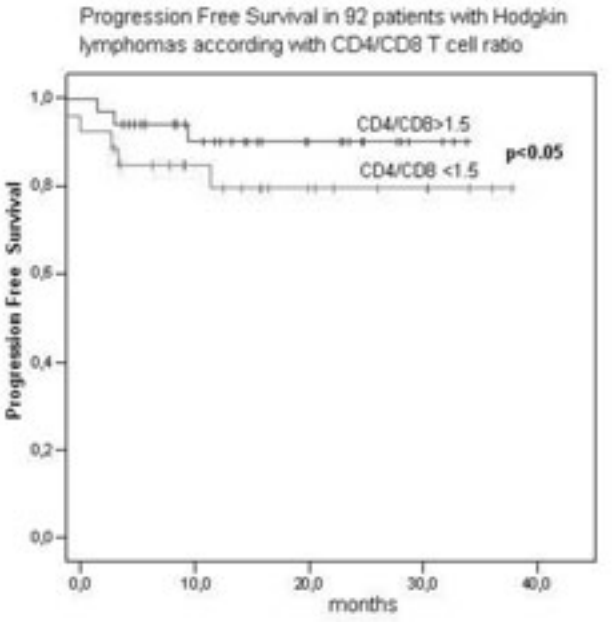

Figure 1.

(14.8% vs 2.85%; p<0.05). At a median follow-up of 24 months, 15 (16.3%) pts developed progressive/relapsed disease; 70% of these patients had a CD4/CD8 t cells ratio <1.5. The incidence of positive PET after two courses of chemotherapy was higher in pts with a low CD4/CD8 t cells ratio (40.7% vs. 17.14%; p<0.05). The 24 months progression free survival (PFS) rate was 76.2% in low CD4/CD8 t cells ratio pts and 92.2% in patients with a CD4/CD8 t cells ratio >1.5 (P<0.05). No significant differences were observed for sex, absolute lymphocyte count, VES, LDH and extranodal disease between the low and high CD4/CD8 ratio groups. *Conclusion.* A low PB CD4/CD8 t cells ratio at diagnosis seems to be associated with unfavorable clinical features and a worse prognosis. Further investigations including analysis of t reg, cytokine, chemokine, are needed to confirm these results.

0763

BASELINE SERUM C-REACTIVE PROTEIN LEVELS (CRP) IN HODGKIN LYMPHOMA (HL): CORRELATIONS WITH CLINICAL AND LABORATORY PARAMETERS AND PROGNOSTIC SIGNIFICANCE UNDER ANTHRACYCLINE-BASED CHEMOTHERAPY

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Background. Serum CRP levels are elevated in the majority of patients with HL at diagnosis, reflecting tumor burden and aggressive biologic behavior. Despite being an easily and frequently measured marker, data on its potential prognostic significance are extremely limited. *Aim.* To analyze the correlation between baseline CRP levels and clinical-laboratory findings and outcome of patients with HL treated with anthracycline-based chemotherapy with or without radiotherapy (RT). *Patients and Methods.* Baseline CRP levels were recorded in 496 patients with HL, who were treated with anthracycline-based chemotherapy with or without radiotherapy (RT) in 2 Centers. Baseline CRP levels were correlated with other baseline clinical-laboratory features (Spearman correlation coefficient or Mann-Whitney test), Failure Free Survival (FFS) and Overall Survival (OS) (Kaplan-Meier curves, log-rank comparison). Multivariate FFS analysis was based on Cox's proportional hazards model. CRP levels ≥5 mg/L were considered elevated. *Results.* The median value of CRP levels in the 496 evaluable patients was 21.10 mg/L; 27% and

73% had normal and elevated CRP levels respectively. Baseline CRP levels correlated with virtually all other parameters reflecting tumor burden or disease aggressiveness: There were strong correlations with advanced stages and B-symptoms (p<0.001) as well as specific extranodal sites (bone marrow, lung, liver). Baseline CRP presented a very strong correlation with ESR (Spearman's rho 0.74, p<0.001), strong correlations with haemoglobin (inverse), platelet counts and albumin (inverse) (S-rho 0.40-0.60, p<0.001) and looser correlations with white blood cell counts, serum LDH, and absolute lymphocyte counts (inverse) (S-rho <0.35, p<0.001). A trend towards a dose-response effect with FFS was observed: 5-year FFS for patients with CRP levels <5, 5-21.09, 21.1-69.99 and ≥70 mg/L (roughly the CRP quartiles) was 81%, 78%, 68% and 66% (p=0.02). The difference was more pronounced for patients with normal versus elevated CRP levels (81% versus 71%, p=0.006). Differences in OS were not significant (p>0.10). The prognostic impact of CRP on FFS was borderline in early stage patients (IA,IIA: 5-year FFS 83% versus 75% for normal versus elevated levels, p=0.09), but no difference was detected in advanced disease (68% versus 66%, p=0.48). CRP was an independent adverse prognostic factor for FFS, when adjusted for stage (III/IV versus I/II), B-symptoms and IPS factors (except of lymphocytopenia). However, baseline CRP was not analyzed along with ESR in the same model, because of their very strong correlation, raising issues of collinearity. *Conclusions.* CRP levels are elevated in ~70% of HL patients at diagnosis and correlated with FFS independently from other established prognostic factors included in the IPS. The very strong correlation between baseline CRP and ESR probably suggests that only one of them should be included in a given prognostic model. Even larger patient series should be analyzed in order to draw definite conclusions, but the final selection might be relied on specific biologic and technical advantages of either marker.

0764

BCL2 IMMUNOHISTOCHEMICAL EXPRESSION IN CLASSICAL HODGKIN'S LYMPHOMA AT DIAGNOSIS AS AN INDEPENDENT BIOLOGICAL PROGNOSTIC MARKER

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Background. Most patients with classical Hodgkin's Lymphoma (CHL) are cured with primary treatment. However, a significant proportion of them do not achieve a complete response (CR) or relapse after completion of initial therapy and need to be rescued with a second line of chemotherapy (2LC) and/or autologous or allogeneic stem cell transplantation (auto-SCT and allo-SCT, respectively). The identification of clinical and biological characteristics of these patients at diagnosis is still a challenge and most prognostic systems fail to identify a proportion of patients with worse prognosis. In this context, different groups are currently analyzing several biological markers as determinants of clinical outcome. It has been reported that Bcl2 immunohistochemical overexpression in Hodgkin's Reed Sternberg cells (HRSC) might confer a worse prognosis. Objetive: To analyze clinical outcomes following 1st line chemotherapy according to Bcl2 expression at diagnosis. *Patients and Methods.* 72 CHL patients, older than 16 years old, receiving at least 1 line of treatment, were retrospectively studied for Bcl2 expression in diagnostic samples. For this purpose, tissue sections were immunostained and semiquantitatively assessed for this marker. Cumulative incidence (CI) of 2LC was defined as primary outcome. 2LC was considered when a different treatment regimen was set up due to lack of response, relapse or failing to achieve CR following 1st line. 2LC-free survival and overall survival (OS) were also analyzed. *Results.* Main patient and clinical features are shown in Table 1. At a median follow up of 32m [30m (2-140) for Bcl2 negative patients and 35m (4-221) for Bcl2 positive patients] CI of 2LC was 17% and 54.4% for the negative and positive cohorts, respectively (p=0.04). Median 2LC-free survival was 39.5m and 30m for the negative and positive cohorts, respectively (p=0.1).Within the cohort of Bcl2 positive patients, 20/35 (57%) underwent SCT (13 auto-SCT, 4 allo-SCT and 3 allo-SCT for the treatment of post-auto-SCT relapse) while 9/37 (24%) of Bcl2 negative patients received an auto-SCT (p=0.02). OS 4 years after diagnosis was 85% negative and 75% for positive patients (p=NS). *Conclusions.* According to these preliminary results, Bcl2 expression in HRSC at diagnosis may constitute an independent biological prognostic marker in CHL patients, seemingly associated with worse outcome and need of 2LC. A longer follow up is needed in order to confirm that aggressive treatment strategies such as SCT may overcome the negative impact in survival of Bcl2 expression in this population.

Table 1.

Main patient and clinical features.		
CLASSICAL HODGKIN´S LYMPHOMAS:	Bcl2 EXPRESSION:	
	POSITIVE:	NEGATIVE:
N (72):	35	37
SEX (M/F):	20/15	21/16
MEDIAN AGE (R):	30 (17-74)	39 (18-78)
HISTOLOGY:		
Nodular sclerosis	28 (78%)	17 (46%)
Mixed cellularity	6 (19%)	12 (32%)
Lymphocyte predominant	1 (3%)	7 (19%)
Lymphocyte depleted	0 (0%)	1 (3%)
Ann Arbor stage at diagnosis:		
I-II	14 (40%)	20 (54%)
III-IV	21 (60%)	17 (46%)
Patients with unfavorable EORTC prognostic factors (stages I-II CHL): n (%)	8 (57%)	10 (50%)
Patients with IPS >2 (stages III-IV CHL): n (%)	6 (28%)	5 (29%)

0765

LYMPHOID IMPAIRMENT IS AN INDIPENDENT FACTOR USEFUL TO PREDICT INTERIM-PET ASSESSMENT IN HODGKIN'S' LYMPHOMA PATIENTS

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Background. Lymphoid suppression plays a key role in determining the tumor growth and progression in Hodgkin's' Lymphoma (HL) and this inflammatory dysregulation seems to be linked with the interim-PET assessment. CD62L reduction on the surface of lymphocytes represents a marker of immune-suppression. *Aim.* In the present work, we evaluated the CD62L expression on lymphocytes surfaces in HL patients at diagnosis and related these findings with the interim-PET outcome. *Methods.* 27 HL patients, 18 at stage I-II and 9 at stage III-IV, treated with ABVD as first line, were enrolled and PET assessment was performed at diagnosis and after two cycles (interim-PET). PET images were interpreted visually according to Dann *et al.*, 2010. 2 out of 18 patients (11.1 %) at early stage and 3 out of 9 (33%) at stage III-IV had a positive interim-PET. CD62L has been evaluated from peripheral blood at diagnosis on CD4 and CD8 T-lymphocytes and expressed as intensity mean fluorescence (IFM). T-test has been performed in order to assess if there is a statistical difference in CD62L levels between different groups. Pearson correlation has been used to define the relationship between CD62L expression and other clinical-laboratoristic parameters. Receiver-operating-characteristic (ROC) curves has been used in order to determine cut-off levels. Informed consensus has been signed. *Results.* We observed reduced levels of CD62L expression on CD8+ lymphocytes surface between HL patients at diagnosis and healthy controls matched for gender and age (57.2 vs 42.48) (p=0.007), but not on CD4+ lymphocytes (average 62.4 vs 61.7) (p=0.88). No statistical differences have been found in CD62L expression on CD4+ and CD8+ lymphocytes at early stage versus advanced stage (p=0.06 for CD62L on CD4+ and p=0.8 for CD62L on CD8+). Interestingly, CD62L expression levels on CD8+ cells was not related with interim-PET assessment in any stage, whereas CD62L reduction on CD4+ lymphocytes related with the interim-PET assessment only in patients at early stage, being in patients with interim-PET negative higher than in patients with interim-PET positive (average 68.69 vs 54) (p=0.03). Additionally, CD62L expression on both CD8+ and CD4+ lymphocytes did not relate with prognostic and inflammatory factors. *Conclusion.* On the whole, our findings suggest that HL patients have a reduced expression of CD62L on CD8 compared to healthy controls and patients at early stages who have also a reduction of CD62L on CD4+ lymphocytes have a poorer prognosis.

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HODGKIN LYMPHOMA (HL) IN PATIENTS =60 YEARS OLD: CLINICAL AND LABORATORY FEATURES, OUTCOME AFTER ANTHRACYCLINE-BASED TREATMENT AND COMPARISON WITH YOUNGER PATIENTS: A SINGLE CENTRE EXPERIENCE

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Background. Published data regarding the effect of advanced age on the outcome of patients with HL are contradictory. Reported differences in prognosis between older and younger patients may have been due to differences in treatment intensity, inadequate staging, inferior efficacy of salvage therapy and presence of comorbid conditions. *Aims.* To compare the clinical and laboratory features and the outcome of patients with HL ≥60 years treated with anthracycline-based chemotherapy (CT) or combined modality therapy with the subgroup of younger patients. *Methods.* Patients ≥60 years old, who were diagnosed with HL and treated with anthracycline-based CT±RT in our Department, were evaluated and compared with younger patients for their demographic, laboratory features and outcome. Older patients were further subdivided in 3 age groups: 60-66, 67-74, ≥75 years old, which were also compared. *Results.* Among 1004 patients with HL treated with anthracycline-based CT±RT, 113 (11%) were ≥60 years old. When compared with younger patients, they had a higher incidence of advanced disease (55% vs 45%, p=0.03). Several potential adverse prognostic features were significantly more prevalent in older patients, including B-symptoms (p=0.01), bone marrow (p=0.005) and liver (p=0.001) involvement, low serum albumin (p=0.003) and elevated β2-microglobulin levels (p<0.001). IPS was clearly higher in older patients (p<0.001). In contrast, lung involvement (p=0.004) and significant leukocytosis (p=0.057) were more prevalent in the younger age group. This probably reflected the much higher incidence of nodular sclerosis in younger patients (70% vs 41%) with corresponding lower incidence of mixed cellularity (19% vs 44%) (p<0.001). The 10-year failure free survival (FFS) was not significantly different in older patients compared to young ones (76% vs 76%, p=0.76). However, older patients had a higher incidence of deaths during treatment (0.2% vs 4.4%, p<0.001), inferior 10-year overall survival (86% vs 46%, p<0.0001), HL-specific survival (91% vs 73%, p<0.0001) and 5-year survival after failure (62% vs 14%, p<0.0001). Among the 113 patients aged ≥60 years, 47, 45 and 21 were 60-66, 67-74 and ≥75 years old respectively. These subgroups had similar baseline characteristics with the exception of worsening anaemia and more β2-microglobulin elevations with increasing age. However, deaths during treatment were 0%, 6.7% and 9.5% respectively (p=0.14), while the 5-year survival after failure was 27%, 0% and 0% respectively (p=0.0008). No significant differences were observed for FFS, while OS and HL-specific survival were marginally inferior in more advanced age groups. *Summary/Conclusions.* Despite differences in baseline clinical and laboratory features, patients with HL ≥60 years old have similar failure rates with younger ones when treated with anthracycline-based CT±RT. However, the higher incidence of deaths during treatment and the poor results of salvage therapy resulted to inferior overall and HL-specific survival rates. Within the ≥60 years group, the latter problems were pronounced in more advanced age subgroups.

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HODGKIN'S DISEASE AND HIV INFECTION (HD-HIV): PROGNOSTIC FACTORS IN 596 PATIENTS (PTS) WITHIN THE EUROPEAN GROUP FOR THE STUDY OF HIV AND TUMOURS (GECAT)

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Background. Hodgkin's disease (HD) is the most common non-AIDS defining tumour diagnosed in HIV setting. The introduction of highly active antiretroviral therapy (HAART) has opened a new prospective in the treatment of pts with HD-HIV since the better control of the underlying HIV infection allows the use of more aggressive chemotherapy regimens, including high dose chemotherapy. However, up to now prognostic factors on overall survival (OS) or time to treatment failure (TTF) have not yet been identified. *Methods.* in order to identify prognostic factors, we analyzed data on 596 pts with HD-HIV diagnosed and treated in 90 different Institution from 6 European countries from October 1983 to March 2010. All factors were analyzed for OS and TTF. *Results.* 86% of pts were male and the median CD4 cell count was 224/dl (range 3-1274); 52% of pts had mixed cellularity subtype, stages III-IV were diagnosed in 72% of cases and 55% of pts had extranodal involvement (bone marrow 35%, spleen 21%, liver 14%). *Conclusion.* We identified a new "European Score" for HD-HIV able to predict different outcomes in these patients. This score should be considered for future prospective studies.

0768

VEBEP REGIMEN IN PATIENTS (PTS) WITH HD AND HIV INFECTION (HD-HIV): FINAL RESULTS OF A PHASE II STUDY OF THE ITALIAN COOPERATIVE GROUP ON AIDS AND TUMORS (GICAT)

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Background. The outcome of pts with HD-HIV is still poor, because the duration of complete remission (CR) is generally short. To improve the prognosis of HD-HIV, a feasibility study with the VEBEP regimen and HAART was started in previously untreated HD-HIV pts. *Methods.* CT included epirubicin 30 mg/m2/day (days 1-3), cyclophosphamide 1000 mg/m2 (day 1), vinorelbine 25 mg/m2 (day 1), bleomycin 10 mg/m2 (day 3) and prednisone 100 mg/m2/day (days 1-3). HAART was given concomitantly to CT. *Results.* From September 2001 to December 2008, 73 pts have been enrolled. The median age was 41 yrs. The median CD4+ cell count was 248/mm3 and 51% of pts had a detectable HIV viral load. Stage III-IV was present in 50/71 (70%) pts. Histologic subtypes were: MC 70%, NS 20%, LD 4%, LP 2%, unknown 4%. Four toxic deaths (5%) were observed (septic shock, PCP, hepatic failure and pneumonia during neutropenia). An absolute neutrophil count <500 was noted in 60% of pts. Grade 3-4 anaemia was observed in 38% of pts and severe thrombocytopenia in 22% of pts. Twenty-two per cent of pts had febrile neutropenia with 19 documented infections in 16 pts (4 varicella, 4 bacterial pneumonia, 3 bacterial sepsis, 2 PCP, 1 cerebral toxoplasmosis, 1 oesophageal candidiasis, 1 HBV reactivation, 1 HCV reactivation, 1 prostatitis, 1 salmonellosis). CR was obtained in 49/73 pts (67%) and PR in 8/73 pts (11%). With a median follow up of 40 months (range 2-106), only 5 of CR pts have relapsed. The 3-yr OS and TTF at 24 months were 66% and 63%, respectively. An IPS greater than 2 (HR 2.87, 95%CI 1.08-7.63, p=0.03) and a ECOG-PS greater than 1 (HR 2.79, 95%CI 1.21-6.44, p=0.02) were significantly associated with a higher risk of death. *Conclusions.* Our data demonstrate that VEBEP regimen in combination with HAART is feasible and active in pts with HD-HIV. As observed in HD of the general population, the IPS is able to stratify patients with different outcome.

This study was supported by ISS grants.

0769

EARLY POSITIVE FDG-PET SCAN DO NOT CONFIRM ITS PROGNOSTIC IMPACT IN BULKY DISEASE HODGKIN LYMPHOMA PATIENTS

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Background. Hodgkin's lymphoma is a malignant diseases with the highest rate of cure particularly if diagnosed in early stage. Neverthe-

less a small proportion of patients with localized stage do not respond to therapy and progressed. *Aims.* To explore the predictive value on therapy outcome of an early evaluation of treatment response by 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) scan performed after two corses of ABVD in pts with localized Hodgkin's disease. *Methods.* From 2002, 263 localized stage Hodgkin's lymphoma pts were consecutively admitted to 13 Italian hematological centers on behalf of Fondazione Italiana Linfomi (FIL). Pts with stage I-IIA according to Ann Arbor stage, independent of presence of bulky disease were evaluated. Bulky disease pts were the object of our analysis. FDG-PET was mandatory at baseline, after two cycles and at the end of therapy. Mediastinal blood pool activity is recommended as the reference background activity to define PET positivity according to International Harmonization Project (IHP). We evaluated the progression free survival of pts starting from the time of diagnosis to relapse or progression of disease or last follow-up. No treatment variation based only on PET-2 results was allowed. *Results.* 78 pts presented bulky disease, the median age was 32 years (13-66). All pts but three were treated with combined modality. The FDG-PET performed after two cycles (PET2) was positive in 18/78 pts (23%): 8 (44%) progressed or relapsed and 10 manteined CR. By contrast 53/60 (88%) pts with a negative PET2 remained in CR. Thus the positive predictive value of a PET2 in bulky disease was very low (44%) and the negative predictive value was 88%. The sensitivity and specificity of PET2 were 53% and 84%, respectively. Radiotherapy was performed in 17 PET2 positive pts, 10 did not fail (59%), one pt with PET2 positivity did not perform radiotherapy and progressed. A FDG-PET was performed at the end of therapeutical program (PET6), all pts (10) with positive PET6 and 5/68 with negative PET6 progressed. In univariate analysis negative FDG-PET performed after two cycles (p .002) and in particular negative PET6 (0.000) were statistically correlated with a better progression free survival. With a median follow-up of 37 months (range 4-103) 73 pts are alive and 63 (81%) are free from progression. The 2-yr PFS probability for PET2 negative and for PET2 positive patients were 92% and 40% respectively (p: .002). *Conclusions.* With the IHP interpretation criteria we observed a large number of false positive PET2 in mediastinal bulky early stage Hodgkin disease. For this reason new PET evaluation methods in this subset of pts are mandatory. Moreover in bulky disease pts radiotherapy could permit to overcome the poor prognostic significance. This multicentric study confirms that a negative FDG-PET scan performed after two courses of conventional standard dose therapy in localized bulky disease pts was able to predict a favourable outcome.

0770

IMPORTANCE OF IMMATURE MYELOID CELLS IN THE PROGNOSTIC ASSESSMENT OF HODGKIN LYMPHOMA

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Background. In Hodgkin Lymphoma (HL) the interim PET (after 2 cycles of ABVD chemotherapy) is the most important prognostic factor and it is probably linked to the persistence of the reactive microenvironment that promotes tumor cells survival. CD68+ tumor associated macrophages (TAM) are increased in cases with poor prognosis, thus to be proposed as additional prognostic marker at diagnosis of HL. Their progenitors circulating in peripheral blood are well known in solid tumors as Myeloid Derived Suppressor Cells (MDSC) for their ability to suppress T-cell immune responses. In mice, MDSC are broadly defined as being GR1+CD11b+ cells capable of suppressing antigen-specific or nonspecific T cell activation, but there is no accord on the correspondent phenotype in humans. *Material and Methods.* We evaluated by flow cytometry circulating levels of immature im-MDSC (CD11b+, CD13+, CD14-, CD34+, CD45+) in peripheral blood of 37 HL patients at diagnosis and after 2 cycles of chemotherapy. We correlated MDSC to clinical findings included interim-PET response and treatment outcome, and T-cell subpopulations, including Treg (CD4+CD25+FoxP3+). *Results.* We found that at diagnosis HL patients have higher levels of im-MDSC, when compared to healthy controls (HC) matched for sex and age (p=0.0001) and these cells return to normal values within the first 2 cycles of chemotherapy in five evaluated responders. Absolute number of im-MDSC was positively correlated to Treg absolute count (r = 0,95, p 0,014), in accord to the biologic observation that MDSC are able to expand Treg compartment, but not with other T cell subpopulations. Conversely, no correlation was found with markers of inflammation (ferritin, ESR, C-RP, fibrinogen) tumor burden (stage, IPS, presence of bulky disease) and SUV-PET at diagnosis. Ten patients out of 37 (27%) had increased im-MDSC count at diagnosis (>4.5 cells/μL), with documented positive interim-PET or progression/relapse of disease for 6 of them. Three out of four patients with a positive interim-PET had increased im-MDSC count at diagnosis. The ROC-curve to predict the outcome of interim-PET based on im-MDSC cell count at diagnosis had area=0.96, p=0.0003. PFS in patients with im-MDSC>4.5 cells/μL were comparable to those interim-PET positive (p=0.57) and significantly worse than im-MDSC<4.5 cells/μL (p=0.002). *Summary/Conclusion* im-MDSC are increased in peripheral blood of HL patients at diagnosis and correlate with interim PET assessment (look at the image in attachment). They could represent an earlier and more easily accessible prognostic factor than interim-PET and an attractive therapeutic target to improve HL immunotherapy.

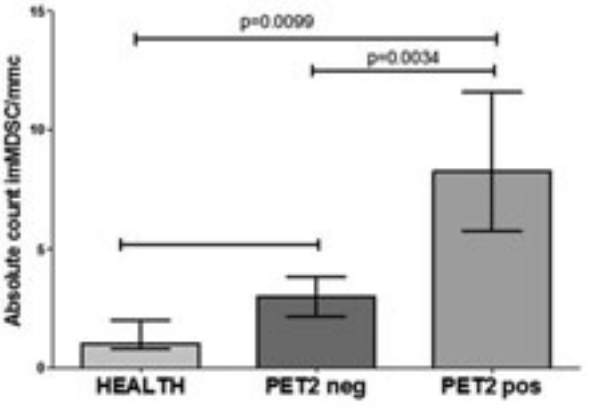

Figure 1. Absolute count of imMDSC in HC and HD.

0771

'EARLY FDG-PET' PREDICTS CLINICAL COURSE OF HODGKIN'S LYMPHOMA ALTHOUGH DOES NOT CORRELATE WITH MACROPHAGES INFILTRATION IN DIAGNOSTIC SPECIMENS

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Background. Hodgkin's Lymphoma (HL) is a highly curable malignancy that mostly affects young adults; however despite satisfactory results, about 20% of patients still die of relapsed/refractory disease and late toxic effects rate, often due to overtreatment, continue to rise with time. Consequently, the optimal treatment should be designed based on prognostic models, but currently all of them predict the outcome with imperfect accuracy. Since "early FDG-PET"and more recently tissue macrophages infiltration in diagnostic specimens emerged as powerful prognostic predictors, we hypothesized that macrophagic infiltration could be the shadow of the inflammatory microenvironment, that FDG-PET identifies in HL and that persists at early assessment in patients who will fail the treatment. *Aims.* the primary endpoint of this study was to verify the prognostic role both of "early-FDG PET" and of macrophagic infiltration, while the secondary endpoint was to test if "early-FDG PET" positivity could correlate with high macrophagic infiltration in diagnostic specimens. *Methods.* A homogeneous cohort of fifty-two patients (M/F: 28/24; median age 33 yrs) diagnosed and treated at Siena and Florence hematology departments between February 2007 and July 2010 was extracted from local databases and retrospectively analyzed. All patients had completed staging with whole body CT scan, FDG-PET and bone marrow biopsy. One patient had stage I, 20 pts. Stage II, 24 stage III and 7 stage IV. Treatment plan

consisted of 4-6 courses of ABVD and, if indicated, involved field radiation therapy. Patients repeated CT scan and FDG-PET after two cycles and after the completion of therapy. Macrophages infiltration in paraffin embedded diagnostic specimen was determined by immunohistochemistry and classified in 3 groups based on the percentage of CD68+ cells, as previously reported by Christian Steidl and coworkers. *Results.* After two cycles of ABVD, FDG-PET was negative in 41/52 patients (79%) and positive in 11 cases (21%). Overall, 40 out of 52 (77%) patients achieved a CR and 12 presented relapsed/refractory disease; interestingly among this patients "early FDG-PET2" was positive in 9/12 cases (75%). CD68 expression in diagnostic specimens was low, intermediate or high respectively in 8(15%), 25(48%) and 19 (37%) cases; moreover among patients with relapsed /refractory disease was low in 2 cases, intermediate in 8 cases and high in 2 cases. Finally, no statistical correlation was found between "early FDG-PET"and CD68 expression by Pearson's chi-square test. *Conclusions.* Our data do not seem to confirm the hypothesis of a possible correlation between "early FDG-PET"and macrophages infiltration in diagnostic specimen, while "early FDG-PET"maintains a high prognostic value.

0772

INTERIM PET-SCAN FOR EARLY RESPONSE ASSESSMENT AND POTENTIAL MODIFICATION OF TREATMENT PLAN AFTER 2 ABVD CYCLES IN ADVANCED STAGE HODGKIN LYMPHOMA (HL)

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Background. Persistent PET positivity following 2 cycles ABVD (PET-2) is a strong unfavorable prognostic factor in advanced HL. Progression Free Survival is approximately 95% vs 15% for patients with negative vs positive PET-2 respectively. The evaluation of PET-2 is based on well defined, though arbitrary, criteria, which require prospective validation. Whether PET-2-based early treatment modification can improve prognosis is unknown. However, it is known that the continuation of ABVD after a positive PET-2 is associated with an unacceptably high risk of treatment failure. *Aims.* (1) To analyze patients with advanced stage HL who were evaluated by PET-2 after ABVD x2 in relation to the final response; (2) To analyze the impact of PET-2 on subsequent treatment plan; (3) To make an attempt to validate current criteria for PET-2 positivity. *Patients and Methods.* We present a retrospective study of 26 patients with advanced stage HL (25/26 younger than 60 years). Advanced stage was defined as stage IIB with mediastinal bulk and/or E-disease, stage III or IV according to the German Hodgkin Study Group(GHSG) definition. PET-2 was evaluated using the established 5-grade scale (see below). *Results.* Median age of the patients was 28 years (19-72), 69% were men, 77% had nodular sclerosis, 3, 10 and 13 had stage II, III and IV respectively, and 70% had B-symptoms. The median value of IPS was 2.5 (0-5). 81% of the patients (21/26) had a negative PET-2: It was completely negative in 10 patients; 8 patients had residual uptake <liver; 3 patients developed new sites with an alternative explanation available and regression of the initial sites of disease (to uptake <liver). PET-2-Negative Patients: A final PET examination was available in 15 patients (too early for 6 patients): 14/15 were PET-neg, but 1/15 developed frankly progressive disease at the end of treatment. At a median follow up of 13 months, only 1/14 PET-neg patients had relapsed. Overall 2/21 patients have failed so far: 1/10 with completely negative PET-2 and 1 out of 8 patients, who had mild (<liver) residual FDG uptake. PET-2-Negative Patients: Among 5 PET-2 positive patients, only one was switched to BEACOPP-escalated x6 (continuous CR for 16 months) and 4 continued n ABVD (2 converted to PET-neg and remain in CR and 2 progressed at 4 and 7 months). If all of the 26 above patients had been treated with BEACOPP-escalated x6-8, they would have had received 156-208 chemo cycles. In fact, only 1 patient received 6 cycles of BEACOPP-escalated; at most 30 cycles would have been administered, if all PET-2 positive patients had been switched to BEACOPP-escalated. *Conclusions.* According to our preliminary data, current criteria for PET-2 evaluation appear to be valid. During this initial phase of incorporation of PET-2 in treatment strategy, a negative result appeared to be reassuring. However, treating physicians tended to be reluctant to make early modifications in the treatment plan, in case of positive PET-2. A more homogeneous strategy according to predefined interim PET positivity criteria might prevent overtreatment and, hopefully result to improved outcome in advanced HL.

0773

LONG TERM RESULTS OF STANFORD V REGIMEN AND HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) IN 59 PATIENTS (PTS) WITH HD AND HIV INFECTION (HD-HIV)

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Background. The introduction of HAART has significantly improved the outcome of pts with HD-HIV. However there are no data on the long term follow-up of HD-HIV pts treated with conventional chemotherapy (CT) regimens. In 2002, we reported the results of a prospective phase II study with the intensive 12-week CT with adjuvant radiotherapy (Stanford V) and concomitant HAART in 59 pts (Spina *et al.* Blood 2002;100:1984-1988). *Methods.* To analyze the long term outcome of patients included in the Stanford V and HAART protocol. *Results.* The median follow-up is 67months (range 3-156 months The 5-yr overall survival (OS), freedom from progression (FFP), disease free survival and event free survival are 54%, 52%, 60% and 37%, respectively. The 5-year OS is significantly different in pts with an international prognostic score (IPS) >2 in comparison to that of pts with an IPS <3 (84% vs 36%, p= 0.0005). Similarly, the percentages of FFP at 5 years in these groups are 72% and 45% (p= 0.03). *Conclusions.* Our data confirm the long term efficacy of Stanford V regimen in combination with HAART in HD-HIV. However, Stanford V is significantly less effective in pts with IPS>2 and therefore new strategies be tested in this setting.

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0774

INTERIM FDG-PET SCANNING IN PATIENTS WITH HODGKIN LYMPHOMA AND HIV PREDICT RESPONSE TO ABVD CHEMOTHERAPY

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Background. There is approximately a 10-fold increase in the incidence of Hodgkin lymphoma (HL) in patients with Human Immunodeficiency Virus (HIV) and patients often present with advanced disease. A number of studies have demonstrated a good outcome after ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) chemotherapy combined with highly active antiretroviral therapy (HAART) in patients with HIV-HL. There is still much debate whether more aggressive regimens such as escalated BEACOPP are more appropriate in patients with advanced disease. The prognostic significance of interim PET in HIV negative patients with HL is now well established. However, there is no published data regarding interim PET scanning in pa-

tients with HL in the HIV setting. *Aims.* Our objective was to evaluate the role of interim FDG-PET imaging during ABVD chemotherapy in determining response assessment for patients receiving first-line treatment for advanced HIV-HL and to assess the outcome in such patients. *Patients and Methods.* 21 patients with HIV-HL from five UK centres treated with ABVD and concomitant HAART were included. Interim PET after two or three cycles of ABVD (PET-2 or PET-3) was carried out in all patients. At the time of therapy, the median age was 42 (range 32-60; 18 M: 3 F). *Results.* Median CD4 count at diagnosis of HL was 187 cells/μl (range 33-995 cells/μl) and majority of patients (76%) had an undetectable plasma HIV viral load (VL). All patients presented with either advanced stage disease (n = 18) or stage 2a disease with adverse prognostic factors (n = 3). Just under half (48%) presented with stage IV disease. All cases were of classical HL and histologic subtypes included: mixed cellularity 8 (38%), nodular sclerosing 7 (33%), unknown 6 (29%). Median Hasenclever IPS was 3 (range 1-6). Of 21 evaluated patients, 20 achieved complete remission (CR) after first-line therapy. Interim PET was negative in 86% (18/21) of patients. Treatment failure was seen in 1 of the 3 interim PET positive patients and none of the 18 interim PET negative patients. The 2-yr progression free survival for patients with a positive interim PET was 67% and for negative interim PET was 100% (p = 0.012). After a median follow up of 20 months (range 5-43), all patients are in continued complete remission (1 after second line chemotherapy for relapse) with no deaths. *Conclusions.* Our findings suggest a negative interim PET maybe highly predictive of successful treatment outcome in Hodgkin's lymphoma even in the setting of HIV disease.

0775

RISK-ADAPTED TREATMENT WITH BEACOPP AND ABVD IN UNFAVORABLE HODGKIN'S LYMPHOMA: A SINGLE CENTER EXERIENCE

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Aims. To describe the results of treatment of 66 patients with Hodgkin's lymphoma (HL) and advanced disease stage or unfavorable prognosis, by using both, BEACOPP and ABVD regimens, in a patient-based, risk-adapted, tailored treatment approach. *Patients and Methods.* Patients were 40 males and 26 females, with a median age of 31 years (range 16-70 years). Sixty-three had classical HL (nodular sclerosis 35, mixed cellularity 22, lymphocyte-rich 5, lymphocyte-depleted 1), two had composite lymphoma and 1 lymphocyte-predominant HL. Disease stage was IIA with multiple (>3) sites of involvement in 12 (18.2%, IIBE: 3), IIB in 11 (16.7%), IIIA in 8 (12.1%), IIIB in 15 (22.7%, IIIBS: 3), IVA in 5 (7.6%) and IVB in 14 (21.2%, IVBE: 5). At presentation 26 patients had B symptoms (39.4%), 17 had bulky mediastinal disease (25.8%), 13 (19.7%) pulmonary- and 8 (12.1%) liver involvement, 40 (60.6%) exhibited anemia (Hb<12 g/dl), 17 (25.8%) leucocytosis >16 x 109/l, 23 (34.8%) thrombocytosis >400 x 109/l, 10 (15.2%) lymphocytopenia (<0.7 x 109/l), 33 (50%) had elevated serum LDH, 13 (19.7%) elevated CRP, 12 (18.2%) elevated beta2-microglobulin and 12 (18.2%) reduced serum albumin (<4 g/dl). The IPS was 0-1 in 20, 2-3 in 36 and >3 in 10. First-line treatment consisted of BEACOPP alone in 46 patients (69.7%), whereas 8 patients (12.1%) were shifted to ABVD, after the achievement of CR, following 2-4 cycles of BEACOPP. ABVD alone was initially administered in 13 patients with stage II (20%), but 7 of them, who did not achieve complete remission (CR) after 4 cycles, they were shifted to BEACOPP. The remaining 6 patients were treated with BEACOPP at their first relapse. *Results.* Overall, 55 patients achieved CR (84.6%), 47 of the 52 evaluable patients, who received BEACOPP as first-line treatment (90.4%, group A) and 8 of the 13, who initially were treated with ABVD (61.5%, group B). Seven additional patients, 5 from group A and 2 from group B achieved partial response (overall response rate 95.4%), whereas 3 patients did not respond. Seven of the 10 patients with refractory or residual disease and additional 5, who later relapsed, received second/third-line treatment followed by high-dose therapy (BEAM) plus autologous stem-cell transplantation (ASCT). Three patients, who achieved CR with BEACOPP, before ASCT remained in continuous CR, additional 3 achieved CR only following ASCT, one is in third CR after relapse, following ASCT, one is alive with active disease and 4 have died. After a median of 62.5 months (range 0.5-154 months) 57 patients are alive, 54 of them disease-free, two have active disease and one is still under treatment. Nine patients have died, 5 of them from infectious complications during chemotherapy-induced aplasia, 3 from disease related complications and 1 from fulminant secondary AML while in remission from the HL. *Conclusions.* Results from this group with advanced or unfavorable HL demonstrated that risk-adapted treatment by using both, ABVD and BEACOPP regimens is feasible and is accompanied by high CR rates, even when CR is not achieved after the initial 3-4 cycles of chemotherapy.

0776

THE PROGNOSTIC SIGNIFICANCE OF SPECIFIC ORGAN INVOLVEMENT AND THE NUMBER OF INVOLVED EXTRANODAL SITES IN ADVANCED STAGE HODGKIN'S LYMPHOMA

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Background. Extranodal dissemination has independent prognostic value within advanced stage Hodgkin lymphoma (HL). It remains controversial whether a specific organ involvement and the number of involved extranodal (EN) sites are prognostically unfavorable. *Aims.* To analyze the prognostic value of specific organ involvement and number of involved EN sites in advanced stage classical HL patients (pts). *Methods.* In a cohort of 100 advanced stage classical HL pts treated with ABVD (1997-2005) we analyzed the prognostic relevance of bone marrow, lungs and liver involvement, as well as two or more involved EN localizations. The median follow up was 7 years. Their significance was tested according to response rate an overall survival (OS). *Results.* The distribution of EN dissemination was: 28 pts with bone marrow infiltration, the lungs involved in 14 pts, the liver involved in 12 pts and other sites involved in 4 pts. Two or more EN localizations involved were found in 14 pts. Complete remission rate was significantly lower in pts with liver involvement (50% vs 83%, p=0.017). A shorter OS was associated with bone marrow (p=0.003), lungs (p=0.022) and liver (p=0.001) involvement. Furthermore, two or more EN sites had adverse effect on OS (p=0.000). Lung involvement and EN two or more localizations had adverse effect on event free survival (EFS) (p=0.000; p=0.001), respectively. Cox's multivariate model revealed EN two or more localizations as a significant independent prognostic factor for OS (p=0.000) and lungs involvement for EFS (p=0.000). *Summary/Conclusions.* Advanced stage classical HL pts with EN two or more localization and lungs involvement are at higher risk of treatment failure and might be eligible for more effective treatment approach.

0777

OUTCOME AND PROGNOSTIC FACTORS IN HODGKIN LYMPHOMA (HL) PATIENTS WHO RELAPSE AFTER HIGH DOSE THERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION (HDT/ASCT)

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Background. Patients with HL who relapse after HDT/ASCT are considered incurable. *Aims*. To evaluate the outcome and prognostic factors of 45 HL patients, who relapsed after HDT/ASCT. *Methods*. Out of 95 consecutive patients with primary refractory/relapsed HL undergoing HDT/ASCT at a single unit from March 1996 to January 2010, 45 patients who relapsed after HDT/ASCT were recorded. Thirty-one out of 45 (69%) were males, their median age at ASCT was 28 years (19-54), 18 (40%) underwent HDT/ASCT at first relapse, 5 (10%) for multiple relapses and 23 (50%) for primary refractory disease. Prior to HDT/ASCT, 14 (31%) were in complete remission (CR), 18 (40%) in partial remission (PR) and and 13 (29%) were chemoresistant. *Results*. Median time between ASCT and the following relapse was 6.3 months (1-54). At relapse after ASCT median hemoglobin was 11.8 g/dL (7.6-14.4), 32%, 18%, 24% και 26% had clinical stage I, II, III και IV respectively and 27% had B symptoms. The median number of involved sites was 3 (1-12), 16% had bulky and 41% extranodal disease. The most common involved extranodal sites were lungs, bones and soft tissue.

Patients received the following therapies: 47% radiotherapy (RT), 25% chemotherapy containing gemcitabine/vinorelbine +/- liposomal doxorubicin, and 22% MOPP-like chemotherapy. Furthermore, 5 patients underwent a second ASCT and 2 patients allogenic stem cell transplantation. At a median follow up of 36 months, 3-year freedom from second failure (FF2F) and 3-year overall survival (OS) after relapse was 23% και 64%, respectively. Thirteen out of 45 patients died of HL, 4 due to secondary myelodysplatic syndrome/acute non lymphoblastic leukemia, 15 are alive with active HL and 13 are alive in CR. Prognostic factor analysis for FF2F disclosed that a short (<12months) interval between ASCT and relapse (p<0.002), chemoresistance prior to ASCT (p<0.002), anemia (p<0.004) and B symptoms at relapse post ASCT (p<0.02) were of unfavourable prognostic significance. Chemoresistance prior to ASCT (p<0.005), anemia (p<0.03) and B symptoms at relapse (p<0.0001) were unfavorable prognostic parameters for OS, as well. In addition, age ≥45 (p<0.03) proved as a poor prognostic factor for OS. Despite the limited number of patients, multivariate analysis revealed that chemoresistance prior to ASCT and B symptoms at relapse post ASCT were independent prognostic factors for FF2F and OS. *Conclusions*. Patients with HLwho relapse after ASCT have a poor prognosis. However, the natural history of the disease remains long, with 2/3 of the patients remaining alive at 3 years after relapse. Chemoresistance prior to ASCT, B symptoms and anemia at relapse post ASCT, as well as, relapse in <12 months after ASCT, are unfavorable prognostic parameters.

0778

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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Aim. To compare the efficacy and toxicity of the treatment with B EACOPP-14 and B EACOPP-esc regimens in patients with Hodgkin's disease (HD) from high risk group in prospective randomized study. *Methods*. since September 2008 103 patients in 6 Ukrainian centers from 18 to 65 years old (median 29 years), 48 male and 55 female with stage B with ≥1 unfavorable factors and stage III-IV were randomized to receive the treatment with B EACOPP-14 (48 pts, 301 cycles, 6.27 cycles per patient) and B EACOPP-esc (55 pts, 315 cycles, 5.95 cycles per patient). The treatment efficacy in both groups was evaluated after 4, 6 and 8 cycles by heson criteria (1999, 2007). Toxicity rate was evaluated with NCI-CTC. 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. *Results*. Maximal observation period in both groups is 29 months. Overall response rate (ORR) after the completion of the treatment was 97.9% in the group of BEACOPP-14 and 98.1% in the group of BEACOPP-esc; p>0.05 (Table). In the group of historical control ORR was 80.39%; that is significantly lower than in both studied groups; p<0.05. The therapy in 4 patients in the group of BEACOPP-14 and 6 patients in the group of BEACOPP-esc was changed to ABVD according to the toxicity. The most frequent toxicity type in both groups was hematological toxicity of different grades (72.8% in the group of BEACOPP-14 and 67.6 in the group of BEACOPP-esc, p>0.05). The special feature of BEACOPP-14 treatment was significantly higher rate of anemia: 25% compare to 12.5% in the group of BEACOPP-esc. In 7.5% the BEACOPP-14 cycles were not completed due to neutropenia of 4th grade. 1 patient in the group of BEACOPP-esc died because of infectious complication. Nonhematological toxicity was observed in 45.6% cycles of BEACOPP-14 and in 51.5% cycles of BEACOPP-esc. The most frequent nonhematological complications were nausea and vomiting (Table). *Conclusion*. The therapy efficacy in both groups of BEACOPP-14 and B EACOPP-esc was higher than in the group of historical control (treatment with ABVD). Both comparative regimens show almost equal treatment efficacy and toxicity rates in patients with HD of the poor prognosis group. However, the results are preliminary and should be confirmed in larger number of patients and with a longer follow-up.

Table 1. Efficacy and toxicity rate.

Data	BEACOPP-14, %	BEACOPP-esc, %	Significance
Early relapse	0 patient	2 patient	
Progression during the therapy	1 patient	1 patient	
ORR, 6 cycles	97.9	98.1	
ORR, 8 cycles	97.9	98.1	
CRR, 6 cycles	86.6	81.25	p>0.05
CRR, 8 cycles	97.9	98.1	
Neutropenia	35.5	37.3	
Febrile neutropenia	8.3	6.6	
Nausea and vomiting	33.3	45.6	
Mucositis	12.0	6.9	
Anemia	25	12.5	p<0.05

0779

GNRH-ANALOGOUS IN YOUNG PATIENTS AFFECTED BY HODGKIN DISEASE (HD) TREATED WITH ABVD

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Background. The mechanism by which chemotherapy causes long-term gonadotoxic effect is not completely understood. Few data are available on features predicting infertility risk and gonadic dysfunction. Despite there aren't standardized methods evaluating fertility in this subset of patients, resumption of the menstrual cycle is considered as expression of the ovarian reserve. This resumption represents a desirable goal in long-term survivals of treated Hodgkin's Lymphoma, in order to protect from unfavorable effects of premature amenorrhea. There are currently no approved procedures certainly effective in trying to preserve fertility (ovarian suppression by treatment with estro-progestinic or gonadotropin-releasing hormone agonist analogue, ovum or ovarian tissue cryopreservation). The gonadic function in patients with HD seems to be adversely affected by therapies containing alkylating agents or procarbazine, by employed doses (BEACOPP and BEACOPP esc.), by advanced stage disease and by age. Conversely there is no evidence on the gonadotoxic potential of ABVD protocol. Matter under discussion is the induction of ovarian suppression by administration of GnRH analogue (GnRH-a) in order to reduce the risk of ovarian failure associated with chemotherapy. *Aims.* Aim of this study was to evaluate the gonadotoxic potential (High/Intermediate/Low/No risk) of ABVD protocol and to examine the ovarian reserve after chemotherapy in a group of patients with HD treated according to protocol ABVD alone or in combination with GnRH-a, defining as primary end-point recovery and regularity of menstrual cycles. *Methods.* We studied 31 patients aged between 16 and 35 years (mean age 23.5 years), affected by HD (70% nodular sclerosing; 30% mixed cellularity; 93% stage I-II, 15% of which with bulky disease; 7% stage III-IV) homogeneously treated with 4-6 cycles ABVD and not receiving pelvic radiotherapy. At diagnosis no patients had disorders of the menstrual cycle. Twenty-two patients received prophylaxis with GnRH-a (triptorelin / DecapeptylTM). Amenorrhea, irregularity and regularity of menstrual cycles were evaluated as end-points events in both groups (GnRH-a +/-): analysis was performed using Fisher exact test and evaluation of the relative risk (RR). Gonadotoxic potential of ABVD protocol was compared to homogeneous historical group of HD (17 pts) receiving protocol MOPP/ABVD (GnRH-a +/-) and efficacy of prophylaxis with GnRH-a evaluated on historical group too. *Results.* In our group of patients the incidence of amenorrhea after ABVD protocol was 1/31. The analysis for the event amenorrhea between the groups GnRH-a +/- showed no significant differences; the analysis conducted for the events irregularity and regularity of menstrual cycles showed differences within the limits of statistical significance between groups GnRH-a +/- (p = 0.07). Significant was the assessment of the relative risk: for amenorrhea RR 3.75 (CI 2.1-6.8), irregular cycles RR 6.3 (CI 1.1-8.9) and regular cycles RR 0.32 (0.1-0.9). ABVD group vs historical group had lower risk for amenorrhea (p=0.01) and GnRH-a decreased gonadotoxic effect (amenorrhea) of high-risk chemotherapy (MOPP/ABVD/GnRH-a vs MOPP/ABVD p= 0.04). *Con-*

clusion. These data suggest low-intermediate gonadotoxic potential of ABVD protocol and indicate a protective effect (RR>1) of inhibition of the hypothalamic-pituitary axis by the administration of GnRH-a on the risk of amenorrhea and irregular menstrual cycles after treatment with ABVD protocol.

0780

SALVAGE THERAPY FOR RELAPSING OR RESISTANT HODGKIN LYMPHOMA: RETROSPECTIVE ANALYSIS OF RESULTS UTILIZING THREE DIFFERENT DEBULKING REGIMENS INCLUDING DHAP, IGEV OR IGEV FOLLOWED BY ESCALATED BEACOPP

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Background. Peripheral blood autologous stem cell transplant (PBASCT) is the standard therapy for patients with relapsed or refractory Hodgkin lymphoma. The aim of second-line chemotherapy regimens utilized in this subset of patients before transplant is to mobilize peripheral blood stem cells and reduce tumor burden, but no consensus has yet been reached on which scheme is the best one. *Aims.* To retrospectively evaluate our experience with different debulking regimens including DHAP, IGEV and IGEV followed by escalated BEACOPP. *Patients and Methods.* We reviewed 90 patients treated from April 1998 to December 2009 for resistant or relapsed Hodgkin lymphoma. Second-line chemotherapy changed over-time from DHAP x 2 cycles (group A) to IGEV x 1 followed by escalated BEACOPP x 2 (group B) and IGEV x 4 cycles (group C). Patient features are illustrated in the Table.

Table 1.

Features	DHAP (A)	IGEV + BEACOPP (B)	IGEV (C)
Total	28	23	39
Median age (yrs)	31 (16-67)	24 (16-61)	31 (15-61)
Early relapse (< 12 mos)	5	4	12
Late relapse (> 12 mos)	4	6	13
Resistant	19 (68%)	13 (57%)	14 (36%)

Overall, first-line CT was ABVD in 83 and MOPP alternated to ABVD in 7 patients. Involved-field RT had been given in 32 patients. PBSC mobilization was carried-out after the first cycle of DHAP or the first cycle of IGEV, both in group B and C. Survival was calculated with the actuarial method. *Results.*The overall response rate (ORR) of group A was 57% (CR in 8 of 28 pts: 29%), of group B 82% (CR in 15 of 23 pts: 65%) and of group C 77% (CR in 11 of 39 pts: 28%). No patient failed to mobilize PBSC; the median number of harvested CD34+ cells after DHAP (group A) was 11.8 x 106/Kg (range: 2.6-48), and after IGEV (groups B and C) 12.3 x 106/Kg (range: 3.7-50.8). Overall, 81 patients underwent PBSCT (25 in group A, 21 in group B, 35 in group C); 34 of them (42%) were given additional chemotherapy before BEAM conditioning: 14 (50%) in group A, 4 (17%) in group B, 16 (41%) in group C. The 2-yr PFS was 35%, 56% and 34% in groups A, B and C; in the same groups, the 5-yr overall survival was 81%, 78% and 63%, respectively. *Conclusions.* The retrospective nature of this survey does not allow firm conclusions on the best second-line therapy in Hodgkin lymphoma. Altogether, the three different approaches allowed an overall response rate of 72%, with a 2-yr PFS of 42%, and no difference in the 5-yr overall survival between the three groups of therapy was observed. In our experience, both IGEV and DHAP showed a good mobilizing capacity, while the inclusion of escalated BEACOPP produced a better CR rate compared to DHAP and IGEV and a higher 2-yr PFS rate.

0781

PATIENTS WITH REFRACTORY AND RELAPSED HODGKIN'S LYMHOMA COULD BENEFIT FROM AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. The autologous haematopoietic stem cell transplantation (AHSCT) in the last two decades has became the optimal therapeutic method in patients (pts) with refractory or relapsed Hodgkin's lymphoma (HL). *Aims.* The aims of our study was the estimation if refractory and relapsed Patients with Hodgkin's lymhoma could benefit from autologous haematopoietic stem cell transplantation. *Methods.* We report the results of a retrospective analysis of 78 patients (pts) with HL (37 female and 41 male; mean age 29 years) treated with AHSCT after failure of conventional chemotherapy between November 1997 and September 2010. Among them there were 77.4% of pts with nodular sclerosis, 19.4% with mixed cellularity and 3,2% with lymphocytic depletion. HL stage at diagnosis by Ann Arbor criteria was IV in 53.0% of pts, III in 22.7% and II in 24.3%. The median lines of chemotherapy was 3.5 and the standard scheme of first line chemotherapy was ABVD. 47.7% of pts were transplanted due to refractory (non-responders pts, NR) and 52.3% due to relapsed lymphoma (29.2% of relapse after complete remission and 23.6% of relapse after partial remission; CRR and PRR respectively). The standard regimen of first-line mobilization was ETO (1.6 g/m^2). The Ara-C regimen (2.4 g/m^2) was applied in remobilization and in first-line of mobilization in heavily pretreated pts (radiotherapy or more than 3 line of chemotherapy). Pts with refractory, active disease were collected after ESHAP regimen. The dose of G-CSF was 5-10 mcg/kg. The conditioning regimen was BEAM in all pts. The median of CD34+ transplanted cells was 3.5x10^6/kg. All patients engrafted. There was no case of death during mobilization and transplantation procedure. *Results.* Patients were evaluated on day 100 after AHSCT. There were 72.3% of pts with CR, 14.3% with PR and 12.5% with disease progression (DP). The median overall survival (OS) of all pts was 42.5 months, those with CRR was 47.2 months, those with PRR was 52.6 months, and those with NR was 34.5 months. There was no significant difference between those three groups (p=0.19). The median overall survival (OS) of pts with CR in 100 days after AHSCT was 46.0 months, those with PR was 47.1 months, and those with DP was 25.1 months. The difference between tree groups was significant (p=0.001). *Conclusions.* In conclusion, AHSCT is safe and have a high efficacy in pts with refractory and relapsed HL, who achieve remission in 100 days after transplantation.

Immune thrombocytopenia and other platelet disorders

0782

DETERMINANTS OF PERSISTING THROMBOCYTOPENIA IN PATIENTS WITH TYPE 1 GAUCHER DISEASE TREATED WITH IMIGLUCERASE FOR 4-5 YEARS

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Background. Thrombocytopenia in type 1 Gaucher disease (GD1) may result in surgical, obstetrical and spontaneous bleeding. Thrombocytopenia generally responds rapidly to imiglucerase enzyme replacement therapy but, in rare cases, the platelet response seems to be delayed and thrombocytopenia persists. *Aims.* To identify patient characteristics associated with (and potentially predictive of) persisting thrombocytopenia despite therapy. *Methods.* Patient characteristics associated with persisting thrombocytopenia were investigated by retrospective analysis of data from the International Gaucher Group (ICGG) Registry. A total of 1,016 GD1 patients with an intact spleen, for whom date of diagnosis, therapy initiation, and platelet counts were known and who received continuous imiglucerase therapy for 4-5 years, were classified into four groups by last platelet count: >120 x 10^9/l (n = 772); >100 to <120 x 10^9/l (n = 94); >80 to <100 x 10^9/l (n = 80); and <80 x 10^9/l (n = 70; 20 with <60 x 10^9/ l). Patients were characterized by initial and cumulative average imiglucerase dose, BMI, platelet count, anemia, hepatomegaly, splenomegaly, and skeletal assessments at baseline and after 4-5 years of therapy. Possible associations with persisting thrombocytopenia were tested using a multivariate proportional odds regression analysis, which adjusted for age at diagnosis and therapy initiation, genotype, gender, year of diagnosis, and year of therapy initiation. A statistical analysis of the relationship between spleen volume and platelet count was performed across all patients with reported spleen volumes (n = 660; 2,299 observations) using scatter plots with a smoothing curve calculated through the loess method. Because methods used to determine spleen volume are not always captured by the ICGG Gaucher Registry and may be based on estimates from ultrasound or palpation, a scatter plot was also performed using data from 50 patients (538 observations) attending the AMC, the Netherlands, irrespective of treatment status and for whom spleen volume had been determined accurately by CT or MRI. *Results.* Correlations were found between persisting thrombocytopenia and baseline platelet count (<80 x 10^9/l), splenomegaly, and anemia (all p<0.0001). After 4-5 years, correlations were found with splenomegaly (p<0.0001), anemia (p<0.0001), WBC (p = 0.049), hepatomegaly (p = 0.004) and bone pain (p = 0.035). Analysis of the relationship between spleen volume and platelet count across patients in the ICGG Gaucher Registry suggested an exponential relationship between the two parameters. Analysis of spleen volume versus platelet counts in patients attending the AMC, demonstrated a similar curve indicating that platelets increase only when spleen volume has decreased substantially. *Summary/conclusions.* The strong association between splenomegaly, anemia and thrombocytopenia at the initiation of therapy and persisting thrombocytopenia after 4-5 years of treatment in a minority of patients suggests that extensive spleen involvement before therapy initiation may be predictive of persistent thrombocytopenia. The exponential relationship between platelet number and spleen volume suggests that the initial platelet response may be slow in cases of extensive splenomegaly.

0783

PREOPERATIVE USE OF ROMIPLOSTIM IN THROMBOCYTOPENIC PATIENTS WITH CHRONIC HEPATITIS C AND LIVER CIRRHOSIS

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Background. Romiplostim is a thrombopoietin mimetic "peptibody" comprising a human immunoglobulin IgG1 Fc domain covalently linked at each of its two C-terminals to two 14-amino-acid peptides that bind to and stimulate the thrombopoietin receptor. Continuous treatment with Romiplostim increases platelet counts in patients with immune thrombocytopenia for up to 5 years, with few adverse effects. Hepatitis C virus (HCV) represents the second most common blood-borne illness in the world, affecting up to 2% of the world's population. Egypt reports the highest prevalence of HCV worldwide, ranging from 6% to more than 40% with an average of 13.8%. Thrombocytopenia, usually from Hypersplenism (and possibly from altered thrombopoietin metabolism or antiplatelet antibodies), is common in patients with cirrhosis. *Aim.* To detect the efficacy of Romiplostim use in thrombocytopenic patients with chronic hepatitis C and liver cirrhosis preoperatively. *Methods.* Our study was performed on 12 patients in the Electricity Hospital, Cairo, Egypt, having chronic liver disease with liver cirrhosis and they were classified as Child-Pugh score C with thrombocytopenia. All the patients started Romiplostim injections at a dose of 2 mcg/kg once weekly for four weeks. CBC, liver and kidney function tests, bone marrow aspirate and biopsy were done to all patients at start of the study and at day 90. CBC was done for all patients every 3 days till day 90. *Results.* The mean baseline platelet count was 31x10^3 /microL (range 21x10^3 - 42x10^3). Eleven of the 12 studied patients showed a highly significant (p value 0.000) increase in the platelet count with a maximum peak of 220x103/microL, occurring between days 15 - 33 in most patients. These patients proceeded to their planned surgery which included cataract, hernia and fracture fixation. No postoperative bleeding complications were recorded. There was no change in the reticulin grade in bone marrow of any of the patients. There were no significant change in liver function but there was a significant increase in the bilirubin level. One patient did not respond by platelet count increase during the time schedule of the study and hence did not undergo surgical intervention. *Conclusion.* Romiplostim can be used under close follow up in chronic hepatitis C patients with liver cirrhosis and severe thrombocytopenia preoperatively in a dose of 2 mcg/kg once weekly for four weeks. Additional studies are necessary to define the optimal dose and schedule of romiplostim.

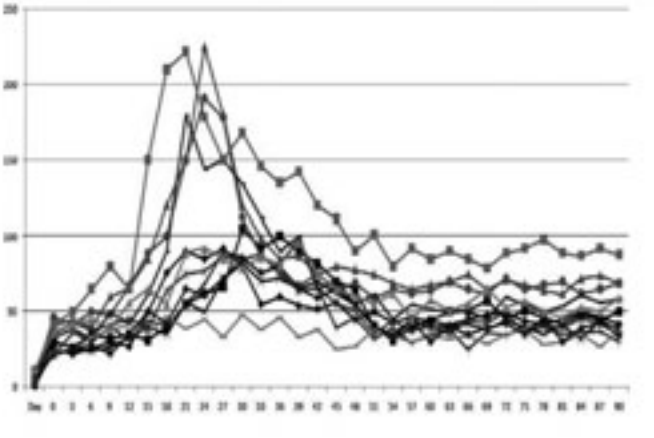

Figure 1. Platelet count in patients.

0784

PRESENCE OF ADAMTS13 AUTOANTIBODIES IN OBESE SUBJECTS AS A POSSIBLE LINK BETWEEN OBESITY AND THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background. Thrombotic thrombocytopenic purpura (TTP) is a rare but often fatal disorder, whose mechanisms are yet to be fully understood. It is characterized by widespread thrombosis in the arterioles and capillaries of multiple organs and is mainly associated with severe deficiency of ADAMTS13, a disintegrin and metalloprotease with thrombospondin (TSP)-1 repeats. This enzyme cleaves high molecular weight von Willebrand Factor1 (ULVWF) multimers freshly released from activated endothelial cells to smaller and less active forms. Recently it has been reported that morbid obesity represents a risk factor for TTP, but the possible mechanisms underlying the phenomenon are still unknown. In obesity a low grade chronic inflammation state exists, with increased levels of adipokines, among them thrombospondin-1 (TSP-1). *Aims.* We aimed to investigate possible mechanisms linking obesity and risk of developing TTP. We also tried to understand the role of TSP-1 as a possible causative factor of TTP in obesity, given its structural homology with ADAMTS13 and its high expression by adipose tissue. *Methods.* 80 obese and 39 lean subjects were characterized by anthropometric, metabolic and inflammatory parameters and compared to 32 patients with TTP in clinical remission. ADAMTS13 antigen levels and activity, ADAMTS13 autoantibodies, TSP-1 and various cytokines' levels were measured. *Results.* 21.3% of obese patients had a positive titre of non-inhibitory ADAMTS13 autoantibodies, while all lean subjects were negative. TSP-1 levels were significantly higher in obese and patients with TTP than in lean subjects. TSP-1 levels in obese patients were inversely correlated with ADAMTS13 activity. Moreover anti-ADAMTS13 antibodies cross-reacted with TSP-1 in obese subjects and patients with TTP in clinical remission. *Conclusions.* The presence of non-inhibitory anti-ADAMTS13 autoantibodies in some obese subjects may be induced by increased TSP-1 levels. Autoantibodies are probably directed against TSP-1 and cross-react with ADAMTS13 thrombospondin-domains. Over time somatic hypermutations could generate inhibitory activity, leading to an acute episode of TTP.

0785

MARKED VARIABILITY IN PLATELET RESPONSE TO ASPIRIN IN HEALTHY INDIVIDUALS: A CROSSOVER STUDY OF PLATELET FUNCTION TESTS

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Background. The Antiplatelet Trialists' Collaboration reported a 25% reduction in death, MI, CVA in high-risk patients treated with aspirin. 'Aspirin resistance' describes recurrent events in aspirin-treated patients with reported rates between 0.4 and 83% depending on the assay used. This study assessed aspirin response in healthy individuals and the performance of various assays. *Aims.* This study assessed the prevalence of aspirin resistance in a cohort of healthy individuals. In addition, the performance of various platelet function tests was examined. A genetic substudy was also performed. *Methods.* A repeated measures, crossover trial was performed in healthy aspirin-naive subjects aged 18-60 years. Written informed consent was obtained. Ethical approval was granted by the Office for Research Ethics Committee NI (ORECNI). The study was funded by Royal Hospitals Trust, Belfast and Northern Ireland Chest Heart and Stroke Association (NICHSA). Subjects were randomised to aspirin dose (75mg or 300mg) and sequence (ABBA, BAAB, ABAB, BABA, A=Aspirin, B=Placebo). The study consisted of 4 3-week treatment periods. Testing (Optical Platelet Aggregation, PFA-100, VerifyNow, serum TXB2 and urinary 11-dTXB2) was performed at baseline and at the end of each treatment period. Standard definitions of aspirin resistance were used. OPA (AA 0.5) was deemed the 'gold standard' (max aggregation ″20%). Compliance was deemed satisfactory at interview. Statistical analysis was performed using Windows SPSS17. *Results.* The overall rate of suboptimal aspirin response was assay-dependant and varied greatly from 2.4% (OPA AA) to 63.5% (OPA ADP). Only 3 subjects were 'aspirin resistant' (via OPA AA) on all occasions. Overall sensitivities ranged from 27.5% (OPA ADP) to 87.5% (serum TXB2). Overall specificities ranged from 85.4% (VerifyNow) to 95% (serum TXB2). In addition, selection of alternative 'cut-off' values chosen for the PFA-100, OPA ADP and serum TXB2 produced marked variation in the calculated prevalence, sensitivities and specificities of these assays. No association was found between aspirin resistance and genetic polymorphisms. *Summary/Conclusions.* Response to aspirin shows significant inter- and intra-individual, inter-assay and temporal variability. The overall prevalence of aspirin resistance ranged from 2.4 to 63.5% depending on assay selection. Testing on multiple occasions using several assays is necessary to reliably predict aspirin response. Selection of alternative 'cut-off' values alters assay performance and caution should be used before categorising patients as 'aspirin resistant'.

0786

ENHANCED P2Y12 INHIBITION IN PATIENTS WITH PREVIOUSLY LOW CLOPIDOGREL RESPONSE AFTER DOUBLING THE DOSE

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Aim. Residual platelet reactivity evaluated by platelet function tests is associated with adverse cardiovascular events in patients at risk on antiplatelet therapy. We compared the *in vitro* efficacy of antiplatelet treatment in patients with double and standard dose of clopidogrel assessing vasodilator phosphoprotein (VASP) P2Y12 specific assay and light transmission aggregometry (LTA). *Methods.* We retrospectively evaluated a cohort of 431 patients by calculating the platelet reactivity index (PRI) based on the VASP assay and by ADP-induced platelet aggregation (ADP max, using 5 µM ADP). Among the patients, 390 were under dual antiplatelet therapy with acetylsalicylic acid and standard dose clopidogrel. A total of 129 patients (33.1 %) showed a PRI >79, indicating a low response to clopidogrel. *Results.* Overall PRI and APD max were 60 ± 41 % and 52 ± 36 %, respectively. There was no significant difference in PRI and ADP max between standard and double dose clopidogrel (student t-test p>0.5). Patients with low clopidogrel response under standard dose revealed PRI and ADP max of 83 ±14% and 61 ± 24%, respectively. In patients with previously poor clopidogrel response, treatment with double dose clopidogrel resulted in significantly lower PRI 60 ± 33% (n=41, student t-test p <0.00001), whereas ADP max showed only a trend to significance (see figure) (ADP max 54 ± 33%, p=0.06). *Conclusions.* In accordance with previous data, we observed no significant difference in an overall cohort of patients between standard and double dose clopidogrel. However, for patients with low clopidogrel response, doubling the dose enhanced the P2Y12 inhibition assessed by VASP and LTA.

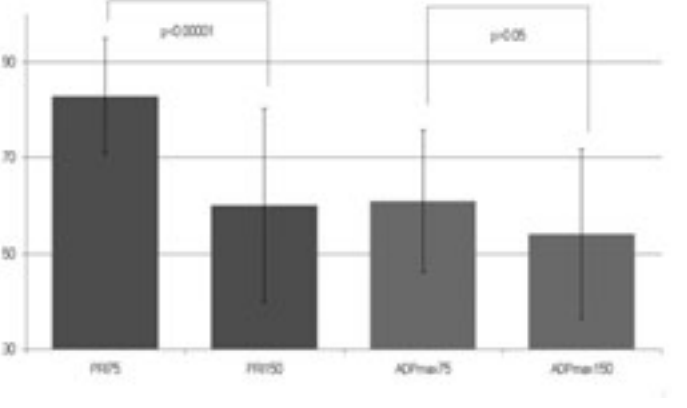

Figure 1. Reduction in platelet reactivity with double dose.

0787

THE USE OF IMMUNOPLATELET COUNTING FOR ESTABLISHING AN ACCURATE PLATELET COUNT DURING PREGNANCY

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Background. The determination of platelet count in pregnancy using automated counters can lead to erroneous results as it excludes the large platelets that are commonly present in both immune and gestational thrombocytopenia. The immunoplatelet count has been validated as an international reference method for determining an accurate platelet count. *Aim.* The aim of this study was to determine if there is a significant difference between automated platelet count and immunoplatelet count in thrombocytopenia in pregnancy and whether this difference would impact upon treatment decisions. *Methods.* A retrospective analysis of patients referred to an Obstetric Haematology Clinic in the past two years with platelet counts less than 100 x 10^9/L during pregnancy or with a history of immune thrombocytopenia (ITP) was conducted. The diagnosis of ITP was established if platelet counts were less than 60 x 10^9/L or if there was a previous diagnosis of primary or secondary ITP. Gestational thrombocytopenia was considered if platelet counts were above 60 x 10^9/L and resolved postpartum. Automated platelet counts were performed on Beckman Coulter analyzers LH750. Immunoplatelet count was performed by flow cytometry using the platelet specific antibodies CD41 and CD61. The thresholds considered for treatment and for epidural anesthesia delivery were based on International consensus report and local guidelines. *Results.* A total of 42 women were referred to our clinic for thrombocytopenia during pregnancy. Immunoplatelet counts were performed in 27 women (total of 68 samples analyzed) at different stages of pregnancy and compared to automated platelet counts. A diagnosis of ITP was established in 14 women and of gestational thrombocytopenia in 13 women. When comparing the platelet count using automated analyzers and immunological methods the immunoplatelet count was greater than the automated platelet count in 97% of samples (n=66/68). Using Wilcoxon matched pairs, signed rank test the difference between automated and immunoplatelet counts was significant for both ITP and gestational thrombocytopenia ($p<0.001$). According to the Mann-Whitney U-test this difference was greater in the ITP subgroup ($p=0.007$). The median increase in platelet count in this subgroup was 68% compared to 38% in the gestational thrombocytopenia subgroup. If we apply the International Consensus Recommendations, using the automated platelet count, 1 woman in the first 33 weeks of pregnancy and 5 women in the late stages of pregnancy would have required treatment. Using the immunoplatelet count all patients had platelet counts above the treatment thresholds. The immunoplatelet count was determined in 8 women at delivery. According to the automated platelet count 5 had a platelet count less than 70 x 10^9/L and may not have been considered suitable for epidural anesthesia. Of these 5 women 4 had an immunoplatelet count greater than 70 x 10^9/L. *Conclusion.* During pregnancy platelet count determination using automated instruments underestimates the platelet count. This may lead to unnecessary treatment as well as preventing the delivery of epidural anesthesia. We suggest further studies to establish the use of immunoplatelet count to guide treatment decisions and interventional procedures in women with thrombocytopenia in pregnancy.

0788

RITUXIMAB WITH OR WITHOUT CORTICOSTEROIDS IS AN EFFECTIVE TREATMENT FOR PATIENTS WITH AUTOIMMUNE HEMATOLOGICAL DISORDERS

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Backround. During the last few years rituximab has been proved effective in the elimination o autoreactive B-lymphocytes and has demonstrated efficacy in many types of autoimmune disorders. However, the most appropriate schedule of rituximab administration in these conditions has not yet been defined. *Patients and Methods.* We have treated with rituximab 41 patients (18 males, 23 females, median age 45 years, range 16-84 years) with various non-malignant hematologic disorders of autoimmune basis, refractory or relapsed to at least a first-line treatment. Rituximab was administered at a dose of 375 mg/m^2 (24 patients) or 200 mg/m^2 (11 patients) or with an initial dose of 375 mg/m^2, then tailored to 200 mg/m^2 (6 patients) on weeks 1, 2, 3, 4, 6, 8, 10, 14, 18, 22, 36, 48 and 60. Patients were diagnosed with autoimmune hemolytic anemia (AIHA, N=9), idiopathic thrombocytopenic purpura (ITP, N=17), thrombotic thrombocytopenic purpura (TTP, N=8), antiphospholipid syndrome (APS, N=2), Evans' syndrome (N=1) and an acquired inhibitor of factor VIII (AIFVIII, N=1). Six patients in total had been previously splenectomized for hypersplenism (3) or refractory disease (3), and three AIHA patients were multitransfused beta-thalassemics. One patient was HBsAg(+), two had anti-HCV(+) and additional 2 with ITP out of 5 tested, were H.Pylorii positive. Previous treatment consisted of corticosteroids (n=41), intravenous immunoglobulin (IV-Ig, N=29), plasma ad/or plasma exchange (N=9), cyclophosphamide (N=7), danazol (N=6), cyclosporine-A (N=6), azathioprine (N=2), vinblastine (N=2) and romiplostim (N=1). Twenty patients (48.8%) were refractory to previous treatment, 19 (46.3%) had relapsed and 2 (4.9%) were intolerant to corticosteroids. All evaluable patients (39) received at least 4 cycles of therapy (median 10, range 4-28). *Results.* Overall, 27 patients achieved a complete response (CR, 65.8%), 5 a partial response (PR, 12.2%) and 7 (17%) did not respond. Response rates were similar among the different nosologic subgroups (CR: AIHA 50%, ITP 64.7%, TTP 75%, other 100%, PR: AIHA 25%, ITP 11.8%, TTP 12.5%). Response rates were similar among patients administered higher or smaller dose of rituximab, as well as among patients younger and older than 50 years old. Median duration of response was 22.4 months for the whole group, and it was 24.5 months for patients with AIHA, 13.6 months for those with ITP, 24.8 months for those with TTP and 24.8 months for patients with other diseases. Overall, 10 of the 32 responded patients (31.3%) relapsed, following treatment discontinuation, and 7 of them were retreated with rituximab alone (2) or in association with pulses of high dose dexamethasone (5). Six of these patients responded again to rituximab +/- steroids and achieved a second CR (4) or a PR (2). After a median follow-up of 30 months (range 1.1-110 months) 39 patients are alive, 31 of them in CR, 6 in PR and 2 have active uncontrolled disease. Two patients have died due to disease-related complications. *Conclusions.* Rituximab alone or in combination with corticosteroids is an effective second-line treatment for patients with autoimmune hematological disorders at a smaller dose than that administered to patients with lymphoproliferative disorders.

0789

ASSOCIATION OF PLATELET INDICES WITH THYROID-STIMULATING HORMONE AND THYROID HORMONES IN EUTHYROIDIC HEALTHY SUBJECTS

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Background. Platelet parameters and especially mean platelet volume, an important determinant of platelet function and morphology, constitutes a novel emerging risk factor for atherosclerosis and its complications such as coronary heart disease. Mean platelet volume (MPV), platelet distribution width (PDW), platelet count and their correlations with thyroid-stimulating hormone (TSH), thyroxine (T4) and triiodothyronine (T3) have not been studied in depth. *Aim.* The aim of this study was to explore the correlation of platelet indices with TSH and thyroid hormones in euthyroidic healthy subjects. *Methods.* We have evaluated 82 euthyroidic healthy subjects (63 women and 19 men) with a mean age: 58 years (range: 22-78 years). None of the subjects had infectious and neoplastic conditions, diabetes mellitus, hypertension and dyslipidemia. To assess thrombopoiesis, we have determined platelet indices using Sysmex 9000 analyser. TSH, free-T3 and free-T4 were determined using electro-chemiluminescence immunoassay intended for use on Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, USA). Statistical analysis of data was performed with SPSS® version 17 for Windows software. Pearson or Spearman correlation coefficients (r) were used for normally or not normally distributed variables respectively. *Results.* From the statistical analysis of the data, the following results emerged: 1) There was a significant negative correlation between TSH and MPV in euthyroidic subjects (Spearman $r=-0.747$, $p<0.001$), 2) There was a significant negative correlation between TSH and PDW (Spearman $r=-0.818$, $p<0.001$), and 3) No statistically significant associations emerged between free-T3, free-T4 and platelet parameters ($p>0.05$). In a multiple linear regression model, con-

trolling for age, gender, body mass index and thyroid hormones, serum TSH is a statistically significant predictor of MPV and PDW levels ($p<0.05$) in euthyroidic healthy subjects. *Conclusion.* Our results indicate that TSH levels play an important predictive role in platelet markers which reflect platelet morphology and function. These results may also suggest that individuals with lower TSH levels tend to present increased platelet activation which could contribute to an increased risk of atherothrombotic complications in the future.

0790

PLATELET PARAMETERS IN PATIENTS WITH SUBCLINICAL HYPERTHYROIDISM: A CROSS-SECTIONAL STUDY

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Background. Subclinical hyperthyroidism (SCH) is often encountered in the general population. Patients may be asymptomatic or may present non-specific symptoms. It has been suggested that SCH could be a risk factor for cardiovascular disease. On the other hand, platelet parameters and especially mean platelet volume, an important determinant of platelet function and morphology, 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. MPV, platelet distribution width (PDW), platelet count (PLT) have not been studied in depth in subclinical hyperthyroidism. *Aim.* The aim of the present study is to compare the platelet count as well as the platelet parameters MPV and PDW in subclinical hyperthyroidism and in euthyroidic healthy subjects and to investigate whether SCH may have a predictive significance in the determination of platelet size. *Methods.* In a cross-sectional study between 2007 and 2010, we have evaluated thirty five patients with subclinical hyperthyroidism prior to any therapeutic intervention (26 women and 9 men) with a mean age: 32.5 ± 8.1 years (range: 18-48 years) and an equal number of euthyroidic healthy subjects (26 women and 9 men) with a mean age: 32.4 ± 7.1 years (range: 20-46 years). Healthy subjects were matched on gender, age (±5 years) 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 Sysmex 9000 analyser. Thyroid-stimulating hormone, triiodothyronine (T3), free-T3, thyroxine (T4) and free-T4 were determined using an electrochemiluminescence immunoassay intended for use on Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, USA). Statistical analysis of data was performed with SPSS® version 17 for Windows software. *Results.* Cases presented significantly higher MPV (mean ± SD: 12.27 fL ± 0.46) and PDW (mean ± SD: 15.8 % ± 1) than controls (mean MPV ± SD: 10.65 fL ± 0.78, $p<0.001$ and mean PDW ± SD: 13.5 % ± 1.34, $p<0.001$). On the contrary, patients with subclinical hyperthyroidism had similar number of platelets per mm3 than healthy euthyroidic subjects (mean PLT in patients: 253 x 103/mm3 ± 34 versus mean PLT in controls 264 x 103/mm3 ± 38, $p=0.18$). In a linear regression model, adjusting for age, gender, body mass index and smoking status, the presence of subclinical hyperthyroidism was the most significant predictor of MPV and PDW levels ($p<0.001$). *Conclusion.* These results suggest that subjects with subclinical hyperthyroidism tend to present increased platelet size and activation. Elevated platelet activation could contribute to an increased risk of atherothrombotic complications observed in SCH. Finally, these findings suggest that platelet morphologic changes observed in subclinical hyperthyroidism, such as higher MPV and PDW could be attributed to metabolic parameters.

0791

HEALTH-RELATED QUALITY OF LIFE (HRQOL) IN ADULT SERBIAN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA

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Background. Multiple available treatments in ITP are aimed at minimizing the risk of bleeding and increasing the patients' platelets. However, these treatments beside bleeding may impact on patients' HRQoL. *Aim.* To evaluate the effect of ITP and its treatment on the patients' HRQoL. *Methods.* Short form SF-36 health Survey was used to measure HRQoL; it was analyzed referring to the most important demographic and clinical characteristics in multivariate regression analysis. Hamilton tests were used for the assessment of anxiety and depression. The patients fulfilled a questionnaire developed from the literature review; it consisted of clinical information including demographics, the course of the disease, previous and current treatments and complications. Non-parametric measures of correlation were performed using the Spearman's rank correlation coefficient. Differences between subgroups were analyzed by $\chi 2$ test and ANOVA test. Predictive value of variables investigated was assessed by multiple linear regression analysis. *Results.* 111 adults with ITP (mean age 47.6 years; female 74%; median disease duration 4 years; median platelet count $14x10^9$/L) were assessed. Throughout their disease 84% patients experienced bleeding and 81.1% patients received some therapy: 95.6% corticosteroids, 24.4% intravenous immunoglobulins (IVIg), 22.2% danazol, 13.3% micophenolate mofetile, 7.8% azathioprine, 6,7% vincristine, 4,4% ciklosporine, 3,3% cyclophosphamide, 1,1% dapsone. A total of 48/111 (43.2%) patients reported a history of transfusions for the treatment of ITP. At the time of the survey the median platelet count was $76x10^9$/L, range 3-$500x10^9$/L; bleeding was present in 18 (16.2%) patients; 44 (39.6%) patients received some therapy: 81.8% corticosteroids, splenectomy 29,7%, 13.6% danazol, 20.5% micophenolate mofetil and 4.5% azathioprine. Herbs/supplements were used by 47 patients (42.3%). Treatment side effects were reported by 85 (96%) patients on corticosteroids and 5 (23.7%) patients on IVIg. At the time of the survey 66.7% reported commorbidities, mainly attributed to the ITP treatment. Regarding the effects of the disease on the patients' life style 90% reported absence from work/school. Patients were bothered the most with reduced work ability (26.1%), fear (25.2%), fatigue (19.8%), bleeding (19.8%), infections (7.2%) and corticosteroid side effects (21.4%). Some degree of fear was reported by 57% of all patients; 20.7% of patients were anxious and 72.1% had some degree of depression. The patients' HRQoL was decreased in the domains of general health (GH), mental health (MH), physical functioning (PF) and vitality (V), as well as in composite score of physical functioning (PCS) and mental composite score (MCS). Disease duration, platelet count, corticotherapy, IVIg administration and side effects of IvIG/ corticotherapy did not impact on HRQoL. On the contrary, treatment with azathioprine and micophenolate mofetile affected GH as a negative predictor, while splenectomy and herbs/supplements improved GH perception. Bleeding significantly decreased HRQoL in 5/8 of SF-36 domains ($p<0.05$). Fear of bleeding increased patients' depression and anxiety and they both significantly decreased all HRQoL domains ($p<0.01$). Age, education, employment and marital status impaired HRQoL, too ($p<0.01$). *Conclusions.* This study shows that the impact of ITP on patients' HRQoL in Serbia is substantial. The most important negative predictors of impaired HRQoL are lower education level, comorbidities and bleeding.

0792

HEALTH-RELATED QUALITY OF LIFE (HRQOL) IN CHRONIC IMMUNE THROMBOCYTOPENIA (ITP) PATIENTS: RESULTS FROM A LONG-TERM STUDY OF ROMIPLOSTIM

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Background. HRQOL is an important outcome for understanding the morbidity of ITP and the effects of treatment. *Aims.* To assess HRQOL in patients treated with romiplostim for 48 weeks. *Methods.* Romiplostim is a novel thrombopoiesis-stimulating peptibody that increases platelet production by stimulating the thrombopoietin receptor. This is an analysis of HRQOL during the first 48-weeks of an open-label extension study of romiplostim treatment in adult chronic ITP patients who had participated in earlier studies. Each patient completed the ITP-Patient Assessment Questionnaire (PAQ) at baseline, and at weeks 4, 12, 24, 36, and 48 in this extension study and also as part of the earlier study. The ITP-PAQ contains 44 items and 10 scales, with scores ranging

Table 1.

ITP-PAQ Scale Scores* by Assessment and Change Scores from Baseline

ITP-PAQ Scale	Baseline Score (n=253)	Scale Score Week 48 (n=208)	Change Score All patients (n=208)	Change Score Splenectomized patients (n=71)	Change Score Patients with no Exposure to Romiplostim within 24 weeks prior to Study (n=73)
Symptoms	74.1 ± 19.1	79.3 ± 17.3	4.2 ± 13.2*	6.8 ± 14.5*	7.7 ± 13.8*
Bother	75.0 ± 24.4	83.1 ± 19.8	6.4 ± 17.4*	12.2 ± 20.8*	9.2 ± 18.7*
Fatigue	70.0 ± 25.3	75.5 ± 24.1	4.0 ± 16.0*	9.2 ± 17.5*	4.8 ± 14.2*
Activity	72.7 ± 30.8	79.2 ± 27.0	5.2 ± 21.1*	11.1 ± 24.8*	7.9 ± 22.6*
Fear	85.2 ± 20.3	89.2 ± 16.4	3.5 ± 12.6*	4.8 ± 13.9*	6.0 ± 13.8*
Psychological Health	78.7 ± 23.9	84.7 ± 21.0	4.0 ± 15.8*	6.5 ± 17.6*	5.1 ± 17.3*
Work**	75.9 ± 30.3	84.5 ± 26.5	2.8 ± 14.5*	5.4 ± 19.7	2.2 ± 19.3
Social Activity	81.5 ± 22.7	85.7 ± 21.4	3.9 ± 13.4*	4.8 ± 14.4*	6.3 ± 14.6*
Reproductive Health (menstrual symptoms and fertility)**	78.9 ± 27.6	80.3 ± 25.6	4.5 ± 16.4*	9.9 ± 21.6*	8.6 ± 19.4*
Overall Quality of Life	63.7 ± 30.1	72.3 ± 27.1	8.3 ± 19.7*	12.0 ± 20.9*	11.8 ± 20.7*

n is the number of patients who completed both the baseline and the week 48 ITP-PAQ.
* Data represent mean ± SD.
* 95% confidence interval did not overlap with zero.
**Sample sizes varied slightly by scale, but were greatly reduced for Work and Reproductive Health since Work included only those who worked for pay, and Reproductive Health included women only.

from 0-100. Previous research on the ITP-PAQ found that 8-12 points reflects clinically meaningful changes for most scales. Scores at each assessment, and change scores from baseline to week 48 for all patients and for subgroups (splenectomized patients and those with no prior exposure to romiplostim) were calculated. 95% confidence intervals were used to determine whether change scores significantly differed from zero, representing statistical significance. *Results.* Results demonstrate a significant improvement with romiplostim treatment in all scale scores, with the greatest improvement for all patients in Bother (6.4 point-change), Activity (5.2 point-change), and Overall Quality of Life (QOL) (8.3 point-change). Change from baseline for the two subgroups for all scales other than Work was higher than change from baseline for all patients (e.g., 6.8 and 7.7 for the two subgroups for Symptoms vs 4.2 for the all patient cohort). The greatest improvement for splenectomized patients was in Bother (12.2 point-change), Activity (11.1 point-change) and Overall QOL (12.0 point-change). Similarly, change scores for patients who had previously received placebo were also highest in these three scales, Bother (9.2 point-change), Activity (7.9 point-change) and Overall QOL (11.8 point-change), with the addition of Reproductive Health (8.6-point change). It is likely that some of these subgroup differences were confounded by the fact that patients that received romiplostim as part of the earlier study (prior romiplostim patients) entered this current study with higher ITP-PAQ scores, therefore resulting in less room for improvement. Some change scores within the subgroups (e.g., Bother, Overall QOL) were also clinically meaningful since they exceeded the 8-point threshold. *Summary/Conclusions.* Adult patients with chronic ITP who received romiplostim for 48 weeks had significant improvement in all aspects of HRQOL. Change scores for both the subgroups (splenectomized patients and patients who were not exposed to romiplostim within 24 weeks) were higher than the change scores for all patients and also reflected significant improvements. Additional research is needed to more fully compare baseline ITP-PAQ scores and change scores during the course of the study.

0793

BONE MASS AND BIOCHEMICAL MARKERS OF BONE TURNOVER IN CHILDREN AND ADOLESCENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA: RELATION TO CORTICOSTEROID THERAPY AND VITAMIN D RECEPTOR GENE POLYMORPHISMS

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Background. Chronic immune thrombocytopenia (ITP) develops in less than 15% of childhood acute ITP. Management is usually conservative, however in compromised patients optional drug therapy includes standard oral steroids, pulsed high dose steroid therapy, IVIG, antiD, and immunosuppressive therapy or thrombopoietin receptor agonists in refractory patients. *Objetives.* As steroids play an important role in the management of ITP whether acute or chronic, this work aimed to study the bone mass in children and adolescents with chronic ITP in relation to biochemical markers of bone turnover, cumulative steroid therapy,and the possible modulating effect of vitamin D receptor (VDR)gene polymorphisms. *Methods.* 36 children and adolescents (mean age 10.67±4.72yrs) with chronic ITP were recruited from the Hematology Clinic, Children's Hospital, Ain Shams University, and the Hematology Clinic of the National Research Centre in Egypt ,and compared to 43 healthy age and sex matched controls (mean age 9.32±2.99 years). The files of the patients were revised and the total cumulative dose of steroids was calculated.After informed consent, patients and controls were subjected to clinical assessment, CBC, bone markers (serum osteocalcin and propeptide I precollagen (CPIP) and urinary deoxypyridinoline excretion). Polymerase chain reaction-restriction fragment length polymorphism (PCR - RFLP) technique was used to analyze VDR gene distribution (Bsm1 and FokI) in patients and control groups. Bone mass was assessed by dual energy X-ray absorptiometry (DEXA) at lumbar and hip regions to measure the bone mineral density (BMD).*Results.* The duration of ITP ranged from 2-10 years(mean 4.4 ± 3.9 years). Compared to controls, ITP patients had higher BMI (P=0.02) and lower height for age SDS (P=0.001). Patients had lower levels of osteocalcin (P<0.001) and CPIP(P<0.001) and higher urinary DPD excretion (P<0.001) when compared to controls. BMD was significantly lower in chronic ITP compared to controls for both spine and hip z-score.BMD was not significantly correlated to serum osteocalcin and CPIP, however urinary deoxypyridinoline excretion was inversely correlated to BMD (P=0.015 and P=0.006 for spine and hip z score,respectively). BMD in ITP was inversely correlated to age (P<0.05), BMI (P<0.01), and cumulative steroid dose (P<0.01),but not to the disease duration. The correlation between cumulative steroid dose and BMD was highly significant in patients receiving frequent oral steroids on daily or alternate days protocols(P=0.014 for spine and P=0.001 for hip z score), while the correlation was non significant in patients receiving monthly pulsed intravenous steroid therapy(P=0.74 for spine and P=0.505 for hip z score). There was no relation between BMD and Bsm1 polymorphism, however FokI polymorphism was significantly related to BMD (P=0.038 for spine, P=0.024 for hip z score). *Conclusion.* High cumulative doses of corticosteroids increase bone resorption in young chronic ITP patients. FokI gene polymorphism could be an accentuating factor in steroid induced bone resorption .Monthly pulse high dose steroids has less effect on bone mass than daily or alternate day oral steroid therapy Protocols of therapy of ITP should restrict chronic steroid use in growing children and favour alternative less harmful therapies.

0794

SINGLE NUCLEOTIDE POLYMORPHISMS OF THE INFLAMATORY CYTOKINE GENES IN ADULT PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA

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Background. Immune thrombocytopenic purpura (ITP) is an autoimmune disease characterized by thrombocytopenia due to platelet autoantibodies, causing an accelerated clearance of opsonized platelets by phagocytes. The etiology of ITP remains unclear, but both genetic and environmental factors are thought to play role in the development of the disease. *Aim.* The aim of our study was to investigate a possible association of some single nucleotide polymorphisms (SNP) in genes for interleukin beta IL-1β (-511 C/T), tumor necrosis factor beta TNF-β (+252 G/A) and tumor necrosis factor alpha TNF-α (-308 G/A) with ITP. *Methods.* We have analyzed 125 unrelated adult patients with ITP (35 men and 90 women) with median age of 47 (range 14-83) and 120 healthy matched controls. The median follow up of the patients was 44 months (12-384). Refractory ITP was defined as platelet count lower than $50x10^9$/L despite treatment with standard dose of corticosteroids and splenectomy. All 125 patients were initially treated with corticosteroids, 38 of which were splenectomized. Forty two (34%) patients were refractory to corticosteroids and splenectomy and were defined as having refractory ITP. Genotyping was performed by using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. The distribution of genotypes and allele frequencies were compared between patients and controls using a chi-squared test or Fisher's exact test. *Results.* Our results demonstrated significantly different distribution of the TNF-β (+252 G/A) genotypes in patients with ITP (n=125; G/G=3, A/G=38, A/A=84) comparing with controls (n=120; G/G=16, A/G=35, A/A=69), p=0.005. Allele frequencies for TNF β (+252 G/A) were also significantly different in patients with ITP (A allele 82.4%, G allele 17.6 %) comparing with controls (A allele 72.1%, G allele 27.9 %), p=0.009 with Yates correction. We didn't found significant differences in the genotype distribution or al-

lele frequencies for two other genes. Allele frequencies for TNF-α (-308 G/A) were 8.4% for A allele and 91.6% for G allele in patients and 11.3% for A allele and 88.7% for G allele in controls, p=0.363 with Yates correction. For IL-β (-511 C/T) allele frequencies were 69.2% for C allele and 30.8% for T allele in patients and 70.4% for C allele and 29.6% for T allele in controls, p= 0.845 with Yates correction. We found significantly different genotype distribution of TNF-α (-308 G/A) between patients with nonrefractory ITP (n=83; G/G=75, A/G=8, A/A=0) and refractory ITP (n=42; G/G=30, A/G=11, A/A=1), p=0.016. Allele frequencies for TNF-α (-308 G/A) were also significantly different in patients with refractory and nonrefractory ITP (A allele 15.5% versus 4.8%), p=0.009 with Yates correction. There was no significant difference in genotype distribution and allele frequencies for TNF-β (+252 G/A) and IL-β (-511 C/T) between these two groups of patients. *Conclusion.* The obtained data indicate that the A allele of TNF-β (+252) is more frequent in patients than in controls and that this polymorphism may play role in disease susceptibility. The A allele of TNF-α was significantly more frequent in patients with refractory ITP, indicating that this gene polymorphisms may contribute to therapy resistance and refractory form of ITP.

0795

INCIDENCE OF ANTIPHOSPHOLIPID ANTIBODIES AND THEIR SIGNIFICANCE IN THE PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA

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Background. The presence of antiphospholipid antibodies (APA) in immune thrombocytopenic purpura (ITP) has been reported, but their pathogenetic role and clinical importance is not clear. *Aims.* In this investigation we estimated the incidence of APA's presence and their influence on development of antiphospholipide syndrome (APS) and systemic autoimmune diseases (SAID) in patients with ITP. *Methods.* This study involved 59 adults patients with ITP. They were pretreatment evaluated for platelet count (<50x10/L vs ≥50x109/L), presence of APA: lupus anticoalgulant (LA) and anticardiolipin immunoglobulin G/M (IgG/M) antibodies (ACA) and antinuclear antibody (ANA). According age, sex, platelet count and APA positivity, the incidence and risk factors for development of APS (thrombosis or fetal loses) and SAID were estimated. The follow-up period was 96 months. Risk factors were identified using the univariate and multivariate analysis. *Results.* The median age of patient was 32 years, range 18-79 years, 69.3% were female and 30.7% were male. At presentation od disease, the paletelet count <50x109/L had 70% of patients and 30% had ≥50x109/L. The presence of APA detected in 48% of patients: ACA and LA in 10.7%, ACA alone in 12% nad LA alone in 25.2% of patients. At presentation of disesase, the incidence of APS was significantly higher in patient with APA positivity and platelets <50x109/L (p<0.05). During the follow-up period, the incidence of development APS was significantly higher in group of patients with LA positivity (p<0.001) and ACA IgG class positivity (p=0.001). The incidence of development SAID was significantly higher in female patients (p=0.004), age over 32 years (p=0.027) and in patient with LA positivity (p=0.039) and ANA positivity (p=0.005). The univariate analysis identified male sex (p=0.010) and both ACA and LA positivity (p=0.007) as a significant risk factors for development APS in patients with ITP. Multivariate analysis proved that most significant risk factor for development of APS was both ACA and LA positivity: p=0.007, relative risk (RR) = 0.063 (95% CI 0.008-0.471). The univariate analysis also identified age over 32 years (p=0.022) and LA positivity (p=0.037) as the significant factors for development of SAID in patients with ITP, while multivariate analysis indicated the LA positivity as the most sifnificant risk factor for development of SAID in patients with ITP: p=0.021, RR=0.047 (95% CI 0.004-0.628). *Conclusions.* The inverse correlation between APS and platelet count, in presens of APA positivity, was found. The most significant risk factor for development of APS in patients with ITP was positivity of ACA and LA mutual, while most significant risk factor for development of SAID was LA positivity. It may be concluded that estimation of APA at presentation of ITP is important for identification of risk group of patients for development APS and SAID.

0796

PROLONGED RESPONSE TO ELTROMBOPAG IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA

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Background. Although difficult to quantify, spontaneous remission of chronic immune thrombocytopenia (ITP) has been estimated at 0.1% per year.[1] American Society of Hematology guidelines refer to 12 case series with observation over 61 years, demonstrating a spontaneous remission rate of approximately 5% of patients after failing to respond to glucocorticoids, splenectomy, and subsequent therapy.[2] Instances of prolonged response have been reported with different therapies; it is not always possible to distinguish a prolonged response from a remission. Treatments for ITP have traditionally addressed the platelet destruction component of the disease; the advent of thrombopoietin receptor agonists has addressed the impaired production of platelets. Eltrombopag is an oral, nonpeptide thrombopoietin receptor agonist that is approved for the treatment of chronic ITP. In 6-week and 6-month placebo-controlled trials in patients with chronic ITP, eltrombopag increased platelet counts, reduced bleeding, and reduced the need for concomitant ITP therapy. Long-term treatment with eltrombopag is being evaluated in EXTEND, an ongoing open-label extension study in chronic ITP patients who completed a previous eltrombopag study. *Aim.* To evaluate prolonged platelet responses to eltrombopag in the EXTEND study. *Methods.* Patients in EXTEND had received eltrombopag or placebo in one of the following studies: two 6-week phase 2 and phase 3 studies (TRA100773A/B), a 6-month phase 3 study (RAISE), or a phase 3 study of intermittent treatment (REPEAT). Dosing in EXTEND is individualized according to platelet counts with a goal of maintaining platelets ≥50,000/μL and <200,000/μL while minimizing the use of concomitant ITP medications. Patients remain in the study until withdrawal or commercial availability of eltrombopag. An ad-hoc analysis was conducted to evaluate the proportion of patients in EXTEND who experienced a prolonged response, defined as a platelet count ≥50,000/μL that was sustained for ≥12 weeks after the last dose of eltrombopag, without any rescue therapies. *Results.* Among 299 patients enrolled in EXTEND between June 2006 and February 2010, a prolonged response was obtained in 14 patients (5%), which is equivalent to 1.4% per year of observation in EXTEND. The median age of these patients was 55 years (range, 20-71 years), and 11 (79%) were female. Five patients had been splenectomized prior to enrollment in EXTEND, and all patients had received at least 1 pharmacologic agent (range, 1-7 prior therapies) before study entry. A median of 26 months (range, 9-128 months) had elapsed since the diagnosis of ITP, and patients had been treated with eltrombopag for a median of 160 days while in EXTEND (range, 14-1107 days). *Conclusion.* Treatment with eltrombopag in the EXTEND study was associated with a prolonged response in 5% of patients over 4 years, which is higher than the expected proportion of spontaneous remission. However, it is not possible to assume that the patients with a prolonged response also experienced remission of chronic ITP, because subsequent follow-up is not available for most of these patients.

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0797

SPLENECTOMY IN CHILDREN WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA -RETROSPECTIVE STUDY-SINGLE CENTRE EXPERIENCE

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Background. The management of chronic idiopathic thrombocytopenic purpura (ITP) and the decision to perform the splenectomy in children with chronic ITP remain two controversial issues. *Aims.* The aim of this retrospective study was to analyze our centre experience with splenectomy in children with chronic idiopatic thrombocy-

topenic purpura. *Methods.* We retrospectively examined the records of 25 children who underwent splenectomy for chronic ITP between 1999 and 2009.We studied the time between diagnosis and splenectomy, long term hematological response, morbidity, mortality and predictors of response to splenectomy. Platelet response are categorized as complete response (CR≥150.000/µl), partial response (PR ≥50.000/µl, but < 150.000/µl) or nonresponse (NR <50.000/µl). *Results.* The median age at moment of the splenectomy was 10.6 (3,5-18,2) years.All patients were treated only with glucocorticoids as first line of therapy (because of the high costs of intravenous immunoglobulin). Five patients had a corticoresistant ITP with recurrent hemorrhagic events and 20 children had a corticodependent ITP.The median time between diagnosis and the moment of the splenectomy was12(4-48)months.Presplenectomy vaccination was administered in all patients. After splenectomy was not used antibiotic profilaxy. The perioperative mortality was zero and the overall morbidity was 2,5%The median postsplenectomy follow-up time was 41(4-96)months.The overall immediate response rate was 92% (22 patients had CR and 1 patient had PR).Two children had NR.An improvement in quality of life was observed in 96% of children. During follow-up 2 children relapsed (at 8 and 12 months respectively after splenectomy) and required intermittently corticotherapy. No correlation existed between CR to splenectomy and age, platelet count at diagnosis and last platelet count before splenectomy. All 5 patients with corticoresistant ITP had CR to splenectomy. One of the two children who had NR at splenectomy obtained a stable PR with Rituximab 100 mg/m2/weekly iv x 4 weeks. *Conclusions.* This study shows that splenectomy is effective, provide long-term control of disease and is associated with low morbidity, important improvement in quality of life and a good cost efficiency. In our centre the splenectomy represents an important option for the treatment of chronic ITP in children.

0798

THROMBOPOIETIN RECEPTOR AGONISTS (TPOA) DO NOT CAUSE ACTIVATION OF THE COAGULATION-FIBRINOLYTIC SYSTEM IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP)

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Background. TpoA stimulates thrombopoietin receptors, increasing platelet counts (PC) in 70-80% of ITP patients. Venous thromboembolism (VTE) was reported in up to 4% of TpoA trials, triggering speculation regarding TpoA activation of the coagulation process, particularly in the absence of evidence for platelet activation. *Aims.* This study used D-dimer (DD) as a marker of coagulation and fibrinolysis to determine the effect of TpoA on this system. *Methods.* We retrospectively evaluated DD in ITP patients on TpoA, performed during routine visits. Two DD assays were used: DD-Dade (cut-off 1.2 mg/L) and DD-HS (cut-off 230 ng/mL). DD-HS was used in 11/108 analyses and these values were not included in estimating median DD. Normal range of fibrinogen (F) was 180-400 mg/dL and CRP <1 mg/dL. *Results.* Median age of 46 patients was 55 years; 40% males. No patient developed VTE during TpoA treatment, in this study. Median time from pre-treatment DD measurement to TpoA initiation was 3 weeks. Median (IQR) pre-treatment DD (n=40) was 1.3 mg/L (0.7-2.2); PC (n=40) 25 x10^9/L (17-53); F (n=12) 315 (199-345) mg/dL; CRP (n=6) 0.47 mg/dL (0.2-3.7). Median duration from TpoA initiation to first available DD on treatment was 12 weeks. Median (IQR) DD on treatment (n=38) was 1mg/L (0.5-2); PC (n=38) 57 x10^9/L (31-165); F (n=7) 319 mg/dl (203-367); CRP (n=8) 0.4mg/dL (0.1-0.7). No significant difference was found between pre- and on-treatment DD values (n=32, p=0.1) although DD tended to decrease with the duration of TpoA treatment (Figure). DD for both assays were positive in 24/40 (60%) pre-treatment and 17/32 (53%), 6/29 (35%) and 5/19 (26%) at 4, 8 and 12 months during continuing treatment, respectively. A comparison of the various ITP therapies for those patients with positive (n=24) vs negative (n=16) DD pre-TpoA initiation was: 42% vs 25% IVIG; 38% vs 19% steroid; 13% (but 8% were also on IVIG) vs 6% rituximab; 0 vs 19% Rigel; and 17% vs 44% no treatment (p>0.05). There was no correlation between paired PC and DD values pre-treatment, 4, 8 or 12 months during treatment. *Conclusion.* This study does not support the evidence for increased VTE in ITP patients treated with TpoA being related to a general activation of the coagulation system. Treatment of ITP with TpoA did not activate the coagulation-fibrinolytic pathway as evidenced by no increase in DD following initiation of TpoA. In fact, DD tended to decrease while patients were receiving ongoing Tpo-A treatment. There is a suggestion, although not a significant difference, that the pre-treatment DD may be related to the therapy, or lack of, received at that time. Further studies are needed to determine the pathoaetiology of the infrequent TpoA-associated-VTE.

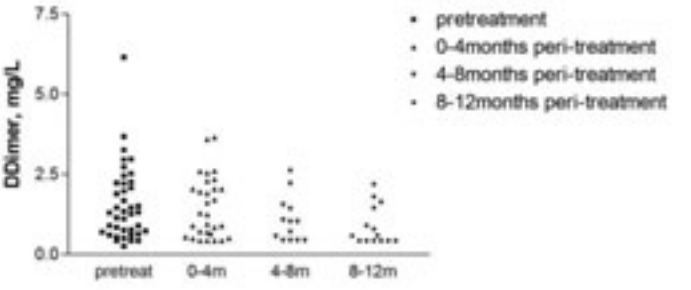

Figure 1.

0799

XENOTROPIC MURINE LEUKAEMIA-RELATED VIRUS (XMRV) AND IMMUNE THROMBOCYTOPENIA (ITP)

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Background. Fatigue in ITP results in decreased health-related quality of life (HRQoL); its pathogenesis is unknown. Emerging evidence suggests that T regulatory cells, natural killer cells, innate immune dysfunction and cytokine imbalance, in addition to organic causes such as hypothyroidism or iron deficiency, may lead to fatigue in ITP. Xenotropic murine leukaemia-related virus (XMRV), a human gammaretrovirus, has been associated with chronic activation of the innate immune system and natural killer cell dysfunction in chronic fatigue syndrome (CFS). XMRV is present in 2-4% of the general population. *Aims.* The aims of this study were to assess the level of fatigue expressed and to determine any associations between fatigue and XMRV in patients with ITP compared to healthy controls. *Methods.* Blood samples were collected prospectively with consent at routine clinic visits from ITP patients and from healthy controls, older than 15y, for complete blood count and blinded XMRV detection via: serological techniques to detect XMRV/MRV Env antibody protein; RT-PCR detecting viral RNA; and isolation in culture. Fatigue was tested concurrently for every subject, using the standardized Multidimentsional Fatigue Inventory (MFI) questionnaire: a high score reflects severe fatigue. *Results.* 36 patients with ITP, mean age 50y (range 19-84y),50% female. Eight of 20 (40%) patients with ITP were positive for XMRV compared to 3/13 (23%) controls (p=0.419). Mean MFI score for every scale was worse for ITP patients than controls (Table). There was no difference in fatigue on any scale between ITP patients XMRV positive and negative. Three patients were on immune suppression, of whom

Table 1. MFI scores, mean (+/- SE).

	General fatigue	Physical fatigue	Reduced activity	Reduced motivation	Mental fatigue
All patients, n=22	11.10(+/-0.82)	9.62(+/-0.89)	8.52(+/-0.79)	9.38(+/-0.79)	7.67(+/-0.65)
All controls, n=13	7.69(+/-0.81)	6.31(+/-0.71)	7.00(+/-0.93)	5.77(+/- 0.48)	6.00(+/-0.75)
p	0.014	0.009	0.166	0.002	0.059
Patients +ve XMRV, n=8	12.29(+/-1.49)	9.50(+/-1.77)	8.75(+/-1.28)	10.10(+/-1.26)	7.70(+/-0.99)
Patients -ve XMRV, n=12	10.92(+/-1.10)	9.69(+/-1.00)	9.15(+/-1.11)	9.15(+/-0.93)	8.23(+/-0.90)
p	ns	ns	ns	ns	ns
Control +ve XMRV, n=3	8.00(+/-1.00)	7.00(+/-1.00)	7.00(+/-2.08)	5.67(+/-0.33)	4.00(+/-0.00)
Control -ve, n=10	7.58(+/-0.87)	6.33(+/-0.77)	7.08(+/-1.00)	5.75(+/-0.52)	6.17(+/-0.79)
p	ns	ns	ns	ns	ns

2 (67%) were positive for XMRV, compared to: 3/10 (30%) with a splenectomy; 6/12 (50%) on thrombopoietin receptor agonists (TPO-A); 1/3 (33%) on IVIG; none of 2 on GMA161 (an anti-FCγRIII antibody); and none of 3 on no treatment. Among those patients testing positive for XMRV, the mean platelet count was not significantly lower. The platelet count, median 207 x109/L (range 8-1160), was associated with fatigue for Reduced motivation (p=0.022) and showed trends for General fatigue (p=0.081) and Physical fatigue (p=0.105). Increasing disease duration, median 17 years (range 3.25-32), tended to have worse scores for Mental fatigue (p=0.128). *Summary/Conclusions.* ITP patients in this study population expressed higher levels of fatigue than controls across all MFI scales, with an association between lower platelet counts and worse scores for 3/5 scales, illustrating again the importance of fatigue as a manifestation of ITP. This study demonstrates a 40% incidence of XMRV in patients with ITP and 23% in healthy controls. There was no difference in fatigue expression between patients who were XMRV positive and negative, thus XMRV may not be involved in the pathogenesis of fatigue in ITP. Given the small numbers and preliminary nature of the results there may be a potential association between immune suppression and XMRV infectivity. Studying more patients may clarify clinical associations of XMRV in ITP. Table: MFI scores, mean (+/- SE) (higher scores = worse fatigue), p values representing differences between: patients and controls; and positive and negative XMRV results, for each MFI scale.

Infectious diseases, etiology and supportive care

0800

IDENTIFICATION AND CHARACTERIZATION OF ASPERGILLUS-SPECIFIC IMMUNE RESPONSES TO DIAGNOSE INVASIVE ASPERGILLOSIS IN HIGH RISK PATIENTS: A MULTICENTER STUDY

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Background. The mortality of Invasive Aspergillosis (IA) still affects from 27% to 55% of high risk hematologic patients. The reasons of such a poor outcome also rely on several drawbacks limiting the diagnostic accuracy of non cultural based diagnostic methods (NCBDM) and hampering the opportunities for an early intervention. Studies in mice model of IA and in healthy subjects have shown that Aspergillus-specific T-cells producing interferon-gamma (IFN-gamma-T1) are protective, while Aspergillus-specific T-cells producing interleukin-10 (IL-10-T2) are non-protective to IA. *Aims.* We have investigated whether the identification of Aspergillus-specific IFN-gamma-T1 and/or IL-10-T2 through an *ex-vivo* enzyme linked immunospot (ELISPOT) assay may be effective in the diagnosis of IA in high risk patients. Furthermore, in the proven IA patients, we have functionally and phenotipically characterized such T cells through the cytokine secretion assay (CSA). *Methods.* 180 patients (168 hematologic and 12 solid organ transplant patients) have been enrolled. They were classified, according the revised EORTC/MSG criteria, as follows: 18 proven, 35 probable, 17 possible IA cases and 110 controls. The control patients were divided in two groups: group 1 included 86 (78.2%) patients with hystological and/or cultural verified infectious/inflammatory/neoplastic diseases, but other than IA; group 2 included 24 (21.8%) patients without clinical and/or microbiological features of IA. ELISPOT has been performed, as described [Potenza *et al.* Leukemia 2007; 21: 578-81], by using as antigens Aspergillus either conidia or recombinant antigens, namely CRF1p, GEL1p, PEP1p, SOD1p, α1-3 glucan, β1-3 glucan and galactomannan (GM). *Results.* The patient and sample positivity rates were 94.4%/89.5% in proven, 45.7%/35.3% in probable, 35.3%/50% in possible IA cases and 1.8%/4.5% in the controls, respectively. The sensitivity and specificity of ELISPOT for the diagnosis of IA resulted 94.4% (95% CI, 73%-99%) and 98.2% (95% CI, 93%-99%), respectively. The PPV of the test was 89.5% (95% CI, 67%-99%), the NPV was 99.1% (95% CI, 94%-100%) and the efficiency was 97.6% (95% CI, 92.3%-99.4%). The positive likelihood ratio (LR) resulted 51.89, the negative LR was 0.06 (Table 1A,B). In proven IA patients, CSA demonstrated that Aspergillus-specific IL-10-T2 were predominantly central memory (CM) CD4+ T cells (median frequency 0.37%/0.22%), while Aspergillus-specific IFN-gamma-T1 were either CD4+ or CD8+ cells of either effector memory (EM) or CM phenotype (median frequencies 0.24%/0.20%). Also lower frequencies of Aspergillus-specific either CD4+ or CD8+ T cells producing IL-4 (0.11%/0.19%) of EM phenotype, and EM CD8+ cells producing IL-17 (0.18%), were detected. Moreover, although CRF1p, GEL1p, α1-3 glucan and SOD1p resulted the antigens eliciting the highest number of Aspergillus-specific IFN-gamma-T1, only GEL1p and α1-3 glucan were those most constantly targeted by protective immune responses along the entire course of the IA. *Conclusions.* Our findings demonstrate the potential of ELISPOT in the diagnosis of IA, suggesting that it may complement the other NCBDM, enabling a more consistent diagnosis of IA. Furthermore, this study describes for the first time the Aspergillus-specific immune responses in patients with proven IA, identifying

Table 1.

A,B. Diagnostic Accuracy of the ELISpot assay for the diagnosis of Invasive Aspergillosis (IA).

A. Rate of positivity of the assay per-patient and per-sample. B. Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Efficacy of the assay matching proven IA with control cases.

A.

Classification	N° of Patients with positive ELIspot: Single positive result	Serial positive result	Patient positivity rate, % (95% CI)	N° of samples tested (n° positive)	Sample positivity rate, % (95% CI)
Proven IA (n = 18)	10	7	94.4% (73%-99%)	38 (34)	89.5% (75%-97%)
Probable IA (n = 35)	8	8	45.7% (29%-63%)	85 (30)	35.3% (25%-46%)
Proven/probable IA (n = 53)	18	15	62.3% (48%-75%)	123 (64)	52% (43%-61%)
Possible IA (n = 17)	3	6	35.3% (14%-62%)	34 (17)	50% (32%-68%)
Controls (n = 110)	9	0	1.8% (0.3%-6.4%)	200 (9)	4.5% (2%-8%)

B.

	PROVEN	CONTROLS	
ELISPOT +	17	2	19
ELISPOT -	1*	108	109
Total	18	110	128

* The proven IA patient did not resulted negative but udetermined, because T cells responded neither to the Aspergillus antigens nor to common mithogens (phytohaemagglutinin and antiCD3 antibody)

also the antigens predominantly targeted by protective IFN-gamma-T1, with possible consequences in designing strategies of either adoptive cell infusion or vaccine therapies.

0801

SURVEY ON ANTIFUNGAL COMBINATION THERAPY FOR TREATMENT OF PROVEN OR PROBABLE INVASIVE FUNGAL DISEASES IN ITALIAN HEMATOLOGICAL CENTERS. THE SEIFEM-COMBO STUDY (NCT 00906633)

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Background. This prospective observational Clinical Trial (NCT 00906633) evaluated the feasibility, efficacy and toxicity of Antifungal Combination Therapy (Combo) as treatment of proven or probable Invasive Fungal Diseases (IFDs) in Hematological patients (pts). *Materials and Methods.* Between Jan 2005 and Dec 2009, 84 cases of Combo were reported from 20 Hematological Centers in Italy. Median age of pts was 34 yrs (range 1-73) and 37% had less than 18 yrs. Acute Leukemia was the most common underlying hematologic disease (68/84; 81%). The status of hematologic disease was: onset 21/84 (25%), remission 18/84 (21%), refractory/relapse 45/84 (54%). The main site of fungal infection was lung with or without other sites. The etiological agents were: Aspergillus sp 68 cases (81%), Candida sp 6 cases (8%), Zygomycetes 4 cases (5%), Fusarium sp 4 cases (5%) and other (2 cases). *Results.* The most used Combo were: Caspofungin + Voriconazole 35/84 (42%), Caspofungin + Liposomal Amphotericin B (L-AmB) 20/84 (24%), and L-AmB+Voriconazole 15/84 (18%). The median duration of Combo was 19 days (range 3-180). The Overall Response Rate (ORR) was 73% (61/84 responders) without significant differences between the Combo regimens. The most important factor that significantly influenced the response rate (in univariate and multivariate analysis) was Polymorphonuclear (PMN) recovery during Combo ($P < 0,0001$). Only one patient discontinued therapy (voriconazole related neurotoxicity) and 22% experienced mild and reversible adverse events (hypokalemia, ALT/AST increase, creatinine increase) without differences between pediatric and adult pts. The IFD attributable mortality rate was only 17%. *Conclusion.* This is the first multicenter, prospective, observational study exploring feasibility, efficacy and toxicity of Antifungal Combination Therapy (Combo) as treatment of proven or probable Invasive Fungal Diseases (IFDs) in Hematological patients (pts). The results of this study indicate that: 1) Combo was well tolerated in both children and adults hematologic pts. 2) The Overall Response Rate was 73% and the mortality IFDs related was only 17%. 3) The most used Combo regimens were Caspofungin+Voriconazole (ORR 80%) and Caspofungin+L-AmB (ORR 70%). 4) In univariate and multivariate analysis PMN recovery during Combo predicts a better outcome.

0802

ADOPTIVE IMMUNOTHERAPY TO PREVENT AND TREAT HHV-6 REACTIVATION POST ALLOGENIC STEM CELL TRANSPLANT

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Background. Viral infections cause morbidity and mortality in allogeneic hematopoietic stem cell transplant (HSCT) recipients. Antiviral drugs are costly, often have severe adverse effects, and are frequently ineffective. As an alternative treatment option our group has shown that adoptive transfer of *in vitro* generated trivirus-specific cytotoxic T lymphocytes (CTL) targeting EBV, CMV and Adv antigens is effective and protective *in vivo*. Reactivation of Human Herpes Virus type 6B (HHV6B) also plays a critical role in the outcome of patients after HSCT. HHV6B is detected in >50% of HSCT recipients and associated with severe clinical symptoms including encephalitis, acute GvHD, bone marrow suppression and increased overall mortality. However, to date, production of CTLs directed against this virus has been hindered by lack of information regarding immunogenic/protective T-cell target antigens. *Aims.* The goals of this study were to identify immunodominant HHV6B T-cell antigens which can be used for T-cell studies and as targets for T-cell therapy. *Methods.* We assessed the cellular immune response directed against 5 HHV6 antigens (U71, U90, U11, U14 and U54), whose counterparts in the significantly homologous CMV or HHV7 viruses were found to be immunogenic. For T-cell stimulation we isolated PBMCs from HHV6 sero+ve, CMV serove healthy donors (thus eliminating the possibility of cross-reactive T-cell recognition) pulsed them with peptide libraries spanning each antigen (pepmix) or infected them with the HHV6B wild type virus strain Z29 and then expanded them *in vitro* for 10 days. *Results.* Pepmix-stimulated lines were polyclonal as confirmed by V-beta repertoire analysis with a predominant antigen response in the CD4+ T-cell compartment (mean CD4+: 62+/-6%, n=15). Using IFNg ELIspot we identified a hierarchy of immunodominance, with all donors responding to U90 (median 216; range 53-812 SFC/1x105 cells), U14 (164; 43-666), U11 (143; 25-209) and U54 (138; 44-247) whereas only 8/15 donors responded to U71 (50; 25-80). Furthermore we mapped several MHC class I-restricted T cell epitopes using individual peptides derived from each of the antigens. T cells cultured with HHV6B Z29 wild-type virus-infected PBMCs as a stimulus showed comparable phenotype and frequencies of antigen specific T-cells, confirming that our chosen antigens are relevant targets. The CTLs were polyfunctional, producing multiple cytokines (IFN-γ, TNF-α, IL-2) and effector molecules (Granzyme B) upon stimulation with cognate antigen. They demonstrated cytolytic activity against pepmix-pulsed and HHV6B Z29 wild-type virus-infected autologous monocytes in a standard 4hr Cr51 release assay. Finally, we were able to detect U11, U14, U54 and U90 specific T-cells in peripheral blood of patients who controlled their HHV6 reactivation post-HSCT, proving the *in vivo* relevance of T cells specific for these antigens. *Summary.* In summary we were able to identify a hierarchy of immunodominance against 5 HHV6 antigens in both healthy donors and patients after HSCT. Generated HHV6 CTLs were functional in cytokine production and cytolytic activity. Pepmixes can now be used to further delineate the tempo of endogenous T-cell recovery in patients after allogenic HSCT and to generate effective and protective HHV6B-specific CTL for adoptive transfer.

0803

IMMUNOGENICITY OF A NOVEL INFLUENZA A (H1N1) VACCINE IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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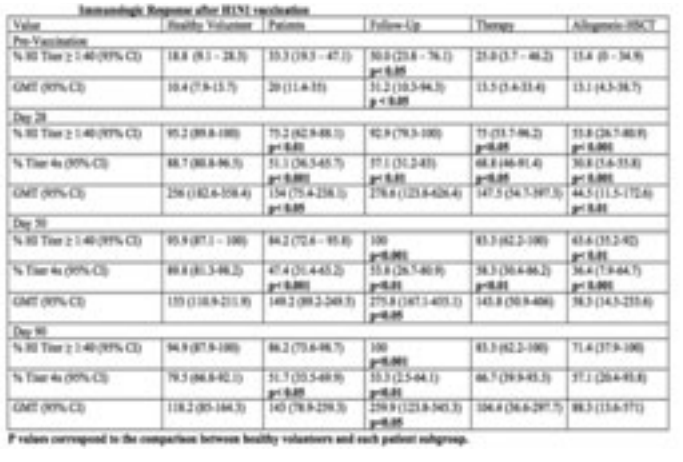
Background. Immunocompromised patients are particularly at risk for developing fatal complications after infection with influenza A (H1N1). A novel vaccine against the virus strain A/California/07/2009 (H1N1) was proved to be protective in healthy volunteers, while its efficacy in immunocompromised patients, in particular those with cancer, is unknown. *Aims.* The aim of this study was to determine the safety and efficacy of the 2009 H1N1 vaccine in patients with hematologic malignancies. *Methods.* We prospectively evaluated the humoral and cellular immune responses after one injection of monovalent adjuvated influenza A/California/2009(H1N1)-like strain surface antigen vaccine in 47 adults with hematologic malignancies and 77 adult controls. Antibody titers were measured by hemagglutination-inhibition assay and virus-specific T-cell responses by flow-cytometry on days 0, 28, 50 and 90 after injection. *Results.* Of the 47 patients, 15 had lymphoma, 21 multiple myeloma, 9 myeloproliferative disease; 15 patients were in follow-up, 17 were receiving treatment, 13 were recipients of allogeneic hematopietic stem cell transplant (HSCT). By day 28, immunologic response was lower in the patient cohort relative to controls by all parameters ($p< 0.05$). At subsequent time points, seroprotection (antibody titers ≥ 1:40) rates and geometric mean titers (GMT) increased in the patient group and were not significantly different from healthy volunteers. In marked contrast, seroconversion rates (≥ 4 fold increase in antibody titer) were lower in the patient group also at later time points ($p<0.05$). Subgroups analyses were performed to evaluate the influence on vaccine efficacy of follow-up, treatment, previous HSCT. Patients in follow-up had immunologic responses similar to controls at all time points. Conversely, patients receiving treatment had lower seroprotection rate on day 28 and lower seroconversion rates on day 28 and day 50 relative to controls. Of note, within the treated group, patients receiving immunomodulatory drugs (IMIDs) displayed a trend for improved seroprotection (day 90, 100% vs 60%, p=0.06) and increased GMT (day 28, 246 vs 87, p=0.3) compared to other treatments. Patients vaccinated after HSCT had the lowest seroconversion and seroprotection rates on day 28 and day 50 ($p< 0.01$ relative to controls), with a slight increase on day 90. Accordingly, GMT was lower than in controls on day 28 ($p< 0.001$) and subsequently it slightly increased. We also assed the cellular response to H1N1 vaccine by enumeration of fold increase of virus specific CD4+ and CD8+ cells on day 21, day 50 and day 90 relative to day 0. In the control group, specific CD4+ (6.0 ± 2.4, day 50, $p<0.05$) and CD8+ cells (21.7 ± 12.0, day 50, p=0.08) significantly increased on day 21 and day 50 relative to day 0. A similar trend was observed in the patient cohort for specific CD4+ (28.0 ± 11.0, day 50, $p< 0.01$) and specific CD8+ cells (16.5 ± 5.9, day 50, $p< 0.05$). *Summary/Conclusions.* Patients in follow-up or receiving IMIDS were protected by H1N1 vaccination. In contrast, patients receiving other types of treatment and those treated with HSCT responded poorly. These results may contribute to improve the vaccination strategy especially for poor responders.

Table 1.



0804

PROPHYLAXIS OF INVASIVE FUNGAL DISEASES WITH POSACONAZOLE IN ACUTE MYELOID LEUKEMIA. A REAL LIFE EXPERIENCE

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Background. Acute Myeloid Leukemia (AML) patients are at high risk of Invasive Fungal Diseases (IFDs). We report our real-life experience with POS prophylaxis in AML. We also compare the performance of POS prophylaxis with an historical, well matched, control group of AML pts who received prophylaxis with Fluconazole (FLUCO) or Itraconazole (ITRA). *Patients and Results.* Fifty-five unselected and consecutive AML pts received POS prophylaxis (600 mg daily) between Jan 2009 and Oct 2010. Median age of this population was 47 yrs (range 18-69). All cases were given chemotherapy with anthracyclines and cytarabine. The POS was started when neutrophil (PMN) count was less than 1000 mL and was stopped at PMN recovery. The median duration of severe neutropenia (PMN lower than 500 mL) was 15 days (range 7-41); 10/55(18%) of cases had an oral mucositis grade II-III CTC (common toxicity criteria) and 73%(40/55) of these pts received a proton pump inhibitor. An active diagnostic work up was made in all cases with Galactomannan assay, standard chest X-ray and thoracic CT scan in case of fever (FUO) lasting over 48 hours. The median duration of POS prophylaxis was 15 days (range 7 to 41). Only 4/55(7%) of pts required parenteral empiric or pre-emptive antimycotic therapy and only 2/55(4%) experienced a proven IFDs (Fusarium solanii fungemia and Aspergillus sp pneumonia). Mortality IFDs related was 0%. POS was well tolerated and only 9%(5/55) of pts experienced mild drug related side effects. No cases of POS discontinuation, due to the side effects or intolerance, were reported. When we compare the 55 pts who received POS with an historical control group of 55 AML pts who received FLUCO (45/55) or ITRA (10/55)prophylaxis, between Jan 2008 and Jun 2009, no significant differences were observed for underling disease status, age, IFDs risk factors, days of severe neutropenia and days of prophylaxis.Instead, there were significant differences in breakthrough IFDs (4% in POS group vs 16% in control group; P=0,02), and in days of parenteral antymicotic therapyn (37 vs 163). *Conclusions.* This real-life experience confirms that POS prophylaxis is feasible, safe, well tolerated and effective (prevention of IFDs) in unselected AML patients. Only 7% of these high risk pts required parenteral antimycotic therapy and only 4% experienced breakthrough IFDs. We also confirm that POS is more effective than FLUCO or ITRA as antifungal prophylaxis in AML pts.

0805

ELEVATED COMBINED SERUM FREE LIGHT CHAIN (CFLC) LEVELS ARE SIGNIFICANTLY ASSOCIATED WITH INCREASED MORTALITY

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Background. Though abnormal serum free light chain (FLC) ratios are diagnostically important in almost all plasma cell disorders an absolute rise of polyclonal FLC levels are often discarded as inconsequential. Here we report a striking association between increased combined FLC (summated FLCκ and FLCλ; cFLC) concentrations and mortality. *Aims.* To evaluate and correlate the prognostic impact of elevated cFLC levels and other biomarkers in a haemological referral cohort. *Methods.* 753 serum samples were sent for various haematological investigations. Serum cFLC, albumin, C-reactive protein (Crp), erythrocyte sedimentation rate (ESR), estimated glomerular filtration rate (eGFR determined by the MDRD equation) and total IgG, IgA and IgM immunoglobulins were measured by standard techniques. Patients with abnormal serum protein electrophoresis, and / or abnormal FLC ratio with confirmed positive IFE were removed. The remaining 528 patients with absolute rise of polyclonal FLC levels were followed for up to 4.5 years. Statistical analysis was performed using SPSS (version 19). *Results.* Over the 4.5 years of follow-up there were 99 deaths (18.8% mortality). A Kaplan-Meier survival curve revealed that a large proportion of the deaths were within the first 100 days (29 deaths; 29% of all deaths). As a consequence, Cox regression analysis was performed separately to deter-

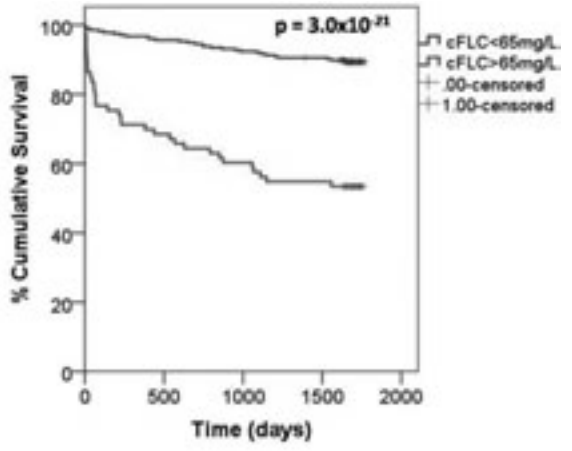

Figure 1.

mine risk factors for all deaths and deaths within 100 days. The relative risk of death increased proportionally with increasing combined FLC (cFLC) concentrations. Patients with cFLC concentration >50mg/L had a significantly increased risk of death compared to patients with cFLC concentration <50mg/L (75%ile overall survival (OS) <50mg/L not reached, >50mg/L 851 days, $p = 8.7x10^{-12}$). Receiver Operator Characteristic Curves (ROC) analysis indicated that cFLC concentrations >65mg/L was the optimum cut off associated with mortality in this population (75%ile OS <65mg/L not reached, >65mg/L 298 days, $p = 3.0x10^{-21}$. Figure 1). Univariate analysis identified albumin <33g/L, Crp >10mg/L, ESR >12, eGFR <30mL/min/1.73m^2, age >75 and cFLC >65mg/L as being significant predictors of mortality for the whole population and for mortality within 100 days. Gender was associated with mortality within 100 days only. Using multivariate analysis only cFLC concentrations >65mg/L, albumin concentrations <33g/L and eGFR <30mL/min/1.73m^2 were independently associated with mortality within 100 days and for the entire duration of follow up. Age >75 years old was independently associated with mortality over the duration of follow up but not for mortality within 100 days. A simple risk stratification model based on albumin <33g/L and cFLC >65mg/L identified 86% mortality within 100 days and 62% over the duration of follow up, compared to 50% and 24% using albumin <33g/L and 73% and 50% using cFLC >65mg/L independently. *Conclusion.* Polyclonally raised cFLC measurements predict significantly high mortality both within 100 days and beyond. cFLC levels can used in a simple risk stratification model to identify 'high risk' patients with increased mortality even in those *without* B cell disorders.

0806

EMPIRIC ANTIBIOTIC STRATEGIES IN FEBRILE HAEMATOLOGICAL PATIENTS IMPACT ON EPIDEMIOLOGY AND LETHALITY OF BLOODSTREAM INFECTIONS

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Background. Adequate empiric antibiotic therapy in febrile neutropenic patients is mandatory for a favourable outcome of infectious complications. Epidemiological surveillance of infections occurring at a Haematology Unit is essential to drive an effective antibiotic strategy. *Aims.* To determine epidemiology, prognostic factors and emerging antibiotic resistance of bloodstream infections (BSI) according to empiric antibiotic strategy adopted. *Methods.* All BSI consecutively occurred at our Institution during a 78 month period (June 2004-December 2010) were evaluated and correlated with type and state of underlying disease, neutropenia, associated pneumonia, previous antibiotic therapy, including prophylaxis with fluoroquinolones, resistance to antibiotics and outcome. Empiric antibiotic therapy was different during time according to epidemiological data collected. *Results.* During the entire period of observation, 502 BSI were recorded. Fungi were responsible in 8 (1.6%) cases; Gram-positive (G+) bacteria in 167 (33.3%), Gram-negative (G-) bacteria in 293 (58.4%). In 35 (7%) cases a polymicrobial (PM) BSI was observed. Globally, no significant differences in the distribution of fungal, G+, G- and PM BSI were recorded over time. Empiric antibiotic strategies adopted were ceftriaxone+amikacin from June '04 to March '06 (21 months: period A), piperacillin/tazobactam+amikacin from April '06 to June '09 (38 months: period B) and ceftazidime+amikacin from July '09 to December '10 (19 months: period C). BSI/month ratio was 5.7, 6.8 and 6.3 during period A, B and C respectively. P. aeruginosa BSI were more frequent during B and C in comparison with A (21% and 19% vs 9% respectively, p=0.005 and 0.04); multiresistant Pseudomonas (MR Pseud) were absent in A and accounted for 12% and 9% in B and C respectively. Vancomycin-resistant enterococci (VRE) accounted for 25% and 22% of all enterococcal BSI in A and B, respectively, whereas they were absent in C. Frequency of extended-spectrum β-lactamases (ESBL+) enterobacteriaceae was lower during B (10%) in comparison with A+C (21%) (p=0.037). Overall mortality was 9.6%; it was significantly higher during B in comparison with A+C (12.3% vs 6.2%, p=0.021). P. aeruginosa BSI lethality was absent during A; during B it was higher than during C (31.5% vs 17.4%), although not significantly. MR Pseud BSI lethality was significantly higher during B in comparison with C (46.9% vs 9.1%, p=0.033). Among other MR pathogens, 3/7 patients with a VRE BSI and 1/33 patients with ESBL+ BSI died. Univariate analysis showed that uncontrolled haematological disease (p<0.0001), P. aeruginosa BSI (p<0.0001) and associated pneumonia (p=0.0014) were statistically significant risk factors for death. Piperacillin/tazobactam+amikacin as empiric antibiotic approach was also associated to increased risk of death at our Haematology Unit (p=0.021). *Summary/Conclusions.* The empirical approach with ceftazidime+amikacin showed higher efficacy in terms of reduced MR Pseud BSI lethality; for this reason at present it is the association still adopted at our Haematology Unit. When piperacillin/tazobactam+amikacin was chosen as empirical antibiotic therapy the frequency of ESBL+ enterobacteriaceae was reduced; however, the impact of ESBL+ BSI on mortality seems to be limited. Further epidemiological surveillance is warranted in order to monitor emerging resistant strains (particularly ESBL+) and related mortality.

0807

RISKS OF POST-SPLENECTOMY SEPSIS: ARE PATIENTS AWARE? A SURVEY

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Introduction. Splenectomy is associated with a lifelong increase in the risk of sepsis. The aim of this survey was to assess the level of knowledge of patients who have undergone splenectomy in Northern Lincolnshire and Goole NHS Trust. *Methods.* Adults undergoing splenectomy between 1993 - 2009 in our trust were invited to participate in the survey. Standardised postal questionnaires were completed by them. *Results.* 245 splenectomies were carried out in that duration. 85 responses were obtained from 145 patients who were alive as per our records in Jan. 2010. Most of them (94%) were aware of increased risks of certain infections. Majority of them (69.6%) didn't think they (or their family) received any counselling before/after the operation. 54% had standby antibiotics readily available to them. 48% of them carried an asplenia card/identifying bracelet or necklace to alert others of their splenectomy. Majority of them were not aware of travel precautions (62.4%) and increased risk of Malaria (73%). *Conclusion.* Although most patients displayed a good knowledge of infection risks of post-splenectomy sepsis, deficiencies were identified. We propose the development of a splenectomy protocol and patient briefing to improve patient education to reduce the risks of post-splenectomy sepsis. We also propose the development of a national database for Asplenic/Hyposplenic patients.

0808

FIRST LINE TREATMENT OF PROBABLE AND PROVEN INVASIVE ASPERGILLOSIS WITH CASPOFUNGIN IN ONCOHEMOPATIC PATIENTS. A SINGLE CENTRE EXPERIENCE

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Background. Infections are the main complication for oncohemopatic patients (pts) with severe neutropenia. Caspofungin (Caspo) inhibits the growth of the fungal cell wall. *Aims.* to evaluate the tolerability and efficacy of Caspo. *Methods.* Between 2004 and 2009 we have treated 70 consecutive adult neutropenic pts with probable or proven invasive aspergillosis with Caspo as first line therapy. In case of persistent fever (3 days) despite broad spectrum antibiotic therapy, a high-resolution CT-scan of the lungs and galactomannan test were performed. According to the revised EORTC criteria and Cornely *et al.* (CID 2007) we defined as probable the infections with clinical criteria and a highly suggestive CT-

scan. The pts were 39 males and 31 females; the mean age was 56 yrs (range 18-77 yrs). The diagnoses were: leukemia 54 (77%), myeloma 2 (3%), lymphoma 14 (20%); the disease's phases were: new onset 30 (43%), remission 18 (26%), relapse 22 (31%). Thirteen pts received an allogeneic and 5 an autologous transplant; the other pts received an induction or consolidation or rescue chemotherapy course. *Results.* Infections were proven in 15 cases (21%: 12 aspergillus spp, 2 aspergillus fumigatus, 1 G. capitatum) and probable in 55 cases (79%). The first site of infection was the lung in 69 pts (99%) and paranasal sinuses in 1 patient (1%). CT scan was positive (halo sign or air-crescent sign) in all the pts with a lung localization, while the chest X-ray was positive in 40% of them. BAL was performed in 36 pts. Galactomannan on BAL and serum was positive in 32/36 (89%) and 30/70 cases (43%) respectively. Caspo was administered at the dose of 70 mg i.v. on day 1 followed by 50 mg i.v. in 1 hour daily. The mean time of treatment was 17 days (range 13-25 days). Caspo was well tolerated and not discontinued for adverse events. No premedication was necessary. The global (partial and complete) response, defined as clinical and radiological, was 60/70 (86%); 10 pts died for fungal infection. The responses were similar for probable and proven infections. No breakthrough fungal infections were found. All surviving patients, upon discharge from the hospital, received oral treatment with Voriconazole or Posaconazole. Among the 60 responsive patients, 30 (50%) died later: 26 for hematologic disease and 4 for sepsis. In 3 cases there was the recurrence of the fungal infection. *Conclusions.* For all the cured pts, there was a concomitant recovery of neutrophils so also in our experience this appears to be crucial for the resolution of the infection. In conclusion the resolution rate of the infections is very high (86%); Caspo seems safe, it does not preclude any other treatment, it is well tolerated and the cost is lower than other antifungal treatments.

0809

THE EFFECTIVENESS OF MEGA-DOSE METHYLPREDNISOLONE AND FRESH FROSEN PLASMA TREATMENTS IN PATIENTS WITH CRIMEAN-CONGO HAEMORRHAGIC FEVER ASSOCIATED WITH REACTIVE HAEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Background. Crimean Congo Hemorrhagic Fever (CCHF) is characterized by a macrophage activating syndrome, which starts during the period when viremia decreases. A cytokine storm initiates and triggers the development of haemophagocytic lymphohistiocytosis (HLH). Cytokines mediating vascular dysfunction causes disseminated intravascular coagulation (DIC). The causes of hemorrhage are DIC, thrombocytopenia and endothelial damage. DIC may develop due to direct damage of endothelial cells by the virus, liver disfunction and cytokine storm (increase in interleukin (IL)-1, IL-6, IL-12, IL-18, tumor necrosis factor (TNF)-α and intereferon (IFN)-γ). *Aims.* The effectiveness of mega-dose methylprednisolone (MDMP) (5-30 mg/kg/d) and fresh frozen plasma (FFP) (15 ml/kg 1-3 doses in a day) was investigated in patients with CCHF associated with reactive haemophagocytic lymphohistiocytosis (HLH). *Methods.* Seven patients with CCHF in association with reactive HLH were included in the study and treated with MDMP and FFP. 2 patients were treated with intravenous immunoglobulin (1 g/kg/d for 2 days) in addition to the MDMP and FFP treatments because the patients had resistant thrombocytopenia. *Results.* All patients were succesfully treated with MDMP and FFP. Fever decreased under 37'C in 1,6 ± 0,9 days, WBC increased above 4.500/ul in 4,0 ± 2,1 days, platelets increased above 150.000/ul in 8,6 ± 5,3 days and D-dimer decreased under 1 mcg/dl in 5,9 ± 3,8 days. *Conclusions.* We suggest that CCHF associated with reactive HLH should be treated with MDMP and FFP to supress the macrophage activation and cytokine storm, and to complement the deficient coagulation factors due to disseminated intravascular coagulation, respectively.

0810

CHARACTERISTICS AND RISK FACTORS FOR ABSCESSES IN A CONSECUTIVE COHORT OF PATIENTS WITH ACUTE MYELOID LEUKEMIA PATIENTS: A TERTIARY CARE CENTER EXPERIENCE

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Background. Abscesses may occur as an infectious manifestation in acute myeloid leukemia (AML) patients. Early reports primarily focused on anorectal infection in leukemic patients only. Recently, articles of leukemic patients developing abscess mostly focused on brain abscesses or fungal infections and were case reports only. There were no systemic analyses done before regarding the pathogens prevalence, risk of abscess, survival, or treatment consensus in leukemic patients diagnosed of abscesses. *Aims.* We focused on the prevalence of abscess in patients with AML and the clinical characteristics, risk factors, and prognosis of AML patients developing abscess. *Methods.* 354 newly diagnosed patients were retrospectively analyzed. Eligible patients were sub-grouped as abscess group (n = 34) and non-abscess group (n = 320). We determined the factors potentially associated with abscess incidence. *Results.* The prevalence of all kinds of abscesses in AML patients is 9.6% with predominant sites at perianal and hepatosplenic abscesses. Bacteria were the major pathogen of abscesses. The two independent risk factors predicting abscess development in AML patients are secondary AML and receiving intensive induction (P = 0.001, HR = 3.739; P = 0.002, HR = 3.773, respectively). We categorized patients according to the numbers of two independent risk factors they possessed (0, 1, and 2 risk factors). Figure 1 showed the cumulative incidence of abscess within 36 months after AML diagnosis according to the risk stratification system (1.7% vs. 12.4% vs. 22.2%, 0 factors vs. 1 factors vs. 2 factors, P = 0.001). Patients developed abscesses in non-remission status of AML are associated with higher post-abscess 100 days mortality (92.3% vs. 42.0%, P = 0.004). *Conclusion.* Abscesses are not uncommon in AML patients. Patients who have secondary AML and receiving intensive induction chemotherapy warrant special attention since they are prone to have abscess. Leukemic free status at abscess diagnosis predicts a better survival in AML patients with abscess.

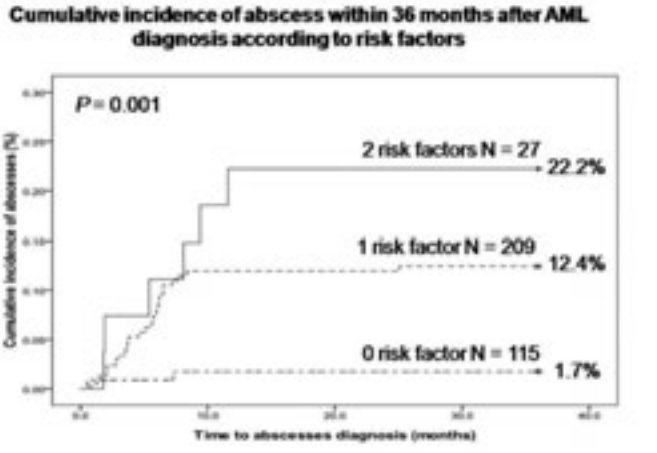

Figure 1. Cumulative incidence of abscess within 36 months.

0811

MORPHOMETRIC NEUTROPHIL DATA FOR EARLY DIAGNOSIS OF SEPSIS IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES

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Background. Early diagnosis of serious blood stream infections is essential to improve treatment and survival of patients with haematological diseases. C-reactive protein (CRP) in combination with neutrophil counts and the manual band counts in blood smears are often used for predicting systemic clinical infections (sepsis). However CRP rises several hours after the onset of infection, so it can be falsely negative; on the other side, the manual myeloid immaturity differential count is a time consuming procedure, requiring well-trained skills of the laboratory technicians. Neutrophils are early activated by mediators of inflammation in systemic infections and rapidly change their volume and granularity. *Aim.* To investigate the clinical usefulness of additional morphometric data from automated hematology analyser of reactive neutrophils for predicting systemic clinical infections (sepsis) in patients with haematological malignancies. *Methods.* Morphometric research population data (Volume, V; conductivity, C; Scatter; S) were obtained by VCS technique of the Beckman Coulter DxH 800 hematology analyser during automated differential counts of peripheral blood of 134 consecutive adult haematologycal patients with positive blood culture and nucleic acid amplification tests for Gram (+) and

Gram (-) bacteria and in 65 patients from the Haematology Unit but without systemic infections. 64 patients investigated were affected by lymphoprolipherative disorders, 40 by myeloproliferative disease, 23 by multiple myeloma, 50 by myelodysplastic syndrome and 22 had undergone stem cell transplantation. *Results.* Statistically significant increase of mean neutrophil volume (MNV) was observed in peripheral blood of septic patients when compared to patients without infections (MNV: 161 ± 15.1 vs 144 ± 8.6, $p < 0.001$) whereas CRP and absolute neutrophil counts did not discriminate between these two groups. With a cut-off of 153 for MNV were achieved a sensitivity and a specificity of 74.3 % and 85 %, respectively. *Conclusions.* MNV is easily and rapidly obtained by routine analysis of peripheral blood on new haematology analyser; it seems to be a promising useful marker for early onset systemic infections.

0812

SAFETY AND EFFICACY OF AN EDUCATIONAL PROGRAM IN REDUCING 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. *Results.* Ninety-five PICCs were in place for a total of 7,295 PICC days (range, 1-331 days; mean, 76,7 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. There were 2 thrombotic complications PICC-related (0,27 per 1,000 CVC days), and only one CRBSI (0,13 per 1,000 CVC days) during neutropenia. Other mechanical complications occurred in 11 catheters, and were accidental dislodgement (4), catheter break (3), catheter inadequate (4). *Conclusions.* Our results indicate that a training and competence assessment program is effective in reducing the main complications PICCs-related in haematological setting.

Table 1.

Complications requiring early removal of peripherally inserted central catheter (PICC).

Complications	n.	per 1000 CVC days (total days 7.295)
Mean duration of PICC	Range 1-331 days	76.79 days
Infective complications:		
Definite CRBSI	1	0.13
Non infective complications		
Thrombosis	2	0.27
Occlusion	5	0.68
Accidental dislodgment	4	0.54
Catheter breakage	3	0.41
Catheter inadequate	4	0.54

CRBSI catheter-related bloodstream infection.

0813

EFECTIVENESS OF SECURING CENTRAL VENOUS CATHETERS IN HEMATOLOGIC PATIENTS WITH SUTURELESS PERCUTANEOUS CATHETER ATTACHMENT DEVICES.

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Introduction. Catheter stabilization devices are becoming standard of care for peripheral venous and arterial lines but scarce data are available about sutureless attachment devices of Central Venous Catheters (CVC). *Objective.* The aim of this prospective observational study was to evaluate the effectiveness of securing CVC in hematologic patients with sutureless StatLock adhesive attachment devices instead of traditional securement techniques like sutures and/or tape. *Methods and Results.* From January 2010 to December 2010, a total of 211 short-term CVC and 5162 catheter days were studied. All CVC were bilumen and secured with a sutureless StatLock attachment devices (Figure 1). No patient was given prophylactic antibiotics or anticoagulation drugs for infection or thrombosis. All patients received close dressing on the CVC insertion site every 3 days. The insertion sites of CVC were: internal jugular vein 156/211 (74%) and subclavian vein 55/211 (26%). The median CVC life span was 24 days (range 1-106 days). We evaluated the success rate of correct implantation of CVC, the dislodgement rate, the incidence of exit site infections and CVC infections related. The success rate of CVC implantation was 98 % (208/211). The incidence of catheter related infections were 3,5/1000 catheter days, that was very low according to the literature data on this topic. The exit site infections were only 14/211 (7%). Premature dislodgement rate of CVC and extravasion rate were 1,4 % (3/211) and 2% (4/211), respectively. *Conclusions.* The sutureless stabilization technique is safe and effective with a low complication rate. These securement devices can replace the traditional suture fixation of CVC in hematologic patients and appear to improve care in this setting by reducing infectious and noninfectious complications.

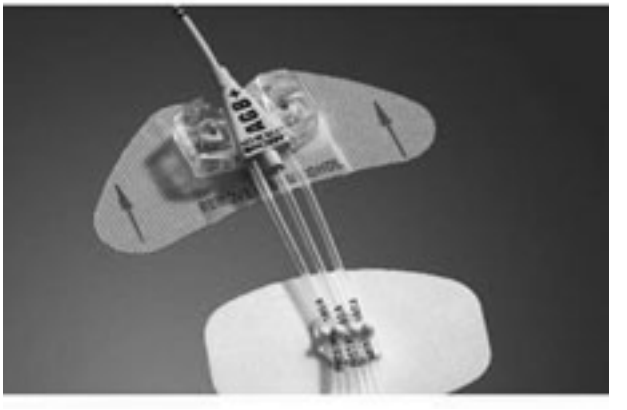

Figure 1.

0814

VORICONAZOLE PLASMA LEVELS AND GENETICS POLYMORPHISM OF CYP2C19 IN HEMATOONCOLOGICAL PATIENTS

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Background. Voriconazole (VORI) is a broad spectrum antifungal which exhibits a wide spectrum of its plasma concentrations. Among others, differences in drug metabolism due to polymorphic gene expression of CYP2C19, is discussed as an possible reason for this variability of VORI plasma concentrations. *Aims.* To evaluate the genotype of

Table 1.

	wild type	heterozygous mutations
number of patients	90	33
number of samples	333	136
median of VORI plasma concentration µg/ml	1.04	1.73
mean VORI plasma concentration µg/ml	1.34	1.44
VORI plasma concentration <1µg/ml	47.7% (n=159)	30% (n=41)
Vori plasma concentration 1-4µg/ml	49.6% (n=165)	65.5% (n=89)

CYP2C19 in hematooncological patients treated with VORI in our institution and its correlation with VORI plasma concentrations obtained during the VORI treatment. *Methods.* Trough VORI plasma concentration were measured using a high performance liquid chromatography assay. A retrospective analysis of laboratory results from patients treated with VORI between August 2005 and January 2010 was preformed. The genotype status of CYP2C19 was determined retrospectively using a polymerase chain reaction. *Results.* From 122 patients included in the study 90 patients have got wild type in both alleles of CYP2C19. 32 patients have got heterozygous mutations. There was no patient with homozygous mutations in our study. In our patient group VORI was administered in 40.7% as prophylaxis, in 13.5% as an empirical antifungal treatment and in 45.8% as a preemptive treatment or treatment of provenof invasive fungal infection. VORI was administered mainly orally; only in 2% of 481 analyzed samples intravenously. The median plasma concentration (after standard daily dose 400 mg orally) was in the wilde type group 1.04 µg/ml and 1.73 µg/ml in the group with heterozygous CYP2C19 mutations. The VORI plasma concentration was < 1.0 µg/ml, below the level associated with a better response to treatment of invasive aspergilosis, in 47.7% in wild type group and in 30% in heterozygous group. There was a significant difference between medians of VORI plasma concentration in group with wild type and group with heterozygous mutations - 1.04 and 1.73 (p=0.014, median test). The results are in Table 1. *Conclusion.* The knowledge of genotype status of CYP2C19 could be an useful guide during the antifungal treatment. Together with VORI plasma concentrations monitoring help us to achieve the plasma and tissue concentrations and improve the outcome of invasive fungal infection in hematooncological patients.

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0815

CLONAL PATTERNS OF X-CHROMOSOME INACTIVATION IN FEMALE PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA USING REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION METHOD

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Background. Chronic idiopathic neutropenia (CIN) is a disorder of granulopoiesis characterized by increased apoptosis of the granulocytic progenitor cells and presence of activated, oligoclonal T-lymphocytes with myelosuppressive properties in the bone marrow. The underlying T-cell activating stimulus remains obscure. The possibility of the clonal origin of CIN, that might induce the immune system activation, has never been investigated so far. *Aims.* To probe the hypothesis of the clonal origin of CIN by looking for clonal patterns of X-chromosome inactivation in peripheral blood (PB) cell subsets of female CIN patients. *Methods.* We have studied the expression of the polymorphism at nucleotide (nt) 1311 of glucose-6-phosphate dehydrogenase (G6PD) and at nt 438 of iduronate-2-sulfatase (IDS) using a reverse transcription polymerase chain reaction (RT-PCR), in 77 females fulfilling the diagnostic criteria for CIN and 18 female healthy controls. Lymphocytes were purified from PB samples by density gradient centrifugation whereas granulocytes were isolated from the red cell pellet by Dextran precipitation. DNA extracted from total PB cells was screened for heterozygocity at nt 1311 of G6PD and/or at nt 438 of IDS. RNA was extracted from the isolated cell subpopulations of individuals showing heterozygocity of at least one of the two genes and assessed further by means of RT-PCR. Samples showing the expression of >95% of one allele in granulocytes and/or lymphocytes were classified as a "clonal pattern". *Results.* Overall, 19 CIN patients and 6 healthy individual heterozygous for G6PD and/or IDS were further studied. Of these subjects, 10 CIN patients and 3 healthy individuals were heterozygous for the nt 1311of G6PD whereas 12 CIN patients and 4 healthy individuals were heterozygous for the nt 438 of IDS. Clonality analysis using the G6PD polymorphism showed that 6 of 10 CIN patients (60%) displayed a clonal pattern in both granulocytes and lymphocytes. Two patients (20%) showed a clonal pattern in granulocytes but a polyclonal pattern in lymphocytes. Two patients (20%) showed expression of both alleles in granulocytes and lymphocytes compatible with polyclonality. Clonality analysis using the IDS polymorphism showed that 7 of 12 CIN patients (58.3%) displayed a clonal pattern in both granulocytes and lymphocytes. One patient (8.3%) showed a clonal pattern in granulocytes but a polyclonal pattern in lymphocytes. Four patients (33.3%) showed a polyclonal pattern in granulocytes and lymphocytes. Analysis of samples that were informative for both polymorphisms showed that the results were concordant in every case. None of the controls showed a clonal pattern in G6PD and/or IDS expression in either granulocytes or lymphocytes. Combining the G6PD and IDS results, clonal patterns were observed in granulocytes of 13/19 CIN patients (68.4%) and 0/6 normal individuals (p<0.01). *Summary/Conclusions.* Clonal patterns of granulocytes occasionally in association with clonal patterns of lymphocytes are identified in a significant proportion of CIN patients. These data suggest the possible involvement of a pruripotent or myeloid committed clonal stem cell in the pathogenesis of CIN, indicating for first time the possible clonal origin of this disease.

0816

PREGNANCY OUTCOME IN GENETIC SUBTYPES OF CONGENITAL NEUTROPENIA

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Severe congenital neutropenia (CN) comprises a heterogeneous group of disorders with a common hematological and clinical phenotype characterized by a maturation arrest of myelopoiesis at the level of the promyelocyte/myelocyte stage with peripheral blood absolute neutrophil counts (ANC) below 0.5 - 109/l and early onset of bacterial infections. Current data on the molecular causes suggest that CN is a multigene disorder. CN follows an autosomal dominant or autosomal recessive pattern of inheritance. To date more than 10 gene mutations have been described which are involved in disorders associated with CN. Genetic analyses in autosomal dominant and sporadic cases of CN indicate that the majority of these cases are attributable to mutations in the elastase 2 (ELANE) gene encoding neutrophil elastase. However, mutations in the ELANE gene do not discriminate between patients with CN and patients with cyclic neutropenia (CyN), another rare congenital disorder with a cycling haematopoiesis of 21 days. In addition, a number of less frequent other mutations has been identified in recessive CN, which are mainly associated with multi-organ involvement such as p14, SBDS, G6PT, G6PC3, TAZ or WAS. Since 1987, recombinant human Granulocyte-Colony stimulating factor (G-CSF) has been available for treatment of CN. More than 90 percent of patients respond well to G-CSF with a sustained increase of absolute neutrophil counts and a prolonged life expectancy. Since our first patients reach adulthood the desire for parenthood is coming up. In this study we assessed the outcome of 22 pregnancies in 12 mothers and 13 pregnancies from 7 fathers with different genetic subtypes of congenital neutropenias or unknown gene mutation as shown in the table below.

Table 1.

	Genotype	Mother/Father (n)	Life births	Miscarriages		
				Newborn with CN	Newborn without CN	Unknown
ELANE	CN	Mother	3	4	1	1
		Father	4	4	1	1
SDS		Mother	1	1		
		Father	0			
ELANE	CyN	Mother	2	3		
		Father	2	3	2	
	CN	Mother	4	3	3	1
	UNKNOWN	Father	1	1	1	
	CyN	Mother	2	2	1	1
	UNKNOWN	Father				
		Total Mothers 12				
		Total Fathers 7				
Total pregnancy outcomes			16	9	7	3

Conclusion. Out of the 32 life births 16 newborns presented with congenital neutropenia documenting the inheritance of the genetic defect from the affected parents, 9 were healthy and in 7 the outcome was not reported. In 3 mothers a miscarriage was documented. No other neonatal abnormalities were reported in our cohort, independent of any cytokine treatment during pregnancy. 32 of the 35 pregnancies resulted in a life birth. However, the proportion of newborns with congenital neutropenia reflects the inheritance of the genetic defects from their affected parents and reveals the need for genetic counseling.

0817

DOCUMENTED INFECTIONS IN NEUTROPENIA: DATA FROM THE ITALIAN NEUTROPENIA REGISTRY

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Background. Infections are important causes of morbidity/mortality in children with severe congenital (SCN), autoimmune (AN) and idiopathic (IN) neutropenia. Few data on the incidence and type of infections are available in the literature *Aims.* to describe incidence and type of infections in a large population of neutropenic patients. *Patients and Methods.* Patients affected with SCN, AN and IN followed from 2000 to 2009 in 8/20 centres of the Italian Neutropenia Registry entered the study. As infections data contained in the INR were not enough detailed for the aims of our study and in order to uniform infection diagnostic criteria,a dedicated CRF was sent to each centre for retrospective data collection.The occurrence of infections was calculated during a period "at risk"defined as person-days at risk (pdr). The infection rate (IR) was calculated by dividing the number of events by the pdr and expressed as episodes/1000 pdr (95% CI). *Results.* Seventy three patients (28 females and 45 males) of whom 12 (16%) with SCN, 38 (52%) with AN and 23 (32%) with IN were analyzed. At least one infectious episode was observed in 100% of SCN and in 30% of both AN and IN. Overall 2/73 patients died (2,7%). They were both SCN subjects of whom one deceased of haploidentical HSCT related complication after of done for loss of response to G-CSF and the other of sepsis after the parents decided to stop G-CSF. From birth to the last follow up, a total of 108 infections occurred in 31/73 subjects (42%): 69 episodes in 12/12 patients with SCN, 25 infections were concentrated in 12/38 patients with AI and 14 episodes in 7/23 IN. Skin/subcutaneous infections (49%) and pneumonia (18%) were the most frequent localizations. Othomastoiditis, tonsillar phlegmons, osteomyelitis (10%) and deep abdominal infections (5%) occurred almost exclusively in SCN. Microbiological characterization was possible in 26/108 episodes and showed a slight prevalence of Gram-negatives (13/26) over Gram-positives (9/26) without differences according to the type of neutropenia. Fungal infections was diagnosed in two different SCN subjects.G-CSF was used in all SCN patients, in 26% of AN and 4% of IN. In the whole population and in each group, the IR in the time-span from birth to the emergence of neutropenia was significantly higher vs IR calculated from the emergence of neutropenia to the last follow up (p<00,1). *Summary/Conclusion*. Among neutropenic children the highest infectious burden was observed in SCN, but was not negligible in AN and IN. Skin was the main involved site. Gram-negatives were the slightly prevalent microorganisms while fungi occurred only in SCN. The significant decrease of IR after diagnosis of neutropenia vs previous period can probably be attributed to the increased awareness of the patients/family, to early antibiotic intervention and to the G-CSF therapy.

0818

DETECTION OF ANTI-NEUTROPHIL ANTIBODIES IN CHILDREN WITH CHRONIC NEUTROPENIA

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Background. The widespread use of automatic blood counters has led to the diagnosis of neutropenia in an increased number of children. Chronic neutropenia in children may be caused by autoimmune mechanisms, mainly related to circulating antineutrophil antibodies. Primary autoimmune neutropenia of infancy and childhood is characterized by moderate to severe neutropenia, exhibits a self-limited course and is usually not associated with serious infections. The treatment of this condition is mainly supportive. *Aims.* To investigate the role of the detection of antineutrophil antibodies in the differential diagnosis process of children with chronic neutropenia. *Methods.* Blood samples from children with neutropenia of at least 3 months; duration were tested for anti-neutrophil antibodies in specialized laboratories by using Granulocyte Immunofluorescence Test (GIFT), Granulocyte Agglutination Test (GAT) and selectively Monoclonal Antibody Immobilization of Granulocyte Antigens Assay (MAIGA). Clinical data on the occurrence of bacterial infections and treatment were collected. *Results.* We evaluated 100 patients who presented with chronic moderate-severe neutropenia defined as an absolute neutrophil count below 1,000/μl for at least 3 months. The mean age at diagnosis was 28 months (median, 15 months; range, 3 months-13 years). The male-to-female ratio was 1:1.2. Neutrophil-specific antibodies were detected in 75 patients (75%) (75% with GIFT, 34% with both GIFT and GAT). To identify the specificity of the detected anti-neutrophil antibodies MAIGA was performed in 21 patients. Most of these patients had antibodies against Fcγ receptor IIIb (20 out of 21, 95%). Positive antibodies were demonstrated in 2 out of 4 patients, who were retested after an initial negative result. Further evaluation in 3 of 24 seronegative patients established the diagnosis of congenital neutropenia (Barth syndrome, Kostmann syndrome, severe congenital neutropenia syndrome, respectively). The clinical course of seropositive patients was, in general, benign. Supportive treatment was required for patients with frequent or serious infections and included antibiotic prophylaxis (15 patients), regular infusion of IVIG (2 patients), and short term G-CSF administration (2 patients). *Conclusion.* Anti-neutrophil antibodies are frequently detected in children with chronic neutropenia. The different methods used have limited and often complementary sensitivity; their results need to be correlated with the clinical course of the patient. Detection of anti-neutrophil antibodies is of significant value in establishing the diagnosis of autoimmune neutropenia and may limit the breadth of necessary workup. Autoimmune neutropenia in childhood has a benign, yet prolonged, course.

0819

CHRONIC NEUTROPENIA: CAUSES, CLINICAL-LABORATORY CHARACTERISTICS AND FOLLOW UP OF 229 PATIENTS

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Background. Chronic neutropenia, usually discovered in routine examination, constitutes a rather common medical condition. Patients often undergo strenuous and repeated tests for this condition which troubles them as well as their physicians. *Aim.* The study of clinical and laboratory characteristics as well as the frequent causes of chronic neutropenia, and the verification of an algorithm for the investigation and follow up of this condition. *Methods.* Patients who attended the Outpatient Hematology Clinics of the University of Athens and Athens Medical Center presenting neutropenia ($1.8x10^9$/l) of >6 months duration were evaluated. Complete blood counts (CBC) and blood smear examination (BSE) as well as laboratory investigation, including BUN, liver function tests and LDH were performed. Patients also underwent the following examinations: Serum thyroid tests and autoantibodies, ferritin, B_{12}, folic acid, antibodies against EBV, CMV, toxoplasma, HBV, HCV, HIV, a direct Coombs test and ultrasonography of liver/spleen. Previous CBC's revealing duration of neutropenia were reviewed. A blood immunophenotype with CD3, CD4, CD8, CD16, CD19, CD20, CD38, CD56, CD57 monoclonal antibodies was performed in all pa-

Table 1. Special causes of chronic neutropenia.

Causes of neutropenia	#	%
Thyroid disorders (Hashimoto's thyroiditis / Other)	61 (30 / 31)	27
T-LGL (Clonal TCR)	56 (27)	24
Autoimmune disorders SLE / Sjogren syndrome / Rheumatoid arthritis/ Sarcoidosis / Polymyalgia rheumatica	10 (3/3/2/1/1)	4
Drug intake	10	4
MDS	8	3
B12 insufficiency	5	2
Viral infections (HBV / EBV / HCV)	5 (3/1/1)	2
No specific cause-(common idiopathic neutropenia / autoimmune neutropenia)	74	32

tients. If T-large granular lymphocytosis was found, T-cell receptor rearrangement (TCR) was examined. Patients with clonal TCR expansion or suggestion of myelodysplastic disorder in BSE underwent a bone marrow examination (BME). Follow up was performed with clinical examination, CBC and BSE every 6 months in patients with neutrophils<1.5x10^9/l and every 12 months in the rest, unless a specific disorder was found. *Results.* During 2004-2010, 229 patients with chronic neutropenia were examined. Male to female ratio was 1:4 and median age at diagnosis 55(15-83) years. Median duration of neutropenia was 4(1-20) years. Median WBC at presentation was 3,68x 10^9/lt(1.62-6.1 x 10^9/l) and median neutrophil count was 1,66x 10^9/l(0,16-1.8 x 10^9/l). Infectious complications were observed in 75 patients (33%). Frequent complications included recurrent urinary infections (37 pts), apthous stomatitis (30), recurrent febrile upper respiratory infections (9) and chronic sinusitis (8), which were usually successfully treated with the appropriate antibiotic therapy. Median neutrophil count of patients with common infections was not statistically different from those without recurrent infections. 45 patients underwent a BME -17 with clonal T-LGL in 13 of whom a scarse lymphocytic infiltration was found and among the rest 28 pts, 8 were diagnosed with myelodysplastic syndrome and 8 with reactive/myelodysplastic-like findings. Neutropenia was attributed or related to special causes as shown in Table 1. In 74 patients no related cause was found and they were classified to the chronic idiopathic neutropenia (CIN) group. Median follow up was 26 (6-75) months. During follow up, GCSF was not administered to our patients except to those with diagnosis of myelodysplastic syndrome. No life-threatening infection was recorded. *Conclusions.* Chronic neutropenia constitutes a common problem of the general population and is frequently attributed to thyroid disorders, T-LGL, autoimmune diseases, drug intake and CIN. Serious complications are very rare and infections, when observed, are recurrent but mild and easily treated. Follow up should include a CBC and BSE. Longitudinal follow up is, however, required to confirm the favorable prognosis of chronic neutropenia.

0820

SEVERE NEONATAL ALLOIMMUNE NEUTROPENIA IN A NEWBORN DELIVERED BY A CD16 DEFICIENT GYPSY WOMAN

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Background. Neonatal Alloimmune Neutropenia (NAN) is an uncommon disease of the newborn due to maternal IgG alloantibodies against fetal human neutrophil antigens (HNA) or leukocyte antigens (HLA) inherited from the father but absent in the pregnant mother. The involved antibodies can readily cross the placental barrier, causing neutropenia in the newborn that can range from mild (1000-1500/µl) to severe (<500/µl). The incidence of NAN has been estimated to 1:1000/6000 live births, mainly due to HNA-1a or1b and HNA-2a antigens. *Aim.* We describe a severe case of NAN due to FcγRIIIb (CD16) alloantibodies occurred in a Roma (Gypsy) family. *Patients and Methods.* A female child was born from second, uncomplicated pregnancy to a healthy 16-year mother, at the 40th week of gestation. Mother had a normal neutrophil count and the first child was healthy. Severe neutropenia (190/µl) and ocular infections, with otherwise normal laboratory findings, was detected on the first day of the newborn's life and persisted for 3 weeks. Direct (D) and indirect (I) granulocyte immunofluorescence tests (GIFT) were performed on newborn's and mother's blood samples. Flow cytometry cross-match of mother serum against father granulocytes was also performed. Family HNA-1, -3, -4 and -5 genotypes were evaluated using BAGene HNA-Type extra 3 kit (BAG Health Care). Anti-CD177 monoclonal antibody was used to determine HNA-2a phenotype. *Results.* A strong positive reaction was observed in the newborn's D-GIFT. As reported in the table, family genotyping indicates that the HNA-1b antigen could be involved in this NAN case. Mother and newborn sera showed a strong reaction against father's neutrophils and against 16 typed HNA blood donor neutrophils (including 6 HNA-1b subjects). Only one donor showing FcγRIIIb deficiency did not react with mother's and newborn's sera, indicating that the alloantibody specificity was not related to HNA-1b but to whole FcγRIIIb. Interestingly, both mother and father resulted HNA-3a negative. *Conclusions.* Alloantibodies against FcγRIIIb were the cause of this case of severe NAN developed in a CD16 null Gypsy mother. The described family had the HNA-3b phenotype that is quite rare in the Caucasian population. It would be interesting to determine the HNA frequencies in Gypsies.

Table 1.

Results of HNA -1, -3, -4, -5 genotyping and of HNA-2a phenotyping

	-1a	-1b	-1c	-2a	-3a	-3b	-4a	-4bw	-5a	-5bw
Mother	-	-	-	+	-	+	+	-	-	+
Newborn	-	+	-	+	-	+	+	-	-	+
Father	+	+	-	+	-	+	+	-	+	+

0821

HUMAN NEUTROPHIL ANTIGEN-3 (HNA-3) EXPRESSION IN BRAZILIANS

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Background. Recently, it was characterized that the HNA-3 arises from a nucleotide polymorphism in the choline transporter-like protein-2(SLC44A2), as a result of a single nucleotide polymorphism rs2288904. The HNA-3a and HNA-3b variants are encoded by a guanine and adenine at nucleotide position 461, respectively, resulting in either an arginine or a glutamine residue at amino acid position 154. The role of the HNA-3 is still unknown, but it is a target antigen in febrile transfusion reactions, immune neutropenias and transfusion-related acute lung injury (TRALI). *Aims.* To develop a technique of genotyping for HNA-3, and determine the prevalence of HNA-3a and HNA-3b in the Brazilian population. *Methods.* We obtained DNA of 500 healthy blood donors, 120 Amerindians, 39 Japanese individuals, and 124 patients with sickle cell anemia (SCA). All individuals were genotyped for rs2288904 by PCR-RFLP assay. The amplified product was digested with enzyme Taqα1, specific to nucleotide guanine (HNA-3a). Genomic DNA of 9 blood donors (3 HNA-3a/a, 3 HNA-3a and 3 HNA-3 was sequenced to confirm the results of the PCR-RLFP. *Results.* The genotyping results showed that 66.2% of blood donors were homozygous for antigen HNA-3a, 30.2% were heterozygous (HNA-3a/b), while 3.6% were homozygous for antigen HNA-3b. All Amerindians typed homozygous for the antigen HNA-3a. Among Japanese subjects 43.6% were homozygous for HNA-3a, 43.6% were heterozygous, and 12.8% were homozygous for HNA-3b. Among African Brazilian individuals 72.6% were homozygous for HNA-3a, 25.8% heterozygous, while 1.6% were homozygous for HNA-3b. Overall, the frequency of the allele HNA-3a in the population of Brazilian blood donors was 0.80 and the allele HNA-3b was 0.20. The frequency of the HNA-3 was significantly different between blood donors and Japaneses (HNA-3a - 66.2% vs 43.6%, p=0.0045; HNA-3b -

Table 1.

Frequency of HNA-3 genotypes in Brazilians

Group	HNA-3a/a (%)	HNA-3a/b (%)	HNA-3b/b (%)
Blood Donors	66.2	30.2	3.6
Amerindians	100.0	0.0	0.0
Japaneses	43.6	43.6	12.8
SCA	72.6	25.8	1.6

3.6% vs 12.8%, p=0.0061); Amerindians and: blood donors (HNA-3a - 100% vs 66.2%, p<0.0001; HNA-3a- 0.0% vs 30.2%, p<0.0001; HNA-3b - 0.0% vs 3.6%, p=0.0057), Japaneses (HNA-3a - 100% vs 43.6%, p<0.0000; HNA-3- 0.0% vs 43.6%, p<0.0001; HNA-3b - 0.0% vs 12.8%, p<0.0001), African Brazilians (HNA-3a - 100% vs 72.6%, p<0.0001; HNA-3a- 0.0% vs 25,8%, p<0.0001); and Japaneses and African Brazilians (HNA-3a - 43.6% vs 72.6%, p=0.0009; HNA-3- 43.6% vs 25.8%, p=0.0346; HNA-3b - 12.8% vs 1.6%, p=0.0026). *Conclusions.* This study describes for the first time the frequencies of the antigens HNA-3 in Brazilians. The prevalence of HNA-3 antigens in Brazilian blood donors is similar to those described in Europeans (63.1% of HNA-3a/a, 32.1% HNA-3a and 4.8% of HNA-3 Greinacher et. al., 2009) and North Americans (59.8% of HNA-3a/a, 35.5% of HNA-3a and 4.7% of HNA-3 Curtis et. al., 2010). However, we found that all Amerindians were HNA-3a/a, while African Brazilians showed a slightly lower frequency of HNA-3b and Japaneses had a significant higher prevalence of HNA-3 The PCR-RFLP assay well validated by DNA sequencing, suggesting that this technique could be useful in transfusion practice to elucidate cases of HNA-3 incompatibility and reduce reactions involving anti-HNA-3.

0822

THE OXIDATIVE BURST OF NEUTROPHILS IS INFLUENCED BY COMBINED HORMONAL CONTRACEPTIVES IN A COMPLEMENT-DEPENDENT AND -INDEPENDENT MANNER

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Background. Neutrophils are crucial in host defense against invading microorganisms through reactive oxygen species (ROS) production that is also known as oxidative burst (OB). ROS are products of normal cellular metabolism and are generated by a tightly regulated enzyme complex, NADPH oxidase. Beneficial effects of ROS involve physiological roles in defence against infectious agents and maintenance of cellular redox homeostasis. However, inherited/acquired dysfunctions involving the OB response may result in oxidative stress and/or contribute to impaired neutrophil functions and inflammatory processes. The use of estrogen as hormonal contraceptives or hormonal replacement therapy is associated with the thromboembolic and chronic inflammatory diseases. In particular, the complement receptors (CR) are important mediators of the OB of neutrophils and are also involved in the inflammation. Of recent interest are the known antioxidant and pro oxidant properties of estrogens and their effects on neutrophil functions, as well as the influence of the sex hormones upon susceptibility to infections. *Aim.* To evaluate the effect of the estrogen and progestagen, as combined hormonal contraceptive (CHC), upon the OB of neutrophils mediated or not by CR. *Methods.* This study was approved by local Research Ethics Committee (protocol #114). All volunteers agreed to provide blood samples and gave written informed consent to participate in this study. Healthy women (n=54; 18-35 years-old), CHC users or not > 6 months, were assigned to one of three groups: control (CHC non-users, n=19); group 1 (30mg ethinylestradiol (E)/3mg drospirenone (D); n=17); group 2 (20mg E/3mg D; n=10); group 3 (30mg E/0.15mg levonorgestrel; n=8). The OB of neutrophils was measured by a luminol-dependent chemiluminescence (CL) assay. Isolated neutrophils were stimulated with Zymosan (Zy), opsonised or not with complement from normal human serum (NHS), hormonal contraceptive serum (HCS) or NHS inactivated by heat (NHI) as control. Cells in the absence of stimulus were also used as negative control. Area under the curve of CL profile was calculated. *Results.* The following differences were observed among the groups: an increased OB in neutrophils from women of the group 1 when stimulated with Zy/NHS and Zy/HCS compared to control group (Zy/NHS) (p<0.05, t-test); and the OB of neutrophils of group 3 was significantly higher than that observed for the control group when stimulated with Zy not opsonised (p<0.05, t-test). No differences were observed among the different CHC. *Summary.* These results suggest that different CHC influence the OB of the neutrophil in a complement-dependent (Zy/NHS and Zy/HCS) or -independent (Zy) manner. These observations may have significant consequences to inflammatory and homeostatic processes involving neutrophils, complement and hormonal regulation and these findings need to be investigated further in future. clenimar@usp.br

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0823

A STUDY OF NEUTROPENIA IN INFANTS AND CHILDREN ADMITTED IN A UNIVERSITY CHILDREN HOSPITAL IN CAIRO,EGYPT

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Background. Neutropenia is defined as absolute neutrophil counts (ANC) <1,000/mm3 in infants and <1,500/mm3 in children. Neutropenia may be congenital or acquired with infection being the most common cause followed by autoimmune neutropenia (AIN) whether primary or secondary. *Aim* of this work was to study the prevalence, severity, and etiological causes of neutropenia in infants and children admitted to a University Children hospital in Cairo, Egypt. *Methods.* 200 patients with neutropenia were recruited from the inpatients in The Children's Hospital Ain Shams University form April 1st 2010 to July 30th 2010, after having parental consent. Patients with a known hematological or immunological disease were excluded from the study. The patient's age ranged from 2 months-15 years (mean 24.4 ± 28 months), with male:female ratio of 1.1:1. Patients were classified according to ANC into mild, moderate and severe groups. Moderate and severe neutropenic children were followed for three months period. Follow up ANC was done till recovery or an underlying cause is uncovered. Viral serology was done for moderate/severe neutropenia patients including cytomegalovirus (CMV), Epstein-Barr virus (EBV),hepatitis B virus (HBV), hepatitis C virus (HCV),and HIV. Anti-Neutrophil Cytoplasmic Antibody (ANCA) tested by enzyme immunoassay and bone marrow aspirate were done for prolonged moderate / severe neutropenia (persisting for 3 weeks or more). *Results.* Out of 200 patients,neutopenia was mild in 90 (45%) moderate in 56 (28%) and severe in 54 (27%). The clinical diagnosis at admission was bronchopneumonia (38%), PUO (17%), bronchiolitis (13%), urinary tract infection (9%), acute gastroenteritis (8%), hepatitis (6.5%), septicemia (5%), others (3.5%). All patients with mild neutropenia recovered within one week of follow up. Among the 110 patients with moderate/severe neutropenia, 80(73%) recovered in <3 weeks,while 30 patients (27%) had prolonged neutropenia. Predictors of prolonged neutropenia were patient's age <18 months (P<0.01), ANC < 500/mm3 (P<0.05), hemoglobin <10 gm/dl (P<0.05) and positive CMV serology (P<0.05). Sex was equivocal.None of the patients with moderate/severe neutropenia had serological evidence of HCV, HBV, or HIV, while CMV serology was positive in 38 patients (34.5%) and EBV serology was positive in 8 patients (7.3%, all had severe neutropenia). ANCA was positive in 9 patients (aged 5-15 months), representing 30% of patients with prolonged neutropenia and 42.8% of prolonged severe neutropenia patients,all had hypercellular bone marrow with normal sequence of maturation. *Conclusion.* Neutropenia is a frequent finding in Egyptian infants and children, usually mild and transient,and mainly associated with infection. Cytomegalovirus and Epstein-Barr virus are important infections associated with prolonged moderate/severe neutropenia. Immune neutropenia is a common cause of moderate/severe neutropenia in the first two years of life. Close clinical and laboratory observation and adequate supportive care of the neutropenic child is of crucial importance.

0824

FCG RECEPTOR IIA AND FCG RECEPTOR IIIB POLYMORPHISMS: FREQUENCIES OF THE ALLELIC VARIANTS AND THEIR COMBINATIONS IN BRAZILIAN SYSTEMIC ERYTHEMATOSUS LUPUS PATIENTS

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Background. Recent data have provided evidence that genetic polymorphism of the receptors for Fc of IgG (FcgR) is associated with im-

mune abnormalities and risk to development of systemic lupus erythematosus (SLE). FcgRIIa and FcgRIIIb display functionally relevant genetic polymorphisms (H/R-131 and HNA-1a/1b, respectively), which allelic variants can influence the biological responses and the susceptibility to and course of infectious diseases. In particular, the presence of the FcgRIIa-R131 allotype results in lower affinity binding to IgG2, a subclass of IgG specific for encapsulated bacteria. In addition, the homozygosis for FcgRIIa-R131 and FcgRIIIb-HNA-1b combination has been associated with impaired phagocytosis and the FcgR polymorphisms have been also implied in the susceptibility to and prognosis of infectious and autoimmune diseases. *Aim.* To assess the frequencies of the alleles for the FcgRIIa and FcgRIIIb polymorphisms and their combinations in Brazilian SLE patients compared to healthy subjects. *Methods.* Committee approval was obtained for the taking of blood samples and all studied patients agreed to provide them. All patients were diagnosed at Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto-USP and fulfilled the American College of Rheumatology classification criteria for SLE (n=47). Ninety one healthy volunteers were included in this study as the control group. Genomic DNA was isolated from EDTA-anticoagulated blood using the salting out method. Determination of FcgRIIa and FcgRIIIb genotypes were performed using polymerase chain reaction (PCR)-based allotyping methods with allele-specific primers and the PCR products were analyzed by electrophoresis (2% agarose gel with gel red dye). *Results.* With respect to FcgRIIa, the heterozygosis FcgRIIa-HR-131 was the genotype most frequent in both SLE (53.2%) and healthy (45%) groups, being higher in SLE group. However, the frequency of the homozygosis H-131 was reduced in SLE (12.8%) compared to healthy (23%) groups. For FcgRIIIb, the heterozygosis HNA-1a, 1b was the most frequent genotype in SLE (44.4%) and healthy (45%) groups with an equal frequency in both groups. In SLE the homozygosis for HNA-1a was more frequent than that observed in controls, 26.7% and 16.5%, respectively; while the homozygosis for HNA-1b was more frequent in healthy (38.5%) group compared to SLE patients (28.9%). It was found the following frequencies for the alleles studied in SLE and healthy groups, respectively: H-131, 0.39 and 0.45; R-131, 0.61 and 0.55; HNA-1a, 0.49 and 0.39 and HNA-1b, 0.51 and 0.61. The most frequent genotypic combination observed in SLE patients was HR-131/HNA-1a, 1b (24.2%) and HR-131/HNA-1b and R-131/HNA-1b in healthy group (17.4%). *Summary.* This study has implications and can contribute for the understanding of neutrophil abnormalities in SLE and identifying genetic markers that would predict patients who are at high risk for infections. clenimar@usp.br

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MDS and other bone marrow failure syndromes - Clinical 2

0825

MYELODYSPLASTIC SYNDROME WITH DEL(5Q) CAN BE DISCRIMINATED FROM OTHER CYTOGENETIC SUBTYPES OF MYELODYSPLASTIC SYNDROME BY IMMUNOHISTOCHEMICAL DETECTION OF P53 OVEREXPRESSION IN BONE MARROW TREPHINES

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Background. Haploinsufficiency for the RPS14 gene that encodes ribosomal protein RPS14 has been implicated in the pathogenesis of the 5q-syndrome, a subtype of myelodysplastic syndrome (MDS). Genetic studies indicate that a p53-dependent mechanism underlies the macrocytic anaemia and bone marrow dysplasia in a murine model of the 5q-syndrome. Consistent with this hypothesis, nuclear p53 staining accumulates in the bone marrow of patients with 5q- syndrome. *Aims.* We set out to test the hypothesis that bone marrow trephine (BMT) biopsy specimens from patients with del(5q) associated MDS may show higher levels of p53 staining than BMT biopsies from patients with other cytogenetic subtypes of MDS. *Methods.* We collected 30 BMTs obtained at diagnosis from patients with *de novo* MDS (26), MDS/MPN (2), or treatment-related MDS (2) whose bone marrow at the time of biopsy had been analysed by *fluorescence in situ hybridisation (FISH)* for the most common MDS associated cytogenetic abnormalities (trisomy 8, monosomy 7, del(5q) and del(20q)). The immunohistochemical detection of p53 (antibody clone DO-7, Novocastra) in BMT sections was performed on a BondMax automated immunostainer using an immunoperoxidase method, diaminobenzidine (DAB) substrate and haematoxylin counter-stain. Fourteen lymphoma staging BMTs that were negative for marrow involvement were used as normal controls. Light microscopy was used to visually assess the p53 expression levels in 200 cells in each of 5 representative fields of view (x400 magnification), totalling 1000 cells per BMT. Cellular p53 expression was graded 0-3 based on stain intensity (0 = negative, 1 = positive, punctate/light staining, 2 = positive, uniform, medium staining, 3 = positive, uniform, dark staining). The percentage of cells in each grade category was multiplied by its grade (0-3) and the value of all grades accumulated to give an aggregate score for each BMT section (0=lowest possible score and 300=highest) (adapted from the neutrophil alkaline phosphatase scoring system - Rutenberg *et al.*, 1965). Aggregate scores were plotted against the MDS cytogenetic abnormality. *Results.* Aggregate scores for p53 expression levels were shown to be higher in MDS cases with del(5q) either as the sole abnormality or with additional cytogenetic abnormalities (ranges = 75-190.2 and 88.4-204.6 respectively), compared to MDS cases without del(5q) and normal controls (range = 18.3-75.6). *Conclusion.* Our results suggest that a high p53 expression score (>76.0) can discriminate del(5q) MDS from other cytogenetic subtypes, and supports the hypothesis that haploinsufficiency of *RPS14* and possibly additional genes within the del(5q) region causes overexpression of p53. Longitudinal studies and current investigations into which cells are responsible for this p53 overexpression may help determine the diagnostic and prognostic significance of these findings.

0826

EPIDEMIOLOGY AND LONG-TERM FOLLOW-UP OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES FROM A TERTIARY CENTER. DATA FROM 1990 TO 2010

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Background. Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders characterized by impaired bone marrow (BM) function, peripheral cytopenia/s and eventually progression to acute myeloid leukemia (AML). Incidence of MDS varies among registries, significantly increasing with age, estimated 22-45/105/year over age of 70. We report preliminary analysis of MDS patients (pts) at Hospital Virgen del Rocío (Seville, Spain) in a two-decade time period. *Patients*

Table 1.

Age (mean; range)	71 (17-93)
Sex	Male: 257 (57,4%) Female: 191 (42,5%)
Type of MDS	Lower-risk MDS: 335 (74,6%) Higher-risk MDS: 100 (22,3%) Lost: 14 (3,1%)
FAB/OMS	RA/RARS: 198 (44%) RCMD: 67 (14,9%) RAEB-1: 49 (10,9%) RAEB-2: 66 (14,7%) RAEB-t: 25 (5,5%) CMML: 41 (9,1%) Lost: 3 (0,6%)
Hemoglobin (g/L)	91 (35-154)
Platelet (x10⁹/L)	109 (5-879)
ANC (x10⁹/L)	>1,5: 221 (49,2%) 1-1,5: 65 (14,5%) 0,5-1: 59 (13,1% <0,5: 81 (18%) Lost: 23 (5,1%)
Karyotype (IPSS)	Favorable: 100 (66,6%) Intermediate: 21 (14%) Poor: 29 (19,4%)

Baseline characteristics

Table 2.

	OS (months)	P
BM blasts	<4%: 44m (33-54) 4-9%: 19m (10-27) 10-20%: 6m (3-8)	0,001
Hemoglobin (g/L)	<100: 13m (8-17) >100: 55m (43-66)	0,001
Platelet (x10⁹/L)	>150: 46m (32-59) 50-150: 23m (11-34) <50: 7m (3-10)	0,001
Transfusion dependency (at diagnosis or 6m after)	No: 50m (41-58) Yes: 13m (9-16)	0,001

Overall survival

and Methods. Four hundred and forty nine MDS pts have been analyzed for demographics, stage and evolution during follow-up. Characteristics are presented in table 1. Most pts (335/449; 74,6%) were lower-risk MDS (<10% BM blasts), being refractory anemia and refractory cytopenia with multilineage dysplasia the more frequent subtypes. Karyotype was available in 150 pts, mainly those diagnosed from 2005. *Results.* Incidence rate of MDS pts increased over time: 1990-95: 66 (14,7%), 1996-2000: 59 (13%), 2000-2005: 138 (30,7%), 2005 to present: 172 (38,3%). Overall, 346 pts are dead (77,1%). For lower-risk MDS, 24 and 48-month survival were 56,9% and 42,8%, compared to 22% and 10,5% for higher-risk MDS (p<0,001). Variables associated to overall survival (OS) were (all p>0,001): percentage of BM blasts (<10% vs >10%), hemoglobin (>100 g/L vs <100 g/L), platelet (>150x109/L, 50-150x109/L, <50x109/L) and transfusion dependency. Transfusion-dependent anemia was documented in 63,5% of pts at diagnosis. Supportive care including blood transfusion was the most common approach followed by observation until progression. Evolution to AML could be evaluated in 254 pts. 96/254 (37,7%) progressed to AML. Age at diagnosis (>75y) and BM blasts (>10%) were variables related to AML evolution. Causes of death were identified in 219 pts from medical charts and electronic records. Comorbidity was the main cause of death. Progression to AML (20,9%) and second neoplasia (9%) were identified as other major causes of death. *Conclusions.* Epidemiology of MDS remains unknown as many pts are underdiagnosed as observed by year of diagnosis. This analysis reflects preliminary approach to a large cohort of MDS pts from a tertiary center, with similar results to other registries. Depth of cytopenias seems to affect outcome together with classic prognostic factors. Detailed study for subgroups of pts and effect of intensive/disease modifying treatment is ongoing.

0827

PSYCHOMETRIC TESTING AND VALIDATION OF THE QOL-E QUESTIONNAIRE - A DISEASE-SPECIFIC QUALITY OF LIFE QUESTIONNAIRE FOR PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS) IN GERMANY

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Introduction. Patients with myelodysplastic syndromes (MDS) have a variably low survival while mainly depending on supportive care. Anemia and transfusion-dependence have been associated with poor quality of life (QoL). Patient- reported outcomes are a valuable measure of efficacy of therapeutic approaches aimed at alleviating symptoms due to cytopenias. Specific tools for the assessment of QoL are necessary and must undergo appropriate linguistic validation. QOL-E v. 2 is a self-administered questionnaire developed for the evaluation of QoL in patients with MDS (Oliva *et al.*, 2002, 2008). It explores four general dimensions (physical, functional, social, sexual), a nearly-specific (fatigue) and a specific disease-related dimension of QOL. Scores are standardized on a scale from 0 to 100, where higher scores reflect better QOL. It has been validated in Italian, Bulgarian and USA English. In the present study, the German translation is being assessed in an epidemiological survey in 100 consecutive MDS patients in 3 German centers. *Aims.* To linguistically validate the QOL-E, to evaluate the psychometric characteristics and stability of the QOL-E tool in Germany, and to identify disease-related disturbances and their associations with disease-specific factors in German MDS patients. *Methods.* The validated QoL-E was translated into German according to EORTC guidelines (2 independent forward translations, 1 reconciliation, 1 backward translation). The quality of the translation was tested in a pilot-test in 10 German MDS patients regarding its comprehensibility and the possibility to make suggestions for reformulation of unclear items. MDS patients > 18 years of age with primary or secondary MDS with at least one form of cytopenia according to IPSS criteria, willing and able to complete the questionnaire, were included. QoL-E v2 was completed by patients before the clinical visit and a retest was performed after 1 month. Psychometric characteristics in terms of reliability (Cronbach's alpha) and validity were performed. *Results.* The mean age of patients enrolled in the pilot-test was 68.6 years (SD=13.1) with a mean Hemoglobin (Hb) level of 9.7 g/dl (SD=1.8). Seven patients had low or Int-1 risk IPSS and very low or low risk WPSS scores. Patients found the German translation comprehensible, but had a problem with the meaning of the examples given for the item "performing heavy activities"('running', 'jumping') which were reformulated. For patients who were already retired the question whether "their health is an impediment for them to keep a paid job"was difficult to answer. Psychometric characteristics of the QoL-E were acceptable to good for all domains of the QoL-E (α=.56-.88), but for the physical dimension, which is probably due to the small number of patients from the pilot-test. Highest impairments in patients' QoL were found in the dimensions 'social' (mean 44.6 ± SD 33.7) and 'functional' (mean 55.6 ± SD 35.6). *Conclusion.* QOL-E v. 2 is the only specific instrument available for the evaluation of QoL in MDS. Its linguistic validation may identify QoL-E as a new tool for the evaluation of QoL in the German MDS population.

0828

LONG-TERM OUTCOME OF ISOLATED THROMBOCYTOPENIA ACCOMPANIED BY HYPOCELLULAR MARROW

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Background. Most of the patients with isolated thrombocytopenia do not undergo bone marrow (BM) examination and a presumptive diagnosis of immune thrombocytopenia is made when the history, physical examination, complete blood counts, and examination of the peripheral blood smears do not suggest other etiologies. Hypocellularity of BM, which is not associated with significant dyshematopoiesis, is often found in patients with isolated thrombocytopenia, but its clinical implications have not been studied. *Aims*. To define the clinical features and natural history of isolated thrombocytopenia accompanied by hypocelluar marrow without apparent etiologies in adults. *Methods.* We prospectively evaluated adult patients with isolated thrombocytopenia accompanied by hypocellular marrow (cellularity < 30% in patients aged less than 60 years; < 20% in patients aged 60 years or more) in the absence of significant dyshematopoiesis, cytogenetic abnormalities, or megakaryocytic hyperplasia. Patients with autoimmune disorders and infections were excluded. *Results.* Between January of 2002 and December of 2006, a total of 224 patients with isolated thrombocytopenia were studied, including by BM examination. Among them, 20 patients (17 males and three females) were consistent with isolated thrombocytopenia accompanied by hypocellular marrow. The median age of the patients was 29 years (range: 18-70 years). At the initial presentation, the platelet counts ranged from 12,000 to 99,000/μL (median 63,000/μL) and were >50,000/μL in 16 patients (80%). BM cellularity ranged from 5 to 25% (median 15%) and was ≤10% in six patients (30%), 11-20% in 13 patients (65%), and 21-25% in 1 patient (5%). A weak positive correlation was present between the BM cellularity and platelet counts ($R2 = 0.2044$, $P = 0.045$). During the median 48-month follow-up (range: 12-90 months), three of the 20 patients recovered platelet counts to normal levels (>150,000/μL) after 12, 56, and 66 months, respectively. Three patients developed pancytopenia after 11, 70, and 90 months, re-

spectively. Two patients were consistent with moderate aplastic anemia, and one was confirmed as refractory cytopenia with multilineage dysplasia. In the remainder of the patients, the platelet counts remained unchanged. *Conclusions*. Isolated thrombocytopenia accompanied by hypocellular marrow encompasses a group of heterogeneous conditions, and its subgroups represent the early manifestations of aplastic anemia, myelodysplastic syndrome, or temporary BM depression of unknown etiology. Regular follow-up is therefore mandatory in patients with this condition and a large-scale observational study is warranted.

0829

CYTOGENETIC FEATURES AND PROGNOSIS IN 496 ARGENTINEAN PATIENTS WITH PRIMARY MYELODYSPLASTIC SYNDROMES

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Background. Myelodysplastic Syndromes (MDS) include a group of heterogeneous hematological disorders with variable risk of evolution to Acute Myeloid Leukemia (AML) and short survival. Around 40-50% of patients show abnormal karyotype at diagnosis. Recently, some isolated deletions have been related with a favorable outcome in MDS (i.e. 9q-, 11q-, 12p-) and Breems *et al.*, 2008, proposed that monosomal karyotypes (MK) are an indicator of poor prognosis in AML. *Aims.* To characterize the cytogenetic profile, to test its prognostic value and to evaluate cytogenetic groups of risk in the Argentinean MDS population. *Methods.* This is a multicenter retrospective study of 496 primary Argentinean patients with primary MDS evaluated from 1984 to 2010 (including 239 patients from the Pilot Study and from the MDS Registry sponsored by the Argentinean Society of Hematology). Refractory cytopenia was defined according to Valent *et al.*, 2007 and patients were classified following FAB and WHO criteria. The median age was 69 (17-93) years with a male/female ratio of 1.3. During the follow-up (mean: 26 months), 111 (22.4%) evolve to AML and 222 (44.7%) died. *Results.* Gender, percentage of bone marrow blast, hemoglobin level, platelets count, number of cytopenias, LDH level and red blood cell transfusion requirements, FAB and WHO classification, IPSS and WPSS prognostic systems were significant predictive variables for prognosis (Kaplan-Meier and Long-Rank test, p<.001). Patients with normal karyotype (n=289, 58%, median survival: 51 months) had better outcome than those with cytogenetic alterations (n=207, 42%, 26 months, p<.001). Among abnormal karyotypes, 137 (66%) showed deletions and/or monosomies, 124 (59%) involved, at least, one chromosome #5, #7, #8 and/or #20. The most common cytogenetic aberrations were: -5/5q- (22% among cases with abnormal karyotype), -7/7q- (15%), +8 (21%), 20q- (9%) and -Y (8%). Karyotypes were divided according to IPSS (p<.001) into Good (n=334, 67%, median survival: 48 months), Intermediate (n=102, 21%, 32 months) and Poor (n=124, 12%, 15 months). In order to find the prognostic value of certain cytogenetic findings, karyotypes were divided as follows: normal, low risk alterations (n=45, 9%, median survival: 43 months), karyotypes with isolated deletions (n=20, 4%, 50 months), trisomy 8 (n=25, 5%, 25 months), other intermediate findings (n=54, 11%, 28 months), chromosome 7 alterations (n=19, 4%, 15 months), MK (n=30, 6%, 16 months) and other complex karyotypes (n=15, 3%, 18 months). No significant differences were observed among MK and other Poor cytogenetic findings (median survival: 15 months, p=.592). Patients with isolated deletions showed a similar behavior than Good cytogenetic findings (p=.437) and a borderline better outcome than the rest of the Intermediate ones (p=.07). An intermediate prognosis was observed when karyotypes included a deletion + other alteration (n=13, 3%, 31 months, p=.523). *Conclusions.* Cytogenetic findings had a clear impact in our population. Results in the present series, the largest in Latin America, suggest that MK are indicators of poor prognosis whilst the presence of an isolated deletion (not including 7q-) would be a good cytogenetic finding. However, the wide spectrum of low frequency aberrations stresses the importance of large study groups.

0830

MOLECULAR ANALYSIS OF RPS19 AND RPL5 GENES IN GREEK PATIENTS WITH DIAMOND BLACKFAN ANEMIA

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Background. Diamond Blackfan Anemia (DBA) is a rare inherited disease characterized by congenital defective erythropoiesis. Patients present early in life with severe normochromic, macrocytic anemia, while about 50% of the patients have also congenital anomalies. Heterozygous mutations in 9 ribosomal proteins genes have been identified in ~50% of patients. More than 30% of these mutations are located in the RPS19 and RPL5 genes. *Aim.* To evaluate the clinical and hematologic phenotype, as well as, the incidence of mutations in the RPS19 and RPL5 genes in Greek patients with DBA. *Methods.* A questionnaire for patient with DBA, requesting data on clinical and hematological phenotype was sent to all the pediatric hematology, hematology and transfusion units in Greece. Informed consent was obtained. Genomic DNA was extracted from peripheral blood lymphocytes from the identified patients with DBA. Molecular analysis with PCR, ECMA (Enzymatic Cleavage Mismatch Analysis) and direct sequencing was performed allowing detection and characterization of disease-causing mutations. PCR primers were specifically designed to amplify the whole coding region and the flanking intron/exon junctions of RPS19 and RPL5 genes. *Results.* 17 Greek patients (7 females and 10 males, mean age: 11.4±11 years) with DBA phenotype were included in this study. Congenital anomalies in different organs, including craniofacial, upper extremities, hands, eyes, heart and kidneys were present in 71.4% of the patients. Regarding the clinical course of the patients, 4 are treated with steroids and 8 are on regular transfusions. One patient, who had been on regular transfusions, was transplanted at the age of 8 years from an HLA-matched unrelated donor. Four patients are lost to follow up. One patient developed thyroid cancer at the age of 46 years. 6 patients (35.2%) were indentified to carry mutations to either the RPS19 gene (3 patients,) or the RPL5 gene (3 patients). 2 of the 3 mutations detected in the RPL5 gene, c.C390G (p.Y130X) and c.197_198insA (p.Y66X) are novel. *Conclusions.* This is the first report on the clinical and genetic evaluation of DBA patients in Greece. We have observed similar frequencies of mutations in the RPS19 and the RPL5 genes but higher frequency of physical malformations to the ones reported from other countries. Higher rates of transfusion dependency may be related to referral bias. The occurrence of thyroid carcinoma in an adult patient with DBA is worth-noticing.

0831

MOLECULAR ANALYSIS OF SBDS GENE IN GREEK PATIENTS EVALUATED FOR SHWACHMAN-DIAMOND SYNDROME

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Background. Shwachman-Diamond Syndrome (SDS) is a rare autosomal recessive disorder resulting from loss-of-function mutations in the highly conserved Shwachman-Bodian Diamond Syndrome (SBDS) gene. As a multisystem disease SDS is characterized by bone marrow dysfunction, exocrine pancreatic insufficiency, metaphyseal chon-

drodysplasia, short stature and increased risk of neoplasms. SBDS gene, located in chromosome 7q11, is the only gene presently known to be associated with SDS. Roughly 75% of patients with SDS carry mutations resulting from a gene conversion event with the adjacent pseudogene, SBDSP. In a limited number (<10%) of patients who present with clinical indications of SDS no mutations in SBDS are detected. *Aim.* To evaluate the clinical and hematologic phenotype, as well as, the incidence of mutations in the SBDS gene in Greek patients with SDS. *Patients and Methods.* 7 patients, including 2 sisters, (5 females and 2 males, mean age: 12.4±5.9 years), meeting the diagnostic criteria for SDS were screened for SBDS mutations. Genomic DNA was extracted from peripheral blood lymphocytes and molecular analysis with PCR, ECMA (Enzymatic Cleavage Mismatch Analysis), RFLPs and direct sequencing was performed allowing detection and characterization of disease causing mutations. PCR primers were specifically designed to amplify the whole coding region (five exons) and the flanking intron/exon junctions of SBDS gene but not the SBDSP pseudogene. RFLPs used the Bsu36I and AciI enzymes for the detection of the two most common c.183-184 TA>CT and 258+2T>C mutations respectively. Data on clinical and hematological phenotype were collected for each patient after informed consent was obtained. *Results.* Evaluated patients had a significant heterogeneous clinical presentation. 4 patients presented with neutropenia, and 2 with thrombocytopenia. 1 patient had trilinear cytopenia, evident at birth, with 32% of her marrow progenitors carrying an iso (7q) chromosomal abnormality. This patient was successfully transplanted from a matched sibling donor. One patient have had a stable clonal abnormality (46,X,del(X)(q24[ARROWRIGHT]qter)) in around 35% of her myeloid progenitors. 80% of patients showed congenital abnormalities mainly affecting the bones and the heart. Only one patient is receiving therapy with granulocyte colony-stimulating factor, due to chronic neutropenia. Pancreatic insufficiency was mild to severe, with only 2 patients requiring pancreatic enzymes replacement. Growth hormone was used in 2 patients. 4 patients were compound heterozygotes for the common 183-184 TA>CT and 258+2T>C mutations. One of those was a mosaic which explained her very mild phenotype, while another was heterozygous for the 183-184 TA>CT and homozygous for the 258+2T>C mutation. One patient was compound heterozygote for 258+2T>C and c.460-1G>A novel mutation. Finally, one patient was found to carry only the normal c.635 T>C (p. I212T) variant. *Conclusions.* Mutations in SBDS were detected in approximately 85.7% of patients, with 258+2T>C and 183-184TA>CT were the most frequent mutations, consistent with previous reports. Extreme phenotypic heterogeneity was present and varied from severe to normal. No significant association was observed between the different SBDS mutations and phenotypic severity.

0832

GEOGRAPHICAL DIFFERENCES IN IRON OVERLOAD AND IRON CHELATION PRACTICES IN ANAEMIA PATIENTS: PRELIMINARY RESULTS FROM THE TRANSFUSIONAL HEMOSIDEROSIS REGISTRY STUDY (TORS)

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Background. Data describing iron chelation and transfusion practices as well as diagnoses of iron overload in patients with anaemia across geographical regions are limited. Baseline data from the large EPIC trial (with the oral iron chelator deferasirox) however, suggest that these practices differ between the European, Middle Eastern/African and Asia-Pacific regions (Viprakasit *et al. Blood* 2010;116(21):abst 4272). A prospective epidemiological study may provide further insights into clinical practices for these conditions. Here, we report preliminary data from the multinational TORS on iron overload and iron chelation practices. *Aims.* To examine differences in baseline characteristics among anaemia patients requiring chronic transfusion therapy, to assess the extent of iron overload and to gain insight into patterns of care surrounding the use of iron chelation therapy in different geographical regions. *Methods.* TORS is a prospective, multinational, non-interventional study to collect information from patients >2 years of age requiring chronic transfusion therapy with newly diagnosed anaemias (<12 months from diagnosis), including Low and Intermediate-1 myelodysplastic syndromes (MDS), aplastic anaemia (AA), Diamond-Blackfan anaemia (DBA) and other transfusion-dependent anaemias. Patients were recruited from Turkey, Russia and South Africa as well as from countries within the Asia-Pacific and Middle East regions. Patients with secondary or therapy-related MDS; Intermediate-2 or High-risk MDS; or acute leukaemia were excluded. At the time of patient inclusion (baseline), patient demographics and details of iron overload and iron chelation therapy use were assessed. Transfusional haemosiderosis (iron overload) was defined as ≥1 serum ferritin measurement >1000 ng/mL after onset of transfusion therapy; or a liver iron concentration (LIC) >2 mg Fe/g dry weight; or if serum ferritin or LIC measurements were not available, evidence of ≥20 red blood cell transfusions. *Results.* Of 539 patients recruited into the registry, the majority of patients had a diagnosis of MDS (58.8%, n=317), followed by AA (31.9%, n=172), DBA (1.1%, n=6) and other anaemias (5.9%, n=32). The highest numbers of patients were recruited from Turkey (23.7%, n=128), China (18.0%, n=97) and Thailand (15.4%, n=83; Figure 1). The mean age (±SD) of the patients was 51.2 ± 23.7 years (range 2-92); 49.0% (n=264) were male and 50.3% (n=271) were of Asian origin. At study entry, the percentage of patients receiving transfusions ranged from 47.0-100.0%. The numbers of patients assessed with iron overload ranged from 4.1% (4/97) in China to 38.5% (5/13) in South Africa. The numbers of patients receiving iron chelation therapy ranged from 2.4% (2/83) in Thailand to 26.6% (34/128) in Turkey (recordings of iron overload and iron chelation therapy do not have to apply to the same patients). *Summary/Conclusions.* The data suggest that iron overload and iron chelation practices vary between countries in these newly diagnosed patients with transfusion-dependent anaemias. These analyses are limited by the small numbers of patients in some countries and by a potential bias towards recruitment of patients with specific anaemias. These preliminary data nonetheless provide a better understanding of disease-management practices of newly diagnosed patients with anaemia across different geographical regions.

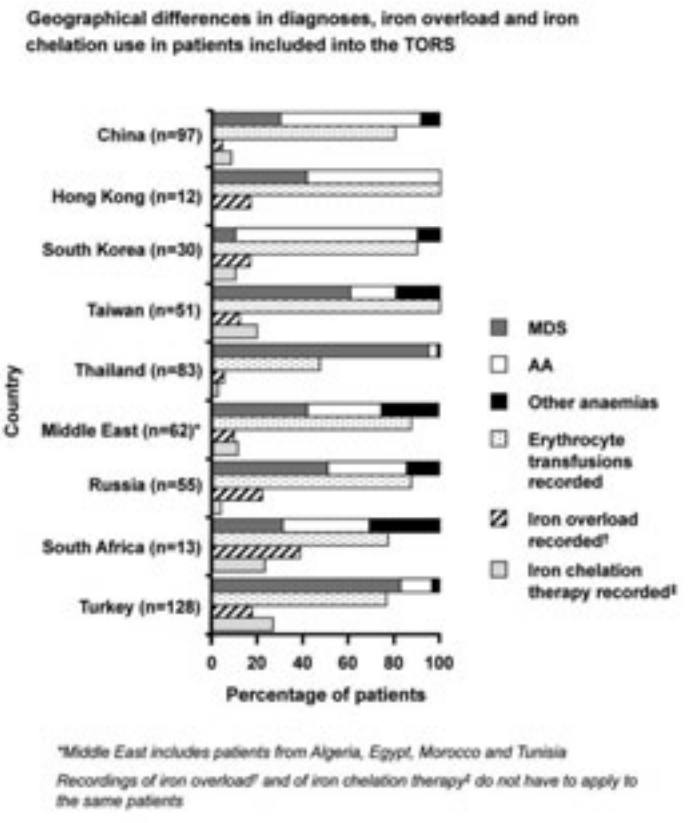

Figure 1.

0833

PEDIATRIC DIAGNOSIS OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA IN THE INTERNATIONAL PNH REGISTRY

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal hematopoietic stem cell disease which can lead to life-threatening complications including intravascular hemolysis, thrombotic events (TE), and kidney disease. Improvements in the understanding and utilization of high sensitivity diagnostic tests and availability of targeted terminal complement blockade treatment have led to improved awareness and prognosis of PNH. There has been little systematic research focused on pediatric patients diagnosed with PNH due to small patient numbers. *Aims.* To describe the clinical characteristics of patients diagnosed with PNH as children and to compare these characteristics to patients diagnosed as adults in a large, international, observational disease registry. *Methods.* Data from 153 clinical sites in 19 countries on 5 continents (as of 31/Jan/2011) were analyzed to evaluate clinical presentation of PNH in patients first diagnosed at age <18 years (pediatric patients N=79) and at age ≥18 years (adult patients N=666). Baseline demographics, laboratory values (including flow cytometry), PNH-related medical history (including TE and kidney disease), and physician-reported PNH symptoms were compared for pediatric and adult patients. Patients (or guardians) gave informed consent prior to enrollment. Analyses were also stratified by percent GPI-deficient granulocytes (clone size) at registry enrollment (<50%, >50%). *Results.* Of the 79 patients diagnosed as children, 25 (32%) were still <18 years old at study enrollment. Approximately half of pediatric and adult patients were female. Years from disease start to enrollment was significantly higher for pediatrics than adults (mean ± SD: 10.7 ± 11.6 vs. 7.8 ± 8.4, respectively, p=.006). Overall, patients diagnosed with PNH as children were similar to those diagnosed as adults. There was no difference between pediatric and adult patients for underlying bone marrow disorders (39% vs. 43%), clone size (mean 63% vs. 64%), LDH fold above normal upper limit (mean 3.4 vs. 3.0), history of TE (10% vs. 18.2%, p=.07), history of renal impairment (10% vs. 13%) or use of transfusions prior to registry enrollment (33% vs. 41%). Physician reported hemoglobinuria and abdominal pain were comparable between pediatric and adult patients (54.5% vs. 56.3% and 44.3% vs. 36.9%, respectively). Pediatric patients had higher total red blood cell counts (mean 3.5 vs. 3.1 x1012/L, p<0.001) and less fatigue (52% vs. 72% p<0.001) than adults. Among patients with clone size ≥50%, pediatric patients were less likely to have a history of TE (7% vs. 22%, p=.02) and less likely to have fatigue (61% vs. 78%, p<0.01) but more likely to have headaches (54% vs. 36%, p=.02) than adults. Pediatric patients were more likely than adults to receive a bone marrow transplant during registry follow-up (5.1% vs. 1.5%, p=.03). *Summary/Conclusions.* PNH patients diagnosed in a pediatric setting present with similar clinical characteristic to adults such as LDH levels, hemoglobniuria, abdominal pain, underlying bone marrow disorders, TE and history of renal impairment. Among patients with clone size ≥50%, pediatric patients had less history of TE than adults, although TE is still an issue for pediatric patients. New clinical sites and geographic regions are encouraged to participate in the Registry (pnhregistry@iconplc.com).

0834

DEFERASIROX REPRESENTS AN EFFECTIVE ORAL IRON CHELATOR IN LOW OR INTERMEDIATE-1 RISK PATIENTS WITH MDS - A COMPARATIVE STUDY TO THE TREATMENT WITH DEFERIPRONE

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Aims. An efficiency of different oral iron chelators was studied in a group of 113 MDS patients with < 10% of bone marrow blasts. *Patients and Methods.* Sixty-five patients were treated with oral iron chelator deferasirox (Exjade®). Median initial serum ferritin level was 2677,5 µg/l (range 780-9923 µg/l), the daily dose of the drug ranged from 10 to 40 mg/kg. Median duration of chelation treatment was 13,7 months (range 4-36 months). The results were compared to previously studied efficiency of deferiprone (Ferriprox®) in 48 patients with the same subgroup of MDS. *Results.* Chelation was effective (maintained or decreased iron stores) in 83% of patients (in 85% of 25 patients with serum ferritin ≤ 2000 µg/l and in 80% of 40 patients with serum ferritin > 2000 µg/l. Incidence of adverse effects was 56,9% and led to discontinuation of deferasirox treatment in 6% of patients. GIT symptoms represented the most frequent adverse effect (33,8% patients) that limited an effective escalation of the daily dose of the drug. An increase in serum creatinine level was observed in 20% of patients, after decease in daily dose or transient discontinuation of the drug serum creatinine level stabilized in all patients. Skin rash was present in 4,6 % as well as oedema and weight increase. When compared to our previous study with deferiprone, deferasirox effectivity was similar to that of deferiprone in patients with serum ferritin ≤ 2000 µg/l (85% v.s. 76% responding patients with deferiprone) but significantly higher in those with serum ferritin > 2000 µg/l (80% responders v.s. only 46% treated with deferiprone). Incidence of adverse effects was similar (56,9% v.s. 62,5% after deferiprone), and GIT symptoms were the most frequent events after treatment with both the drugs. However, symptoms of deferasirox toxicity were mild and mostly transient and no myelosuppresive effect related to the drug administration was observed in contrast to deferiprone where drug related granulocytopenia occurred in 17% of patients and the treatment had to be discontinued in 29% of patients. *Conclusions.* Deferasirox administered in a daily dose of 15-40 mg (depending on initial serum ferritin level) represents an effective and safe treatment for iron overloaded patients with early phase of MDS without excess of blasts.

0835

MYELODYSPLASTIC SYNDROMES (MDS): DOES THE HEPATITIS C VIRUS (HCV) INFECTION PLAY A ROLE?

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HCV infection plays a well-documented role in the aetiology of malignant lymphomas. HCV-infected persons are more likely to have low neutrophil and platelet count; some researches support the hypothesis that HCV infection can itself cause cytopenias, but the mechanism is still unclear. Only a few studies have investigated the relationship between HCV and MDS, and the reports are contradictory. This research is aimed at evaluating the prevalence and clinical features of HCV infection in a population of MDS patients with a monocentric, retrospective analysis. One hundred twenty four patients, 84 males and 40 females, have been studied, mean age 76 years old (range 54-93), mean follow up 31 months (range 3-158); all underwent a complete blood cell count, routine serum chemistry, peripheral blood and bone marrow examination, and were tested for HCV-antibodies, HIV-antibodies and serum hepatitis B virus (HBV) HbSAg antigen. Cytogenetic analysis has been performed in 98 patients. Clinical outcomes were evaluated using Kaplan-Meier analyses. According to the WHO classification, 31 cases were classified as refractory anemia (RA), 28 as refractory cytopenia with multilineage dysplasia (RCMD), 11 as refractory anemia with ringed sideroblasts (RARS), 16 as refractory anemia with excess blasts (RAEB-1), 16 as RAEB-2, 3 as MDS unclassified (MDS_U), and 19 as chronic myelomonocytic leukaemia (CMML). All patients were nega-

tive for HIV infection; only 2 patients were HbSAg positive (1 in the AR group and 1 in the RAEB-2 group, respectively). Nineteen patients (15%) out of 124 were HCV positive; 12 out of 19 HCV positive patients were diagnosed with chronic hepatitis and 5 with mixed cryoglobulinemia. None of them was on antiviral treatment. Noteworthy, the HCV prevalence was significantly different among the WHO subtypes, as follows: RA=0,3% (1 out of 31), RARS=0% (0 out of 11), RCMD= 21% (6 out of 28), RAEB-1=43% (7 out of 16), RAEB-2=0% (0 out of 16), CMML=26% (5 out of 19), MDS_U= 0% (0 out of 3). The median overall survival (OS) was 49 months (mo) for RA patients, 71 mo for RARS, 25 mo for RCMD, 21 mo for RAEB-1, 9 mo for RAEB-2, 36 mo for CMML patients, respectively. In the WHO subgroups with the higher HCV prevalence no significant difference between HCV positive and HCV negative patients has been found either for OS (RCMD: p=0.82; RAEB-1: p=0.41; CMML: p=0.36) or leukemia free survival (LFS) (RCMD: p=0.93; RAEB-1: p=0.19; CMML: p=0.76). *Conclusions.* 1) in this experience the HCV prevalence rate in MDS patients (15%) was slightly higher than the values reported in the general population of comparable age in this geographic area (7-8%); 2) the HCV positive MDS patients are clustered in the WHO subgroups RAEB-1, CMML, and RCMD; 3) in these WHO subgroups the HCV infection has no prognostic significance; 4) the HCV positive cytopenic patients require a careful evaluation for possible MDS.

0836

PROLONGED HEMATOLOGIC AND MOLECULAR RESPONSE AFTER A LIMITED NUMBER OF AZACITIDINE CYCLES IN LOW-RISK MYELODYSPLASTIC SYNDROMES

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Background. Azacitidine (AZA), at a dosing schedule of 75 mg/m2/d subcutaneously for 7 days every 4 weeks, induces high hematologic response rates (60-80%) in patients with Myelodysplastic Syndromes (MDS), and prolongs overall survival in high-risk MDS patients *(Silverman, 2002; Fenaux, 2009)*. However limited data are available concerning the efficacy and safety of AZA in low- risk MDS. Moreover, although continuation of AZA treatment is generally recommended in all responder patients, the optimal duration of therapy has not been clearly defined, especially for low-risk patients. Recently, a community-based study mainly involving low-risk MDS *(Lyons, 2009)*, showed that the lower dose AZA 5 regimen (75 mg/m2/d subcutaneously for 5 days), which avoid week-end dosing, can induce therapeutic responses consistent with the currently approved schedule. *Aims.* These data prompted us to investigate the therapeutic effect of the AZA 5 regimen, administered as induction treatment for a total of 8 courses, in low-risk MDS patients with symptomatic anemia refractory to erythropoietin (EPO). *Methods.* From September 2008 to February 2010, 34 patients with low-risk MDS (IPSS score low or intermediate-1) were enrolled into the study. Age at diagnosis ranged between 56 and 84 years. Moreover, as our group *(Follo, 2009)* previously demonstrated that the inositide signalling pathways, in particular phosphoinositide-phospholipase C (PI-PLC) beta1, may represent a target for AZA, we quantified the degree of PI-PLCbeta1 methylation and gene expression before and during AZA administration. *Results.* According to the 2006 International Working Group criteria *(Cheson, 2006)*, overall response rate (ORR) was 61% (14/23 evaluable patients), including Complete Remission (CR) (22%), and Hematologic Improvement (HI) (39%). Unexpectedly, in 3/14 responder patients we observed a long duration of response, still ongoing, after discontinuation of AZA. Patient 1, a 77 yr male, (WHO diagnosis: Refractory Anemia; IPSS risk: low; karyotype: normal) started AZA on September 2008. He showed a 1st response (HI, erythroid response), with a significant reduction of transfusions, after 3rd course of AZA, and became completely transfusion-independent from August 2009, 3 months after the completion of the 8th course (duration of response: 18 months). Patient 2, a 61 yr female, (WHO diagnosis: Refractory Anemia with Excess of Blasts-1; IPSS risk: intermediate-1; karyotype: normal) started AZA on October 2008, became transfusion-independent after 5th course, and achieved CR after 8th course (duration of response: 24 months). Patient 3, a 70 yr female, (WHO diagnosis: Refractory Cytopenia with Multilineage Dysplasia; IPSS risk: intermediate-1; karyotype: normal) started AZA on February 2009, and achieved HI (erythroid response) after 2nd course (duration of response: 22 months). All the patients showed an increase in PI-PLCbeta1 expression, in association with the hematologic response, and still maintain the molecular response. *Summary/Conclusions.* Our clinical and biological results support the feasibility and effectiveness of AZA treatment in low-risk MDS, especially in the case of EPO refractoriness. After achievement of hematologic response, discontinuation of AZA might be attempted, especially in low-risk MDS, where the risk of leukemic evolution is lower. However, the effectiveness of AZA resumption, in case of relapse, is still unknown.

0837

LENALIDOMIDE IN LOW AND INT-1 RISK MDS: CLINICAL RESULTS OF THE QOL-ESC REVMDS TRIAL

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Background. Lenalidomide induces remissions in MDS patients with del5q. Patient reported outcomes associated with erythroid responses have not yet been reported. *Aims.* We present results of a prospective single-arm trial in 44 low and Int-1 IPSS risk MDS patients with del5q treated with lenalidomide to evaluate safety, efficacy, and changes in QoL. *Methods.* Inclusion criteria required an Hb level < 10 g/dL. All patients received lenalidomide at an initial 10 mg daily dose. Dose reduction was prescribed according to adverse events. Responses were evaluated according to Cheson's criteria (2006). QoL (QOL-E v2 questionnaire) was measured at baseline and weeks 8, 12, 24 and 52. Bone marrow assessment was performed every 3 months. *Results.* Thirty patients were transfusion-dependent. IPSS risk was 0 in 30, 0.5 in 13 cases and 1.0 in 1 case. Eleven cases had an additional cytogenetic abnormality. At 12 weeks, Hb increased from 8.6 ± 0.8 to 11 ± 2.1 ($p<0.0001$) in 40 evaluable patients of which 30 (75%) obtained an erythroid response. There were 11 cytogenetic remissions. Physical QoL scores increased from 40 ± 25 to 55 ± 29 (p=0.007) Drug discontinuation followed by dose reduction was required in all but 4 patients. One patient refused to continue study drug because of progressive anemia. There were 2 progressions observed during the trial. Complete data at 52 weeks will follow. *Conclusions.* Though the starting dose was relatively low, initial hematological toxicity did limit lenalidomide dosing so that most patients required an early reduction. Preliminary results confirm that lenalidomide induces erythroid responses and transfusion independence with cytogenetic remissions and significant improvements in QoL.

0838

INCIDENCE OF APLASTIC ANEMIA AND ITS ASSOCIATION WITH VARIOUS HLA ANTIGENS

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Background and Objectives. Aplastic anemia arises from failure of marrow to produce insufficient quantities of haematopoietic cells, which causes peripheral pancytopenia with fatty or empty bone marrow. The

most severely affected patients have neutrophil counts of less than 200 cubic per millimeters, platelet count of less than 20,000 and reticulocyte counts of less than 60,000 per cubic millimeters along with marrow cellularity of less than 25%. Immunologic events that precede the destruction are less clear. Involvement of the lymphocytes of CD34 or helper cells has been inferred from the over expression of the class II histocompatibility antigen HLA DR2 in white patients with Aplastic anemia and in various studies association of HLA antigens has been established to the response to different modes of treatment. For this purpose we conducted a retrospective study to know the exact epidemiology of Aplastic anemia in our setup as well as its association with various HLA antigens. *Aims and Methods.* Total 315 patients with the confirmed diagnosis of Aplastic anemia were registered in our center from April 1998 till April 2006 and their detailed asseement was performed. After the initial counseling of the illness and symptomatic treatment; these patients were offered the ultimate treatment considering Allogenic BMT as first line treatment option.HLA typing was performed of only those patients who were fulfilling the criteria of bone marrow transplantation. Later on results of HLA antigens were analyzed. *Results.*Total 318 patients were enrolled out of which 237 were males and 71 females. 131patients belonged to pediatric age group (under 15) and 171 above that. The mean age of presentation was 20.79, while the median was 17.21% of the patients under went BMT, 22% patients were given cyclosporin and 5% received ATG with or without cyclosporin. In HLA typing results, there was strong association with HLA B5 (52). *Conclusion.* Aplastic anemia has about 3-4 fold higher incidence in Asian population. Although these results reflect the number of cases from only one tertiary care center, but still the magnitude of disaese can be roughly estimated. Further studies are needed to establish the other causal factors like occupational history and drugs history of last 6-8 months period of time, so that further details regarding this neglected illness could be explored.

0839

EXPERIENCES OF DECITABINE IN ELDERLY PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background. Patients with myelodysplastic syndromes (MDS) are challenging to treat, given the advanced median age and comorbidities of the population. Decitabine, a hypomethylating agent that allows for the re-expression of tumor suppressor genes, represents a treatment option for MDS patients. In phase 2 and 3 studies, decitabine has been associated with durable responses in MDS patients and delayed time to acute myeloid leukemia (AML) transformation or death compared with supportive care. Decitabine has been shown to be well tolerated with a toxicity profile expected for this class of agent. However, the treatment of older patients ≥70 years seemed to be associated with increased toxicity in the first two courses. The delayed cycles of subsequent treatment might be caused by prolonged myelosuppression owing to the continuing presence of underlying disease and the adverse effect of decitabine. And febrile neutropenia occurred frequently (around one third) in patients who received decitabine. *Aims.* We need to confirm the efficacy and safety of decitabine in patients 70 years old and over with myelodysplastic syndrome. *Methods.* We analyzed clinical data of 26 MDS patients ≥70 years who were treated with decitabine. Patients received decitabine 20mg/m2 intravenously daily for 5 days. Decitabine was given for a median of 4 courses (range, 2-11 courses) and median follow-up duration was 13 months. *Results.* Overall response was 57.7% (15 of 26 patients) including bone marrow CR with hematologic improvement in 4 patients. Median time to response was after two cycles in patients achieved any response and four of responding patients showed an effect as late as cycle 6. There were no significant differences in clinical outcomes of patients 70 years old and over to compare to those of patients under 70 years old, except the median time to erythroid response. In elderly patients erythroid response was faster (two cycles). Febrile neutropenia was observed in 15 patients (57.6%) and the majority of infectious complications were happened for first two cycles. Treatment related mortality was 15.4% (4 of 12 patients) at 3 months and was attributed to prolonged myelosuppression and disease progression. *Conclusions.* In elderly patients with MDS, we confirmed that the efficacy of decitabine was comparable with that in younger patients and decitabine was generally well tolerated. However, the treatment of older patients ≥70 years seemed to be associated with increased risk of infectious complications in the first two courses. Therefore, we need the effective strategy to prevent infection at the earlier cycles of decitabine treatment, especially in elder patients with severe neutropenia.

0840

EFFICACY AND TOLERABILITY OF 5-DAY AZACYTIDINE DOSE INTENSIFIED REGIMEN IN HIGHER RISK MDS

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Background. Azacytidine is a hypomethylating agent indicated for treatment of higher risk Myelodysplastic Syndromes (MDS). A recently published phase III trial demonstrated improved overall survival (OS) of MDS patients treated with azacytidine compared to those receiving conventional care regimens, thus establishing this treatment option as first line therapy in those patients for whom bone marrow transplantation is not an option. *Aims.* Evaluate the efficacy of azacytidine regimen in terms of transfusion independence (TI), overall response (OR), time to AML transformation and tolerability in patients with higher risk MDS and AML with 20-30% blasts. *Methods.* Higher risk (International Prognostic Scoring System - IPSS INT-2 and high risk) MDS patients were treated with azacytidine for at least 4 cycles in our institution. OR, including complete response (CR) and partial response (PR) and TI, defined according to the 2000 International Working Group Criteria (IWG), were assessed by blood and bone marrow examination. Treatment cycles were repeated until toxicity or disease progression. *Results.* A total of 48 patients were treated with azacytidine between June 2006 and September 2010; of these, only 26 were treated for at least 4 cycles. Mean age was 65 years old (range 85-36) and male sex was predominant (M:F of 1.3.) Eleven patients had refractory anaemia with excess blasts (RAEB), six had secondary AML, five had chronic myelomonocytic leukaemia (CMML), two had refractory cytopenias with ringed sideroblasts and two had acute erythroblastic leukemia. Most patients were high risk according to IPSS scoring (78%). Azacytidine was used as first line therapy in 35% and as second line in half. An average of 8 cycles (4-22) per patient were administered. The transfusion independence rate was of 50%, with average response duration of 6.5 months. Overall response rate was 31% (7 CR and 1 PR). During the follow-up period, fourteen patients died. Five patients showed disease progression to AML: four of them had never shown any response to azacytidine, while the other one had obtain transfusion independency. Overall survival (OS) from diagnosis was of 26 months, while OS from beginning of treatment was 22 months. There was limited toxicity, mainly grades I and II gastrointestinal and skin toxicity. Eleven patients (42%) had Grade III haematological toxicity and four (15%) suffered Grade IV haematological toxicity. *Conclusion.* The efficacy of azacytidine in achieving TI and prolonging survival in MDS is well recognized. In this study, azacytidine improved quality of life and overall survival regardless of the quality of response. Treatment was well tolerated, with limited toxicity. Our results, though coming from a small group of patients, were comparable to those reported in the literature.

0841

CLINICAL IMPACT OF UNCONTROLLED COMPLEMENT ACTIVITY IN JAPANESE NON-TRANSFUSED PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Introduction and Aims. Paroxysmal nocturnal hemoglobinuria (PNH) is a debilitating and life-threatening hematopoietic stem cell disorder

characterized by chronic uncontrolled complement activation. PNH evolves from the clonal expansion of hematopoietic stem cells with complete or marked loss of the terminal complement inhibitors CD55 and CD59, consequently rendering red blood cells susceptible to systemic lysis. This chronic hemolysis underlies the severe morbidities and mortality associated with the disease: life-threatening thromboembolism (TE), chronic kidney disease (CKD), pulmonary hypertension, end organ damage, fatigue, abdominal pain, dysphagia and dyspnea. While many patients with PNH have anemia, low hemoglobin levels and transfusion requirements may not adequately reflect the burden of disease due to hemolysis since these parameters are influenced by other factors unrelated to hemolysis or complement activation including bone marrow dysfunction, patient specific factors and physician clinical assessment. There is a paucity of reported data in Japanese PNH patients who are not transfused that sufficiently captures their burden of disease. Here, we report the efficacy response of two patients recently treated with eculizumab in the AEGIS study who received no blood transfusions in the 2 years prior to study participation. *Methods.* Eculizumab was administered to 29 Japanese PNH patients (14 men and 15 women; median patient age, 47 years; range 26-70 years) at 9 institutions in Japan for 12 weeks in the AEGIS study. This report summarizes the evaluation of two patients with history of no blood transfusions that enrolled and were treated in the AEGIS study. *Results.* At baseline, the two patients who had received no blood transfusions prior to study participation were hemolytic (LDH approximately 7 and 11 fold above normal), demonstrated significant organ damage with evidence of thrombosis (1 patient with DVT) and renal disease (CKD stage 2 and 1), and suffered disabling quality of life as measured by FACIT-fatigue and EORTC-QLQ C-30 scores. In both patients, eculizumab treatment resulted in substantial 78%-88% reductions in LDH, significant improvements in fatigue as measured by FACIT-fatigue (changes of 5 and 23 points; >3-point improvement is clinically meaningful), improvement in dyspnea in one patient (change of 33 points from baseline; ≥10 point improvement is clinically meaningful), and elimination of CKD with no subsequent thrombotic events in both patients. The LDH reduction in these non-transfused patients compares favorably to the 87% reduction in LDH observed in the entire AEGIS patient population. *Conclusion.* These data demonstrate that despite a history of no transfusions, at baseline these non-transfused PNH patients had significant clinical evidence of disease burden including chronic hemolysis, history of thrombosis, renal disease, and impaired quality of life. These findings underscore the central role of uncontrolled complement activation and the resultant sequelae associated with hemolysis, rather than anemia or transfusion requirements, in guiding the treatment of patients with PNH. Further, inhibition of terminal complement activation with eculizumab in patients with PNH improves hemolysis, fatigue, dyspnea and other significant morbidities of disease whether or not they had received transfusions prior to participation in the AEGIS trial.

0842

LONG TERM SURVIVAL AND TREATMENT RESPONSE IN LOW-INTERMEDIATE RISK MYELODISPLASTIC SYNDROME PATIENTS TREATED WITH RECOMBINANT ERYTHROPOIETIN PLUS 13-CIS-RETINOIC ACID AND DIHYDROXYLATED VITAMIN D3

E Crisà

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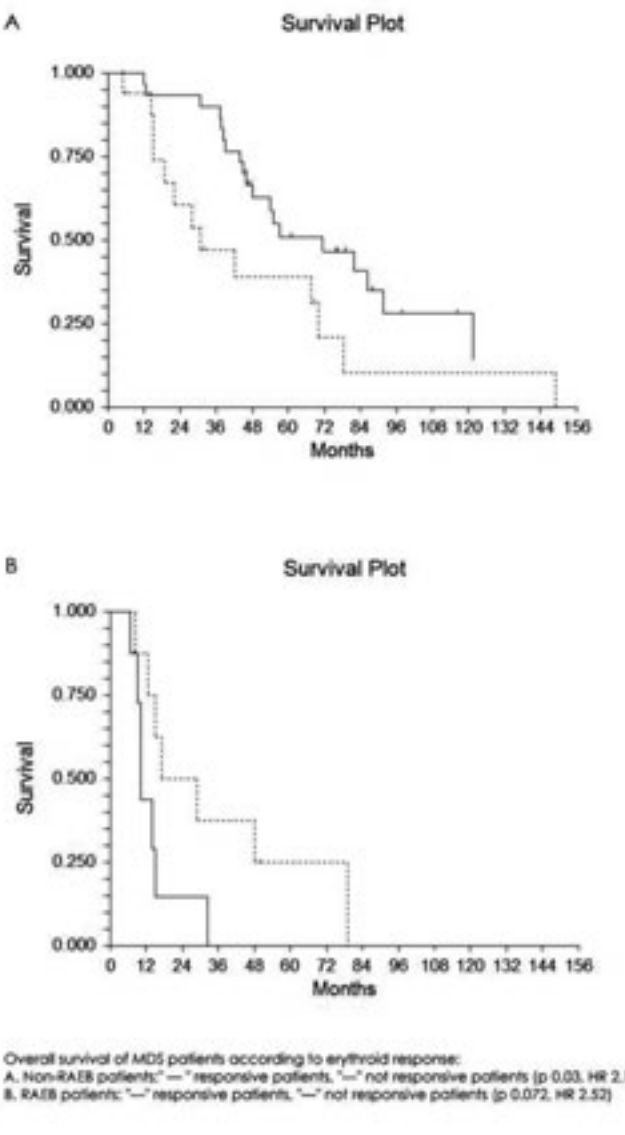
Background. In our previous paper (Ferrero *et al.*, BJH 2009) we reported the treatment of 63 MDS patients (median age 75, 16 RAEB1, 47 non RAEB) with a combination of human recombinant erythropoietin (EPO) (alfa or beta epoetin, 30-80000 U/ week, median 65000U/week),13-cis-retinoic acid (20 mg day) and dihydroxylated vitamin D3 (1 ug day). Eleven of the 16 RAEB1 patients also received intermittent, low dose of 6-thioguanine. In spite of adverse prognostic factors for response to erythropoietin (all patients with Hb <9.5 g/dl, 70% transfusion dependent, 51% IPSS intermediate 1 or 2) 64% of non RAEB and 50% of RAEB1 displayed an erythroid response according to Cheson *et al.* (Blood 2006). At previous evaluation (41 months of follow-up) an overall survival (OS) advantage was evident for non RAEB patients with erythroid response. *Aims.* Now we updated the casistic after 3 years from the previous evaluation. *Methods and Results.* Overall survival and response duration were estimated using Kaplan Meyer method. Median follow up for alive patients is now 65 months (5 months - 13 years). Median duration of erythroid response is now increased to 22 (2-95+) months for non RAEB and 6 (2.5-34.5+) months for RAEB1, 21% of responses in non RAEB patients have lasted more than 3 years. Thirty two of 46 non RAEB and 14 of 16 RAEB1 patients died, with a median survival respectively of 55 and 15 months. Acute myeloid leukemia evolution occurred to 11 patients (5 RAEB1 and 6 non RAEB patients). In non RAEB patients survival was significantly affected by IPSS score (median OS 45 months for intermediate1 plus intermediate2 patients vs 149 months for low risk patients, p= 0.05, HR 2.47) and transfusion dependance (p 0.006, HR 2.93) as expected. These parameters did not significantly modify RAEB patients OS, possibly due to the low patient number. Although the erythroid response did not correlate with known risk factors such as IPSS score, caryotype and transfusion requirement, it confirmed its positive prognostic role for survival in non RAEB patients (p 0.03, HR 2.14): median survival 71.5 months (range 12-156+) for responders, 30.6 months (range 5-149) for non responders. A trend towards a better survival for responder was also observed among RAEB1 patients (median survival 17 months for responders, 10 months for non responders), however, due to the low numbers of patients in this group, the difference was not statistically significant, even if border line (p 0.072, HR 2.52). *Conclusions.* In conclusion our long term follow-up confirmed the positive role of our combined treatment for response duration and survival in a group of non RAEB patients, most of them with unfavorable prognostic features, compared to literature data on EPO alone treatments.



Overall survival of MDS patients according to erythroid response:
A. Non-RAEB patients: "—" responsive patients, "—" not responsive patients (p 0.03, HR 2.14)
B. RAEB patients: "—" responsive patients, "—" not responsive patients (p 0.072, HR 2.52)

Figure 1.

0843

ERYTHROPOIETIN PLUS DANAZOLE, PREDNISONE, B12 AND FOLATE IN REFRACTORY CYTOPENIA WITH MULTILINEAGE DYSPLASIA. MONOCENTRIC PROSPECTIVE STUDY

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Background. RCMD is a well defined entity in MDS. The overall median survival is about 30 months. The appropriate treatment of RCMD is still unclear. *Aims.* To verify if immunosuppressive treatment with danazole and prednisone plus erythropoietins and vitamins is safe and feasible in RCMD treatment. *Methods.* This is a monocentric, prospective, randomized study, regarding the period from July 2008 to December 2010. 30 patients with RCMD were randomized to receive erythropoietin alpha 40000 UI sc/weekly or erythropoietin beta 40000 UI sc/weekly, B12 400 mg/day and folate 5 mg/day (15 patients - group A) or erythropoietin alpha or beta plus B12 and folate at the same dose and danazole 400 mg/day for 1 year and prednisone 50 mg/day for 1 month, then gradually suspended in the following month (15 patients - group B). Median follow-up was 15 months (R 5-29 months). In the group A median age was 67 years (R58-73). M/F was 9/6. 2 patients presented a karyotype with +8, and 1 with +9. In the group B median age was 65 years (R55-70). M/F was 8/7. No patient had an abnormal karyotype. In the group A all patients received erythropoietin beta. In the group B 8 patient received erythropoietin alpha and 7 beta. In group A at diagnosis median Hb level was 9 g/dl (R7-10), PLT 90000 (R70000-98000), ANC 750 (R250-1000). In group B at diagnosis median Hb level was 8 g/dl (R7-9), PLT 45000 (R30000-70000), ANC 450 (R250-900). Every 6 months all patients received a liver ecography and blood liver test, to prevent danazole-related hepatocarcynoma, and only male patients received a transrectal prostatic ecography and PSA dosage, to prevent danazol related prostate carcinoma. *Results.* In group A all patients after therapy achieved a better Hb level with a median increase of Hb of 1.5 g/dl after a median of 2.5 month (R1-5). No improvement was observed in neutrophil and platelets count. In group B all patients achieved a normal Hb (>10 g/dl), PLT (>100000/mcl)and absolute neutrophil count (ANC > 1000/mcl) after a median of 1 month of treatment (R1-3 month). The 8 patients of group B treated with erythropoietin alpha achieved a normal level of Hb (>10 g/dl) with a median of 1 month sooner than the 7 patients of group B and all patients of group A treated with erythropoietin beta (median: 1 month in group with epo alpha, 2 monthin group with epo beta). At December 2010 no patients died for all cause. Patients in group A have a need of platelet transfusion of a median of 1 unit every 2 months and have a median number of hospitalization per year of 6 versus 1 for patients in group B. No patient developed an hepatocarcynoma or a prostate carcinoma during follow-up period. *Summary/Conclusion.* In RCMD treatment with erythropoietin, danazol an prednisone with B12 and folate support seems to be safe feasible and effective. In consideration of the small number of patients treated, these results need confirmation in a larger cohort of patients.

0844

QUALITY ASSESSMENT OF INTERNET MORPHOLOGY EDUCATION PROJECT BASED ON VIRTUAL MICROSCOPY

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Background. Availability of new technologies has expanded the possibilities for education of microscopic morphology on internet. Among others virtual microscopy has enabled evaluation of whole microscopic smears from peripheral blood or marrow aspirate up to a significant magnification. *Aims.* To assess the quality of virtual microscopy internet project used for pregraduate education of medical students. Access to the project is currently restricted only to students and members of project team at www.e-hematologie.cz. *Methods.* Seven experts from the Czech Republic, Finland, Germany and Italy have evaluated the pictures of single normal and pathologic cells and full scope scans of marrow or blood microscopic smears obtained by CellaVision DM 96 (Sysmex) and dotSlide system (Olympus). *Results.* 94 cathegories of normal and pathologic cells on 1-5 pictures depicting single or few cells including special stainings and 58 virtual scans of normal peripheral blood, normal marrow aspirates and marrow aspirates in various hematologic conditions were evaluated. Technical quality of pictures including sharpness and magnification of cells, including lack of the possibility to further magnify the pictures was the most frequent finding (comments raised by 3 to 5 experts on proerythroblasts, promyelocytes, unmature eosinophiles and basophiles; by 4 experts on quality of special staining). Experts agreed on the need to exchange the provided picture or scan for a new one because of poor selection in these cases of: mastocytes (6 of 7 experts); prolymphocytes (4/7); monoblasts, promonocytes, megakaryoblasts, micromegakaryocytes (3/7). A better selection of scaned smears was suggested by 3 experts in cases of APL, AML with inv16, MDS and acute monoblastic leukemia. Scans more than one case of HCL and chronic prolymphocytic leukemia should be provided. *Conclusions.* A very good level of agreement between the authors of the project and external auditors was achieved. Modern tools enabling virtual microscopy have been confirmed as excellent or very good for education of morphology (agreement among all experts). Careful selection of the smears and appropriate use of technologies may avoid scans and pictures of inadequate quality. Implementation of unified morphology classification and links to other national and European projects are mandatory to further improve the quality of the project. The use of these ICT tools is nowadays essential for a consensus morphological diagnosis, that is mandatory in those hematological malignacies where the identification and enumeration of abnormal cells still remain mandatory for the diagnosis.

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0845

LONG-TERM OUTCOMES AFTER IMMUNOSUPPRESSIVE TREATMENT FOR MODERATE APLASTIC ANEMIA

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Background. The clinical course and current management of acquired moderate aplastic anemia (MAA) are variable and few data concerning long-term results of immunosuppressive therapy (IST) are available. *Aims.* To evaluate of IST efficacy and long-term outcomes in patients with MAA. *Methods.* We analyzed the long-term outcome of 59 patients with MAA (28 M and 31 F, age 6-65 years, median 25) treated with ATG and CsA (n=41, including repeated courses in 14 patients) or with CsA alone (n=18) in two centers between April 1994 and February 2011. MAA was defined as hypocellular bone marrow and at least bi-lineage cytopenia lasting more than 4 weeks without meeting the criteria for severe AA. This study includes both retrospective and prospective phases (before and after 2005 respectively). The hematological response was evaluated according to the strict response criteria (Camitta B., 2000). Adult patients or parents of children under 18 years of age signed informed consent. *Results.* A total of 46 patients (78 %) responded to IST but only 10 patients (17 %) achieved complete response. Eleven patients (24 %) relapsed and 9 responded again after retreatment with ATG or/and CsA. Late events included MDS/AML (n=5), rectal cancer (n=1) and hemolytic PNH (n=2). There were 1 early and 5 late deaths. With a median follow-up of 38 months (range 1-201), the probability of 5-, 10- and 15-year overall survival were 93.3 ± 3.7 %, 82.7 ± 7.8 % and 66.2 ± 16.1 % respectively. Due to high incidence of relapse and late events the failure-free survival was much lower: 52.2 ± 8.2 % and 32.3 ± 8.6 % at 5 and 10 year respectively with no apparent plateau in the curve. *Conclusions.* These data demonstrate that despite encouraging short-term results the long-term prognosis of MAA after IST is rather poor and unpredictable. Longer follow-up in a larger cohort and further studies into biologic heterogeneity are warranted to determine optimal treatment strategy in MAA. Careful monitoring of hematologic response would be required in order to clarify the role and timing of allogeneic bone marrow transplantation.

Myeloma and other monoclonal gammopathies - Biology 2

0846

INSULIN GROWTH FACTOR BINDING PROTEIN-3 EXPRESSION AND ITS PROGNOSTIC SIGNIFICANCE IN MONOCLONAL GAMMOPATHIES

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Background. Multiple myeloma (MM) is a clonal malignancy of plasma cells characterized by several genetic and epigenetic aberrations. IGFBP-3 gene is a member of the insulin-like growth factor binding protein (IGFBP) family and it can regulate cell growth and death by its ability to bind insulin-like growth factors (IGFs) as well as its IGF-independent effects involving binding to other molecules. Its role in carcinogenesis in other tumors is still debated. Previous studies demonstrated that in patients with MM the levels of IGFBP-3 protein in serum are decreased. We have recently shown that the gene expression of IGFBP3 in myeloma cell lines were decreased. *Aim.* we analyzed the gene expression of IGFBP3 samples from patients with monoclonal gammopathies at diagnosis and we evaluated the correlation between IGFBP3 gene expression levels and overall survival of patients in order to determine the clinical relevance of this gene. *Methods.* 128 samples of patients at the moment of diagnosis (14 MGUS and 114 with MM) were retrospectively evaluated. The diagnosis was based on standard criteria. IGFBP3 mRNA expression was measured in each samples by real-time PCR using TaqMan Gene Expression Assays and the 7900HT Real-Time PCR System (Applied Biosystems Foster City, CA). *Results.* 128 patients; male 76; female 52; median age 68 years (range 40-89). ISS stage: stage I 41%; stage II 33%; stage III 26%. In 73/128 (57%) patients we found lower levels of IGFBP3 expression compared to the calibrator sample, the remaining 55/128 (43%) patients showed increased level of gene. We analyzed the correlation between overall survival and IGFBP3 levels and we surprisingly observed that patients with lower levels of the gene had a significantly better overall survival (p 0.0215). *Conclusion.* These results suggest IGFBP3 down-regulation as a good prognostic factor. Further analysis of correlation of IGFBP3 gene expression with clinical and biological characteristics in these MM patients is ongoing. More studies are needed to better understand the role of IGFBP-3 in myeloma pathogenesis.

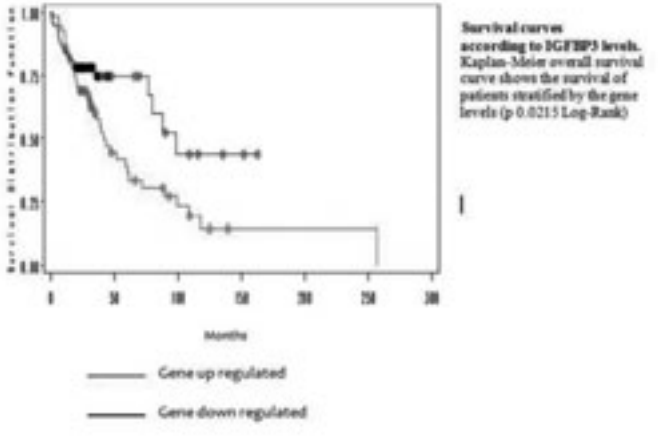

Figure 1.

0847

NEURAL STEM CELL MARKER NESTIN AS A POTENTIAL UNFAVORABLE FACTOR FOR MULTIPLE MYELOMA

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Background. Neural stem cell marker nestin is considered to be a characteristic marker of multipotent proliferative precursors with primitive and undifferentiated phenotype found in some embryonic and fetal tissues. Nestin expression has been also detected in many solid tumors and is proposed to be a suitable diagnostic and prognostic indicator of malignancy and a putative marker of cancer stem cells in solid tumors. Unexpectedly, our previous results confirmed nestin levels in mature $CD138^{+}38^{+}$ plasma cells (PC) of multiple myeloma (MM) patients by flow cytometry; significant differences were found between nestin levels in MM and individuals without any hematological malignancy. One third of MM patients had more than 50% of nestin-positive PC. Nestin seems to be a specific marker only for $CD138^{+}$PC. Expression of stem/progenitor cell marker nestin might be a novel prognostic factor for MM. *Aims.* The aim of this pilot study was to analyze nestin expression in $CD138^{+}$PC of MM and evaluate relationship between nestin expression and cytogenetic aberrations in $CD138^{+}$PC of MM. *Methods.* A total number of 22 MM patients (12M/10F; median age 58 years) were included in this study. Nestin expression was evaluated as $2^{-\Delta\Delta Ct}$ of nestin gene by quantitative real-time PCR in 22 MM patients. As a calibrator was used commercial total bone marrow RNA from healthy donors. $CD138^{+}$PC of MM patients were analyzed for del(13q14), del(17p53), IgH rearrangement (IgH), 1q21 gain and hyperdiploidy/non-hyperdiploidy (HY/non-HY) by interphase FISH. HY was assessed as trisomy of chromosome 5, 9 and 15. Differences of nestin expression between cytogenetic-aberration positive and negative patients were analyzed by non-parametric Mann-Whitney U test. *Results.* The whole group of MM patients had the median $2^{-\Delta\Delta Ct}$ 2.62 (range; 0.49-39.41). Statistical significant differences were found between IgH-positive: median $2^{-\Delta\Delta Ct}$ 5.42 (range; 0.49-39.41) and IgH-negative patients: median $2^{-\Delta\Delta Ct}$ 0.72 (range; 0,46-6,62), p=0.025; HY-patients: median $2^{-\Delta\Delta Ct}$ 0.72 (range; 0.46-24.44), and non-HY-patients: median $2^{-\Delta\Delta Ct}$ 7.00 (range; 0,62-39,41), p=0.024; trisomy 15-positive: median $2^{-\Delta\Delta Ct}$ 0.59 (range; 0,46-4,24) and trisomy 15-negative patients: median $2^{-\Delta\Delta Ct}$ 8.43 (range; 0,99-39,41), p<0.001. *Summary.* Our results confirmed that nestin expression is associated with IgH rearrangement and hyperdiploidy. High nestin expression was related to IgH rearrangement and to non-HY patients. We suppose that nestin might be associated with unfavorable prognosis, since IgH rearrangements and non-HY are unfavorable prognostic factors. However, further studies are required to assess nestin role in multiple myeloma pathogenesis. Supported with research program MSM of Czech republic Nr. LC06027, P304/10/1395 and MSM 0021622434.

0848

INDUCTION BY LENALIDOMIDE AND DEXAMETHASONE COMBINATION INCREASES CD4 AND CD8 REGULATORY CELLS OF NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background. Naturally occurring regulatory cells play an active role in maintaining immune system homeostasis. In various hematological malignancies and solid tumors it was proved that T regulatory cells (Tregs) were increased and functionally active in abrogating immune responses. Few studies documented the expansion of Tregs in the relapsed multiple myeloma (MM) patients after lenalidomide and dexamethasone treatment. *Aims.* To screen the frequency of regulatory (CD4 and CD8 Tregs) and suppressor (Ts) cells between pre- and post-induction cycle by lenalidomide plus dexamethasone combination in newly diagnosed MM patients. *Methods.* Thirteen newly diagnosed

Table 1.

Comparison of regulatory and suppressor cells between pre- and post-induction by lenalidomide and dexamethasone

Pre-induction	Post-induction cycles		P value
CD4 Tregs in median% (range%)			
2.90(0.97-5.42)	1st	4.03 (1.99-7.71)	0.38
	2nd	5.91 (2.57-10.81)	**0.001**
	3rd	6.14 (1.42-7.60)	**0.001**
	4th	6.73 (1.81-8.88)	**0.045**
CD8 Tregs in median% (range%)			
0.32(0.01-1.21)	1st	0.53 (0.16-0.96)	**0.02**
	2nd	0.44 (0.26-1.33)	0.75
	3rd	0.40 (0.12-1.26)	0.9
	4th	0.38 (0.16-1.37)	0.72
Ts cells in median% (range%)			
54.38(19.68-71.25)	1st	39.04 (23.96-65.04)	0.75
	2nd	53.27 (25.34-75.99)	0.75
	3rd	51.53 (32.35-80.04)	0.75
	4th	49.24 (30.47-62.97)	0.72

MM patients were included in this study. Median age of the patient cohort was 60 years (range: 50-64). Based on international staging system (ISS) patients were represented as: ISS1-4/13 (31%), ISS2-7/13 (54%), and ISS3-2/13 (15%). All patients were induced by lenalidomide plus dexamethasone combination for 4 cycles at 28 days interval [lenalidomide (25mg/1-21 days) and dexamethasone (40 mg on days 1,8,15, 22)]. One million of erythrocytes lysed peripheral blood (PB) cells were labeled with following fluorochrome combinations: FoxP3-FITC/ CD28-PE/ CD4-PerCpCy5.5/ CD8-APC/ CD25-PE-Cy7 and analyzed by multiparameter flow cytometry. We compared the PB frequency of CD4 Tregs, CD8 Tregs and Ts cells between pre-induction and post-induction cycles in all 13 patients. Ten age-matched healthy volunteers (HVs) PB samples were also analyzed for comparison. *Results.* CD4 Tregs were strictly identified as CD4+CD25hi+FoxP3+ and CD8 Tregs were characterized as CD8+FoxP3+. Ts cells were identified by absence of co-stimulatory molecule (CD8+CD28-). We observed similar frequency of CD4 Tregs in between MM patients and HVs [median (range) = 2.9% (0.97%-5.42%) vs. 3.0% (1.96%-3.59%); P=0.78]. Whereas, for CD8 Tregs MM patients had significantly elevated level compared to HVs [CD8 Tregs median (range)= 0.32% (0.01%-1.21%) vs. 0.15% (0.04%-0.22%); P= 0.009]. Similarly to CD8 Tregs, Ts cells were also prominently increased in MM patients compared to HVs [median (range)= 54.38% (19.68%-71.25%) vs. 30.36% (12.77%-60.97%); P=0.031]. Post-induction cycle observations showed significant increase in CD4 Tregs compared to pre-induction (Table 1). For CD8 Tregs and Ts cells an increasing trend was observed in post-induction cycles compared to pre-induction, but statistical significance was observed in first post-induction cycle for CD8 Tregs (Table 1). *Conclusions.* In summary, regulatory cells were increased after induction with lenalidomide and dexamethasone combination in newly diagnosed MM patients. This observation should be taken into consideration to efficiently improve the immuno-stimulatory effects for MM patients.
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0849

ROLE OF THE PLATELET DERIVED GROWTH FACTOR-AB IN TUMOR GROWTH AND ANGIOGENESIS IN RELATION WITH OTHER ANGIOGENIC CYTOKINES IN MULTIPLE MYELOMA

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Background. Angiogenesis is a complex process indispensable to growth, invasion and metastasis in various malignant tumors, including multiple myeloma (MM). Various angiogenic cytokines have been implicated in the angiogenic process. Among them, platelet derived growth factor-AB (PDGF-AB), has been reported to be potent stimulator of angiogenesis not only in solid tumors but also in haematological malignancies, including MM. *Aims.* The aim of the study was to investigate the relationship between PDGF-AB, microvascular density (MVD) and various angiogenic cytokines, such as basic-fibroblast growth factor (b-FGF), angiogenin (ANG) and interleukine-6 (IL-6), in patients with newly diagnosed MM. *Methods.* Serum concentrations of the above cytokines were determined in 47 MM patients before treatment, in 22 in plateau phase, as well as in 20 healthy individuals. Serum PDGF-AB, b-FGF, ANG and IL-6 measurements were performed by ELISA with a commercially available kit. We also performed bone marrow biopsies prior and after treatment, in plateau phase. MVD was determined by staining bone marrow vessels with anti-CD31. *Results.* Mean serum values of PDGF-AB and b-FGF were significantly higher in the group of MM patients in comparison to control group ($p<0.001$ and $p<0.001$ respectively). The means ±SD values of PDGF-AB and b-FGF were significantly higher with increasing stage of MM disease ($p<0.001$ and $p<0.001$ respectively). Significant positive correlations were observed between serum PDGF-AB, ANG and IL-6 levels with MVD ($r=0.361$ $p<0.01$, $r=0.0376$ $p<0.001$ and 0.345 $p<0.01$ respectively). Furthermore we found significant positive correlations between PDGF-AB and b-FGF, IL-6, ANG and B2M ($r=0.324$ $p<0.02$, $r=0.491$ $p<0.0001$, $r=0.437$ $p<0.002$ and $r=0.365$ $p<0.01$ respectively). We also found that patients with high MVD had statistically significant higher serum levels of PDGF-AB ($p=0.017$), when as cutoff point of MVD was used the median value 7.7. Furthermore significant difference was found between serum levels of PDGF-AB in pre and post-treatment patients ($p<0.001$). Serum ANG concentrations in the entire group of MM patients ranged from 246.40-1615.4 pg/ml with a mean ± SD of 669.6±332.5 pg/ml. These values were significantly higher than those found in the control group ($p<0.001$), which ranged from 105.78-354.63 pg/ml. Mean values for ANG in the group of MM patients were significantly higher with increasing stage of disease ($p<0.001$). Mean values of MVD were significantly higher in MM patients than in controls ($p<0.0001$). Among the stages in the entire group of MM patients MVD was significantly higher only in stage III in comparison to stage I ($p<0.01$). There was a difference in survival times between patients with high vs. low PDGF-AB levels (51 vs. 66 months) and high vs. low ANG levels (57 vs. 67 months), but the difference could not reach statistical significance in either case. In contrast, only in MVD groups survival time was significantly higher in MVD median group (76 vs. 51 months, $p<0.02$). *Conclusions.* Our results showed that there is a strong positive correlation between PDGF-AB and studied angiogenic cytokines, as well as with MVD, which indicates a role of PDGF-AB in the complex network of cytokines in inducing bone marrow neovascularization in patients with MM.

0850

LENALIDOMIDE TREATMENT POST AUTOLOGOUS TRANSPLANTATION FOR MULTIPLE MYELOMA DECREASES TERMINALLY DIFFERENCIATED CD4 AND CD8 T CELLS AND INCREASES NUMBER OF TREG

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Background. Treatment with Lenalidomide, a structural analogue of Thalidomide, plus dexamethasone (Dex) increases time to progression in relapse or refractory multiple myeloma. However, due to its pleiotropic effect, it's not known if its efficacy is due only to a direct tumor toxicity or benefit also of its immunomodulatory effects. *Aims.* We wanted to assess *in vivo* the changes induced by Lenalidomide treatment on T-cell reconstitution in a cohort of multiple myeloma patients. *Patients.* Thirty-four myeloma patients were treated with the induction combination bortezomib plus Dex, followed by high dose melphalan (140-200 mg/m2) and an autologous transplantation with peripheral blood stem cells. Between 3 to 6 months post autograft, patients were randomized in 2 groups: 12 received 25 mg/day of Lenalidomide for 2 months, 3 weeks per month plus 40 mg of Dex, once a week, then 10 mg/day of Lenalidomide only until relapse. 22, a placebo only. *Methods.* T lymphocyte subpopulation percentage and absolute counts were assessed by multicolor flow cytometry from diagnosis until 18 month after autograft. *Results.* After Lenalidomide plus Dex treatment, we observed a significant decrease in percentage and absolute counts of CD4+ or CD8+, CD45RA+CCR7- effector T cell subpopulations. Surprisingly, CD4+CD25high or CD4+CD25+CD127-/low Treg increased significantly more in treated patients. No correlation was found with documented infections, relapse or survival. *Conclusions.* These data suggest that, *in vivo*, in Human, Lenalidomide plus Dex efficacy on Myeloma tumor is not T cell mediated but this treatment could have a

negative impact on T cell immunosurveillance. Additional studies are required to better assess the respective effects of Lenalidomide and Dex on immune function.

0851

METAPHASE INDUCTION IN MULTIPLE MYELOMA: A NEW CYTOGENETIC APPROACH FOR G-BANDING ANALYSIS

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Background. The usefulness of conventional cytogenetic analysis for the classification and prognostication in acute and chronic leukemia has been demonstrated. In multiple myeloma cytogenetic abnormalities also play an important role as prognostic factor. However, classical cytogenetic fail to detect these abnormalities because the low mitotic index of plasma cells *in vitro*. The analysis of a set of subjects for the most commonly known aberrations is usually done by FISH on interphase cell. *Aims.* The goals of this investigation were the use of the oligonucleotide DSP30 in combination with IL-2, IL-6 and IL-10, as a B-cell mitogen for cytogenetic investigation in MM and the comparison between the karyotype analyses obtained (G-banding) with FISH profile from unstimulated cells. *Methods.* For metaphase induction, bone marrow mononuclear cells of 55 patients with MM were cultured in RPMI 1640 medium with 20% fetal calf serum in the presence of the immunostimulatory CpG-oligonucleotide DSP30 and IL-2/IL-6/IL-10 for 72h. Additionally, two extra set of cell culture was performed for each patient without any stimulant agent (G-banding analysis, when possible and FISH). The FISH panel included probes for the detection of translocations involving IGH gene (14q32), gains associated to 11q23-q25 and 1q21 and deletion of 13q14. The cut off levels for IGH translocations was (>3.2%), gains of 11q23-q25 and 1q21 (2.4% and 2.7%, respectively) and deletion 13q14 (2.8%). All cut off values were established according to the FISH patterns observed in a group of 12 age and sex-matched normal control peripheral blood samples studied with the same probes. *Results.* In the cells treated with stimulus, the cytogenetic analysis was possible in 42 patients (76.5%). On the other hand, in the group without any stimulus, the cytogenetic profile was successful in 20 patients (36.4%), being 8 (40%) with normal karyotype and 12 with chromosomal abnormalities (60%). The group that received stimulus, normal karyotype was found in 16 patients (38%) and metaphases with abnormal karyotype were seen in 26 subjects (62%). Among the cytogenetic profile obtained in both groups were observed aneuploidies involving the chromosomes +3, +5, +9, -13, -14, +15, +16, -18, +19, +20, -22 and structural abnormalities such as add(1)(p21), inv(3)(q21q26), del(13)(q14), add(14)(q32) and t(14;16)(q32;q23). FISH analysis performed in those patients whose bone marrow cells were not stimulated displayed the same chromosomal abnormalities as identified in the group with stimulus. *Conclusion.* These results indicate that the addition of the immunostimulatory oligonucleotide DSP30 in combination with IL-2, IL-6 and IL-10 showed to be effective to induce cell cycle progression of MM cells *in vitro.*

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0852

THE ASSESSMENT OF PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) AND ITS RELATIONSHIP WITH PROINFLAMMATORY CYTOKINES AND PARAMETERS OF DISEASE ACTIVITY IN MULTIPLE MYELOMA PATIENTS

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Background. Multiple myeloma (MM) is a malignant disease of plasma cells that localize to the bone marrow (BM). Clonal plasma cells and BM stromal cells produce several proinflammatory cytokines that are involved in the pathogenesis of the disease. *Aims.* The aims of the study were to investigate circulating levels of proinflammatory cytokines such as Interleukine-1β (IL-1β), Interleukine-6 (IL-6), Interleukine-8 (IL-8), Macrophage Inflammatory Protein-1α (MIP-1α) as well as LDH and B2 microglobulin, in MM patients before treatment, and to discuss its significance in tumor progression. Additionally we analyzed the correlation of measured mediators with the proliferative activity assessed by proliferating cell nuclear antigen (PCNA) immunohistochemistry staining. *Methods.* 44 newly diagnosed MM patients and 20 healthy controls were studied. Patients were staged with Durie-Salmon system. Serum samples were collected at the diagnosis. We also performed BM biopsies prior to treatment. We determined serum levels of IL-6, IL-1β, IL-8 and MIP-1α using enzyme-linked immunosorbent assay (ELISA), as well as PCNA expression in the BM. *Results.* The mean concentrations of the measured cytokines IL-6, IL-1β, IL-8 and MIP-1α in the entire group of patients were 6.2±5.9 pg/ml, 2.8±1.3 pg/ml, 39.3±15.6 pg/ml and 51.7±34.9 pg/ml, respectively. All the above measured parameters were significantly different among the three stages of disease, with higher values with advancing disease stage ($p<0.001$ in all cases). Furthermore in the entire group of patients with MM serum levels of IL-6, IL-1β, IL-8 and MIP-1α were significantly higher in patients in comparison to controls ($p<0.001$, $p<0.001$, $p<0.002$, $p<0.001$ respectively). A positive statistical correlation was found between IL-6 and IL-1β ($r=0.462$ $p<0.002$), IL-6 and IL-8 ($r=0.358$ $p<0.01$), IL-6 and MIP-1α ($r=0.380$ $p<0.001$). Similarly IL-8 and MIP-1α were positively correlated with factors of disease activity such as B2M ($r=0.502$ $p<0.001$, $r=0.413$ $p<0.005$ respectively) and LDH ($r=0.415$ $p<0.006$, $r=0.475$ $p<0.001$ respectively). PCNA immunostaining was identified in the nuclei of the cells in all the cases of the disease. Concerning the three stages of the disease, the proliferation index as assessed by PCNA immunostaining was evaluated in stage I with mean value 6.6±5.2% (range 1-18%), in stage II 24.0±12.4% (range 5-50%) and in stage III 41.4±29.0% (range 10-98%). In the entire group of MM PCNA expression was higher with advancing disease stage ($p<0.001$). Furthermore PCNA expression correlated significantly with parameters of disease activity such as B2M and LDH ($r=0.406$ $p<0.006$, $r=0.581$ $p<0.0001$ respectively). Similarly the number of myelomatous cells positive for the PCNA protein correlated with the measured cytokines IL-6, IL-1β, IL-8 and MIP-1α ($r=0.520$ $p<0.0001$, $r=0.545$ $p<0.0001$, $r=0.320$ $p<0.03$ and $r=0.358$ $p<0.01$, respectively). *Conclusions.* Our results showed that the proliferative activity, as assessed with PCNA, was increased in parallel with disease stage. The positive correlation between PCNA and other measured mediators, such as IL-6, IL-1β, IL-8 and MIP-1α, supports the involvement of these factors in the biology of myeloma cell growth and can be used as markers of disease activity and as possible therapeutic targets.

0853

NEPHELOMETRIC MEASUREMENTS OF FLC κ AND FLC λ FOR MONITORING LIGHT CHAIN MULTIPLE MYELOMA PATIENTS

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Introduction. Serum free light chain (sFLC) measurements are important in multiple myeloma (MM), have replaced urine electrophoresis in diagnostic algorithms and are required to characterise a complete response. However, with the exception of oligosecretory disease, their use is not recommended in current guidelines for routine monitoring. *Aim.* To investigate the use of sFLC measurements for monitoring light chain MM (LCMM) patients. *Methods.* sFLC κ and sFLC λ levels were measured nephelometrically on serial samples from 25 LCMM patients (14 FLCκ and 11 FLCλ); specifically at presentation, after cycles 2 and 4 of therapy and post autologous stem cell transplantation (ASCT). The results were compared with historic urine protein electrophoresis (UPE) plus urine and serum immunofixation electrophoresis (uIFE and sIFE); to identify residual disease sIFE was performed on all patients after 2 cycles of therapy. *Results.* At presentation all patients had a detectable band on uIFE and measurable disease using UPE (>200mg/L). 22/25 patients had a detectable monoclonal band by sIFE, with 1/25 patients having a monoclonal band quantifiable by SPE (11.5g/L). In the FLCκ subgroup of patients, all 14/14 patients had increased FLCκ levels in the serum. The median FLCκ was 3740 mg/L (range, 689-13100 mg/L). Additionally, all 14 FLCκ patients had abnormally increased FLC κ/λ ratios, with a median value of 532 (range, 42- 2400). In the FLCλ subgroup, all 11/ 11 patients had both increased FLCλ levels and abnormally decreased FLC κ/λ ratios in the serum, with the median value of 3000 mg/L (range, 875-22000 mg/L) and a median ratio of 0.001 (0.0001-0.02). Table 1 shows the patient responses as-

Table 1.

	UPE/ UFE		FLC	
	VGPR	CR	VGPR	CR
Cycle 2	25% (5/20)	35% (7/20)	45% (9/20)	10% (2/20)
Cycle 4	14% (3/21)	57% (12/21)	57% (12/21)	14% (3/21)
ASCT	18% (4/22)	59% (13/22)	50% (11/22)	36% (8/22)

sessed using UPE/UFE or sFLC measurements alone; there were no patients with positive UFE results and normal FLC ratios. After 2 cycles of therapy a normal FLC ratio (18/20) correlated well to sIFE (17/20) results in the identification of residual disease (Pearson's r=0.7; p<0.001). The median involved FLC (iFLC) level in these patients was 152 mg/L (range, 52- 202 mg/L). UFE (13/20) under-estimated the number of patients with residual disease and correlated less well to sIFE (Pearson's r=0.5; p=0.03). Post ASCT, uIFE and sIFE indicated CR in 13 patients, however, sFLC measurements detected residual disease in 5/13 patients (median iFLC level of 25 mg/L (range, 15- 25.35 mg/L)). *Discussion.* sFLC measurements correlated well to sIFE measurements in the detection of residual disease. There were no instances when residual disease was detectable by UPE or UFE but not detectable by FLC measurements. In conclusion, FLC measurements could replace UPE and UFE to effectively monitor LCMM patients.

0854

MONITORING SERUM LEVELS ELR+ CXC CHEMOKINES AND RELATIONSHIP BETWEEN MICROVESSEL DENSITY AND ANGIOGENIC GROWTH FACTORS IN MULTIPLE MYELOMA

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Background. The ELR+ CXC chemokines are important mediators of tumorigenesis, related to their angiogenic properties. Angiogenesis appears to be a prominent feature in the progression of multiple myeloma (MM). CXC chemokines have four highly conserved cysteine amino acid residues, with the first two cysteine molecules separated by a single amino acid. The angiogenic potential of this group is determined by the presence of three amino acid residues (Glu-Leu-Arg: the ELR motif) preceding the first cysteine amino acid, in the NH2 terminus. *Aims.* The purpose of this study was to determine serum concentrations of angiogenesis-related chemokines ELR+ motif, such as interleukine-8 (IL-8), epithelial neutrophil activating protein-78 (ENA-78) and growth-related gene alpha (GRO-α), as well the bone marrow microvascular density (MVD) in patients with MM at diagnosis and after treatment, in plateau phase. We also evaluated the relationship among them with other known growth factors involved in angiogenesis, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and tumor necrosis factor-a (TNF-α). *Methods.* Serum levels of the above chemokines were determined in 63 newly diagnosed MM patients, in 28 in plateau phase and in 20 healthy controls. Serum measurements of IL-8, ENA-78, GRO-α, VEGF, HGF and TNF-α were performed with a commercially available kit for ELISA. Bone marrow biopsies were performed before and after treatment, in plateau phase, in order to determine MVD by staining vessels with anti-CD31. *Results.* Serum concentrations of IL-8, ENA-78, GRO-α and TNF-α were significantly higher in the group of MM patients (44.5±25.3pg/ml, 765±572.1pg/ml, 186±129.1pg/ml and 4.2±2.8pg/ml respectively) in comparison to control group (27.3±6.3pg/ml, 335.1±268.6pg/ml, 112.5±76.1pg/ml and 1.3±0.8pg/ml) (p<0.0004, p<0.002, p<0.01 and p<0.0001 respectively). We also found that untreated patients had higher levels of IL-8, ENA-78, GRO-α than post treatment patients, but statistical significant difference was found only for IL-8 (48.36±30.93pg/ml vs. 35.05±19.77pg/ml, p<0.001). Furthermore IL-8, GRO-α, TNF-α, HGF and VEGF were significantly higher with increasing disease stage (p<0.001 in all cases). ENA-78 serum levels were higher in stage III than in stage I and II, but without statistical significance. Additionally we correlated each proinflammatory cytokine with well known angiogenic factors such as HGF, VEGF and TNF-α. A positive correlation was found between serum HGF and IL-8 and GRO-α (r=0.316 p<0.01, r=0.297 p<0.01 respectively). Similarly serum VEGF correlated with ENA-78 and GRO-α (r=0.421 p<0.001, r=0.361 p<0.004 respectively) while a trend to correlate was found between TNF-α and IL-8 (r=0.242 p<0.056). In the pretreatment group of patients a positive correlation between bone marrow MVD and serum levels of GRO-α was found (r=0.304 p<0.01) while MVD did not correlate with IL-8 and ENA-78. There was a difference in survival times between patients with higher than median versus low IL-8, ENA-78 and GRO-α levels, but the differences could not reach statistical significance in either case. *Conclusions.* These findings support the hypothesis that ELR+ motif CXC chemokines, such as IL-8, ENA-78 and GRO-α correlate with angiogenic growth factors and may play a role in the progression of MM. Further studies are needed to determine their prognostic and predictive significance.

0855

LIGHT-CHAIN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE IN GERMANY

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Background. Light-chain monoclonal gammopathy of undetermined significance (LCMGUS) is thought to be the precursor condition leading to light-chain myeloma. *Aim.* To determine 5-year incidence, prevalence and mortality rate of LCMGUS we utilized the biobank of the ongoing population-based, prospective Heinz Nixdorf Recall study. *Methods.* The Heinz Nixdorf Recall study comprises 4814 men and women aged 45 - 75 from 3 large adjacent cities in Germany. Subjects were randomly selected from statutory lists of residence and gave informed consent. Free light-chain (FLC) κ and λ measurements were performed on a Dade Behring BNII automated nephelometer (Siemens, Germany) utilizing a commercially available kit (FREELITE, The Binding Site Ltd, Birmingham, UK). To identify monoclonal proteins, a sensitive approach using standard serum protein electrophoresis combined with screening immunfixation was used (Hydragel 12IF, Sebia). We screened serum samples from both the baseline examination which took place from 2000 until 2003 and the 5-year follow-up. LCMGUS was defined as abnormal FLC ratio (<0.26 or >1.65), increase in the FLC that caused the abnormal ratio and no detectable intact immunoglobulin. Age-standardized prevalence was obtained by direct standardization to the U.S. 2000 population. 5-year cumulative incidence was estimated from samples with available FLC measurements at baseline and follow-up. Standardized mortality ratio (SMR) was calculated comparing LCMGUS cases against the whole Heinz Nixdorf Recall cohort as reference using 10-year age-intervals. *Results.* 4702 and 4059 baseline and follow-up samples were analyzed, respectively. 3986 samples had FLC measurements at both time points. 34 LCMGUS cases were identified among the baseline samples (prevalence 0.7%, 95%-CI 0.5 - 1.0; age-standardized prevalence 0.8%, 95%-CI 0.5 - 1.1). Median age of LCMGUS cases was 66.5 years (range 47 - 74), 29 (65%) were male. As observable for MGUS, the LCMGUS prevalence increased with age and was higher in males. Of these cases 4 (12%) died before follow-up examination. While 13 (38%) cases were still classified as LCMGUS at follow-up, 14 (41%) previously identified LCMGUS cases showed a subsequent normal FLC ratio. All but one of the latter cases involved FLC κ with ratios < 2. Using a reference range adapted to renal function did remove 2 of these cases. Between baseline and 5-year follow-up 9 new cases developed yielding a 5-year cumulative incidence of 0.2% (95%-CI 0.1 - 0.4). The SMR was 1.88 (95%-CI 0.62 - 5.66). In one LCMGUS case a monoclonal protein was identified at follow-up leading to the diagnosis of MGUS. None of the LCMGUS cases progressed to myeloma during a median observation time of 7.2 years. *Conclusion.* We present for the first time incidence, prevalence and mortality rate estimates of the recently defined clinical entity LCMGUS. Using the published FLC ratio reference range of 0.26 - 1.65 in our cohort resulted in a considerable number of potential false-positives, suggesting that a more stringent reference range should be used to define LCMGUS. Additionally, our findings suggest that an abnormal FLC ratio can also precede conventional MGUS. As follow-up continues updated data will be presented at the conference.

0856

IMMUNE-RELATED CONDITIONS INCREASE THE RISK OF MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE: RESULTS FROM A POPULATION-BASED STUDY

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Background. The etiology of multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS) is largely unknown. There is evidence for genetic factors in the etiology of MM and MGUS. Several study groups have shown that immune dysregulation plays a major role in lymphomagenesis. Much less is known regarding immune-related conditions and risk of plasma cell disorders, and results from previous smaller studies have been inconsistent. *Aims.* The aim of the study was to determine whether a previous personal history of immune-related conditions (autoimmune diseases, infections and inflammatory conditions) is associated with an increased risk of MM and/or MGUS. *Methods.* Using national Swedish registries we identified 19,112 patients with MM, 5,403 patients with MGUS, and 96,617 matched control subjects. The patients were diagnosed from 1965 through 2004. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) for each immune-related condition (from the centralized Inpatient register) and for categories of conditions using logistic regression. To avoid detection bias, immune-related conditions occurring less than one year prior to diagnosis of MM/MGUS were excluded. *Results.* A personal history of all autoimmune diseases combined was associated with a significantly increased risk of MGUS (OR = 2.1, 95% CI = 1.9-2.4) but not of MM. Several specific autoimmune diseases elevated the risk of MGUS, however polymyalgia rheumatica (OR = 1.9, 95% CI = 1.4-2.6 for MM, OR = 2.9, 95% CI 2.1-4.1 for MGUS), giant cell arteritis (OR = 7.8, 95% CI = 3.3-18.2 for MM, OR = 11.3, 95% CI = 4.8-26.7 for MGUS), and autoimmune hemolytic anemia were associated with an increased risk of both MM and MGUS. A personal history of all infections combined was associated with a significantly increased risk of MM (OR = 1.2, 95% CI = 1.1-1.3) and of MGUS (OR = 1.6, 95% CI = 1.5-1.7). A personal history of pneumonia, septicemia, sinusitis, meningitis, myocarditis, and other conditions, was found to significantly elevate the risk of MM and/or MGUS. A personal history of all inflammatory conditions combined was associated with a significantly increased risk of MGUS (OR = 1.4, 95% CI = 1.2-1.5) but not of MM. A personal history of nephrotic syndrome, chronic osteoarthritis, diverticulitis, and other conditions, was found to significantly elevate the risk of MM and/or MGUS. The increased risk of MM and MGUS that was associated with these diseases remained statistically significant for the majority of these conditions at more than five years of latency. *Summary/Conclusions.* In this large population-based case-control study including almost 20,000 MM patients, more than 5,000 MGUS patients, and their close to 100,000 matched control subjects, we found that a personal history of several specific immune-related conditions was associated with an increased risk of MGUS and to a lesser degree MM. Our findings suggest that immune-related conditions or the treatment of them are of importance in the etiology of MGUS and possibly MM.

0857

EXPRESSION OF CCL3 BY NEOPLASTIC CELLS IN PATIENTS WITH WALDENSTRÖM'S MACROGLOBULINEMIA: AN IMMUNOHISTOCHEMICAL STUDY IN BONE MARROW BIOPSIES OF 67 PATIENTS

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Background. C-C motif ligand 3 (CCL3) chemokine, previously known as macrophage inflammatory protein-1 alpha is a member of the C-C chemokine family and has chemotactic function against monocytes/macrophages, T-lymphocytes, mast cells, dendritic cells, eosinophils and natural killer cells. Waldenström's macroglobulinemia (WM) is a distinct B-cell lymphoproliferative disorder characterized by lymphoplasmacytic bone marrow infiltration along with an IgM monoclonal gammopathy. Our group has previously shown that CCL3 is elevated in the serum of WM patients and correlates with adverse disease features. However, there is no information for the source of the production of CCL3 in WM. *Aims.* The aim of this study was to evaluate the expression of CCL3 by WM cells in bone marrow biopsies of WM patients to test the hypothesis of production of CCL3 by WM cells. *Methods.* We evaluated bone marrow biopsies from 67 patients with WM (39M/28F; median age: 72 years, range: 39-85 years) who were diagnosed, treated and followed-up in a single center (Department of Clinical Therapeutics, University of Athens, Greece) between 1999 and 2009. Forty-six patients had newly diagnosed WM (4 with asymptomatic disease), while 21 patients had active disease after treatment. Bone marrow biopsy sections were immunochemically tested for the expression of CCL3 (using an anti-CCL3 monoclonal antibody by Santa Cruz Biotechnology, Santa Cruz, CA, USA), CD20, CD79alpha, CD138, MUM-1, as well as for mu, alpha, gamma heavy and kappa and lambda light immunoglobulin chains. The immunoreactivity of CCL3 was examined on the basis of positive lymphoplasmacytic and/or plasma cells with a cut-off value of >20% positive cells to be defined as positive expression. *Results.* In all WM cases, either at diagnosis or at relapse, the whole number of the neoplastic cells, including CD20(+)/CD138(-)/MUM-1(-) small B-lymphocytes, plasmacytoid lymphocytes and rare immunoblasts as well as CD20(-)/CD138(+)/MUM-1(+) plasma cells revealed strong cytoplasmic positivity for CCL3. Due to such strong positivity for CCL3, no definite correlations between CCL3 expression by the neoplastic cells and disease features were able to be established. Regarding the putative role of CCL3 overproduction in WM, only hypotheses can be made. In chronic lymphocytic leukemia (CLL), an abnormally high number of infiltrating CD68+ monocyte/macrophages has been described in the CLL-involved areas of trephine biopsies from CCL3/CCL4-producing CLL cells. Thus, we performed a CD68 (PGM-1) staining in the trephine biopsies of our symptomatic WM patients and found a high number of CD68+ cells in the infiltrating areas. These cells may contribute to the WM cell survival in a similar way observed in CLL. CCL3 is also a chemoattracting factor for mast cells and it is well established that the mast cells support lymphoplasmacytic cell growth in WM. In all our cases, there was a high number of mast cells in the infiltrating areas, supporting a possible role of CCL3 in this accumulation. *Summary/Conclusions.* CCL3 is overexpressed by WM cells. This result supports a possible role of CCL3 in WM biology through interactions of the malignant clone with the marrow microenvironment and reveals CCL3 as a potential target for developing novel drugs against WM.

0858

ALTERED FIBRIN CLOT PROPERTIES IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS AND AFTER TREATMENT WITH THALIDOMIDE

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Background. The risk of venous thromboembolism (VTE), including deep vein thrombosis and pulmonary embolism, is high in newly diagnosed patients with multiple myeloma (MM). The use of thalidomide containing regimens seems to be associated with further increase of thrombotic complications. *Objective.* To investigate fibrin clot properties in MM patients prior and after induction remission with thalidomide. *Patients and Methods.* We studied 38 consecutive MM patients, 19 men and 19 women, aged 49 to 81 years (mean 63.5 years), with no history of VTE. There were 18 cases of IgG MM, 10 of IgA MM and 10 with light chain disease (LCD). Staging according to Durie and Salmon disclosed 5 subjects with IIA stage, 2 with IIB, 17 with IIIA, and 14 with IIIB. Fifteen patients completed treatment according to CTD, 2-6 cycles, mean 3.7 (cyclophosphamide 500 mg/m2/d i.v., day 1, thalidomide 100 mg/d, dexamethasone 10 mg/d on days 1-4 and 9-12). All MM patients received aspirin 75 mg/d. The control group comprised of 38 healthy subjects matched for age and gender. Plasma fibrin clot permeability, turbidity and fibrinolysis were assessed at baseline and after treatment with thalidomide. *Results.* Fibrinogen plasma concentration was higher in MM patients compared with controls (4.03 ±

1.48 vs 2.62 ± 0.43 g/L, P<.0001). The highest fibrinogen was found in LCD subgroup. Fibrin clots in MM patients compared with those obtained from controls had lower clot permeability (5.19 ± 1.28 v 8.89 ± 0.77 10-9cm2, respectively, P< .0001), prolonged clot lysis time (12.5 ± 2.2 v 8.06 ± 0.94 min, P<.00001), and lower baseline maximum clot absorbancy at 405 nm (0.56 ± 0.1 v 0.79 ± 0.06, respectively, P<.0001). Baseline maximum velocity of D-dimer release from plasma clots subjected to tissue plasminogen activator was lower in the MM group than in controls (0.066 ± 0.006 v 0.077 ± 0.006 mg/L/min, P<.00001), while the initial maximum D-dimer level was higher in MM patients (4.76 ± 0.62 v 3.48 ±0.27, mg/L, P<.00001). MM patients with renal insufficiency (n= 16) had significantly lower clot permeability than the remainder (n=22;P<.0013). Comparison of fibrin clot obtained from patients after treatment with thalidomide with that prior the treatment did not show changes in fibrinogen concentrations (3.9 ±1.2 v 4.03 ±1.48 g/L, P<.2), in clot permeability (5.49 ±1.3 v 5.19 ± 1.28 10-9 cm2, P <.4),or in clot lysis time (12,3 ± 1,78 v 12,5 ± 2,2 min, P<.7) but maximum clot absorbancy (0.72 ± 0.1 v 0.56 ± 0.1, P <.003) and the rate of D-dimer release from clots (0.073 ± 0.008 v 0.066 ± 0.006 mg/L/min, P<.0069) was higher. Out of 38 MM patients treated with thalidomide 6 subjects developed venous or arterial thrombosis and they had lower clot permeability compared to the remaining individuals (P<.05); other fibrin clot variables were similar in both subgroups. *Conclusion.* Substantial prothrombotic alterations of fibrin clot properties, including markedly impaired fibrinolysis occur in patients with MM and thalidomide-containing regimens further modify clot phenotype, which seems to contribute in thrombotic complications.

0859

IMPACT OF NOVEL M-COMPONENT BASED BIOMARKERS ON TO PROGRESSION FREE SURVIVAL AFTER TREATMENT IN MULTIPLE MYELOMA PATIENTS

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Background. The depth of response has been associated with longer Progression Free Survival (PFS) in Multiple Myeloma (MM).Serum free light chains (sFLC) and ratio (sFLCR), are used for the evaluation of sCR. They constituted the background for developing specific antibodies that bind the junction of the heavy and light chains on individual immunoglobulin isotypes (Hevylite®), making possible to quantify the IgGκ, IgGλ, IgAκ, IgAλ and their ratios IgGκ/IgGλ, IgGλ/IgGκ, IgAκ,/IgAλ and IgAλ/IgAκ (HLCRs) separately. *Aim.* To investigate the importance of sFLCR and HLCRs normalisation at plateau on PFS. *Patients and Methods.* 51 intact immunoglobulin MM patients were studied from diagnosis to last follow up. All patients were symptomatic. Their sera samples (n=312) were analyzed for sFLC by immunoassay (Freelite®) and for IgGκ, IgGλ IgAκ, IgAλ with Hevylite® antibody, nephelometrically. Serum FLCR and HLCRs values above the 95%-ile of normal individuals were considered as abnormal. File data were reviewed. *Results.* Median lines of therapy were 2 (range 1-11). Median follow up was 28 months (4-135). Retreatment was initiated in all patients according to standard criteria. HLCR was abnormal in all patients at diagnosis. As expected the quality of response correlated to PFS and patients in sCR, CR and nCR had a longer PFS than the others. Both sFLCR and HLCRs normalization were strong parameter of increased PFS after treatment at any line (p=0,029 and p=0,016 respectively). *Conclusion.* Both sFLCR and HLCRs normalization reflect prolonged responses.

0860

HLA-DRB1*13 AND *15 ARE ASSOCIATED WITH RESPONSE TO THALIDOMIDE IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Thalidomide (T) has been approved for treatment of myeloma. There is no generally accepted predictor for response. *Aims.*

Table 1.

	T responders	T non-responders	p
N=46	16 (35%)	30 (65%)	
Age (median,range)	50 (35-73)	50 (31-64)	0.38
Sex (M/F)	8/8	23/7	0.1
Paraprotein IgG Non-IgG	 11 5	 22 8	0.74
Prognosis ISS-I ISS-II or III	 6 10	 15 11	0.34
Number of previous CT cycles (median, range)	6 (0-9)	6 (1-13)	0.31
Number of previous CT lines (median, range)	1 (0-3)	2 (1-3)	0.02
Previous ASCT Present Absent	 9 7	 25 5	0.07
Del13q Positive Negative	 7 0	 8 6	0.06
p53 Positive Negative	 1 1	 7 2	0.42

Table 2.

	T Responders	T non-responders	p
HLA-DRB1*15 Positive Negative	 5 11	 4 26	0.24
HLA-DRB1*13 Positive Negative	 8 7	 4 25	0.01
HLA-DRB1*11 Positive Negative	 2 13	 16 13	0.01

The aim of this study was to analyze the impact of HLA antigens on response to T. *Methods.* Patients (n=46) who received T either as monotherapy (n=36) or combination therapy with dexamethasone (n=10) were retrospectively analyzed. Patient characteristics were: median age 50 (31-73), male/female 31/15, IgG/IgA/IgM/light chain 33/6/1/6 and ISS-I/II/III 21/10/11. Numbers of patients given T for induction/consolidation/relapse were 2/18/26. Patients were given T at doses 50-400 mg for a median duration of 10 (2-72) months. Thalidomide was stopped whenever disease progression or side effects grade≥3 occurred. Response to T was classified as responders ≥ partial remission (PR) or non-responders (disease progression on T). *Results.* 16 patients had response, 18 patients had disease progression and 12 patients had stable disease on T. Parameters which may have contributed to response to T were compared between responders and non-responders (Table 1-2). There were no statistically significant difference between the responders and non-responders in terms of age, sex, paraprotein, prognostic subgroup or the number of previous chemotherapy cycles. The only significant differences were the number of previous chemotherapy lines and the HLA frequencies. *Summary/Conclusions.* In a recent study (Beksac *et al.* 2008), it was demonstrated that HLA-DRB1*15, HLA-DRB1*13 and HLA-DRB1*11 were observed 7.2%, 18.2% and 21.1% in MM population, respectively. In this study, the percentage of response to T in HLA-DRB1*15 positive and HLA-DRB1*13 positive patients were 55.5% and 66.6%, respectively. There were 4 patients who were HLA-DRB1*15/*13 and all of them responded to T. On the other hand, HLA-DRB1*11 was associated with refractoriness to T (11% response, p=0.01). Host related factors such as HLA may have impact on response to T similar to that observed in aplastic anemia-immunosupression-HLA-DRB1*15.

0861

CORRELATION OF SERUM FREE LIGHT CHAIN QUANTIFICATION WITH SERUM AND URINE IMMUNOFIXATION IN MONOCLONAL GAMMOPATHIES

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Objectives. Standard methods for detection and monitoring of monoclonal proteins include electrophoresis and immunofixation (IFE) of

serum and urine samples. Recently, automated quantitative immunoassay for serum immunoglobulin free light chains (FLCs) has become available for clinical use. The aim of this study was to assess the efficacy of serum FLC assay in detection and monitoring of monoclonal proteins in different variants of monoclonal gammopathy. *Material and Methods.* Diagnostic value of serum FLC κ/λ ratios was compared with serum and urine protein electrophoresis and IFE in 106 multiple myeloma, 4 solitary plasmacytoma patients and 8 patients with monoclonal gammopathy of undetermined significance (MGUS). FLCs were measured using Siemens BNII nephelometer with the FreeliteTM Kit (Binding Site). *Results.* Of 37 light chain (LC) myeloma patients, 21 were positive for κ LC and 16 for λ LC. All of these patients had monoclonal LC detected by IFE in urine and 31 also detected in serum. Although 27 patients had M spike in urine, only 9 had M spike in serum. All patients in κ subgroup had an increased κ FLCs in serum and increased FLC ratio. In λ group all patients had both increased λ FLCs and decreased FLC ratio in serum. In 5 of 8 patients with nonsecretory myeloma and negative serum and urine IFE, the FLCs were increased. In all 38 myeloma patients with intact immunoglobulin (G 26, A 8, M 1, D 2, E 1) assessed at diagnosis or in disease progression, the FLC ratio was abnormal; in 3 patients in CR after bortezomib therapy the FLC ratio was normal and in 2 of 3 patients with PR the FLC ratio was abnormal. In 13 of 29 patients treated with autologous stem cell transplant the normal FLC ratio documented stringent complete response. Discrepancy between IFE and FLC results was found in 5 patients. Abnormal FLC ratio in 3 patients was ahead of the disease progression that was revealed in IFE and histology. In contrast, in one case, the appearance of M-protein in IFE and increased plasma cell rate in bone marrow was observed earlier in relation to FLC level which remained normal. In all 4 patients with solitary extramedullary plasmacytoma the FLC ratio was normal. Three of 8 MGUS patients with follow-up from 10 to 26 years had abnormal the FLC ratio. Multiple myeloma progression developed in one patient with normal FLC ratio. *Conclusions.* Serum FLC assay allows improved detection rates of light chain multiple myeloma and nonsecretory multiple myeloma. This test enables also monitoring of these diseases, and allows more precise definition of remission status.

0862

EVALUATION OF IGG, IGA AND IGM MONOCLONAL AND BICLONAL GAMMOPATHIES BY NEPHELOMETRIC MEASUREMENT OF INDIVIDUAL IMMUNOGLOBULIN κ/λ RATIOS - HEVYLITE ASSAY VERSUS IMMUNOFIXATION

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Background. Availability of antibodies which bind to conformational epitopes spanning the junctional regions between bound κ or λ light chains and their respective heavy chain partners has allowed the specific measurement of serum IgGκ, IgGλ, IgAκ, IgAλ IgMκ and IgMλ concentrations. In turn, this has enabled the calculation of IgGκ/IgGλ, IgAκ/IgAλ and IgMκ /IgMλ ratios (heavy/light chain or HLC ratios) for individual patients (Bradwell *et al.* Clin Chem 2009; 55: 1646, Avet-Loiseau *et al.* Blood 2009;114:722). *Aim.* In this study, the diagnostic value of HLC ratios was compared with the serum protein immunofixation (IFE) results. *Methods.* Fresh and archived, frozen sera from 51 patients with monoclonal and biclonal gammopathy including 34 with multiple myeloma and 11 with Waldenstrom's macroglobulinemia were assayed. Serum protein electrophoresis and IFE were performed using HYDRASYS 2 apparatus (Sebia, France) and antisera from the same company. Igκ/λ pairs concentrations were measured on a Siemens BNTMII nephelometer, using reagents from the Binding Site, UK. *Results.* IFE and HLC ratio results were concordant for 44 of the 51 IFE - monoclonal protein positive samples. IFE detected 2 "minor"IgGκ bands (1 oligoclonal after autologous stem cell transplantation, 1 reactive) and one IgAλ with polyclonal background that were not detected by HLC assay. In 5 cases with biclonal IgGκ + IgGλ gammopathy revealed in IFE, HLC ratios were normal and in 4 cases with biclonal IgG + IgA gammopathy, including 3- IgGκ + IgAκ cases and one IgGκ + IgAλ, HLC ratios were concordant with IFE. In one patient with Waldenstrom's macroglobulinemia and 3 homogenous IgG+IgA+ IgM in serum IFE analysis, HLC assay correctly revealed only IgMκ/IgMλ abnormal ratios while IgGκ/IgGλ and IgAκ/IgAλ ratios were normal. *Conclusion.* HLC and IFE are complementary methods. IFE is more sensitive than HLC assay but the latter provides numerical results.

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NORMAL RANGES AND REFERENCE INTERVALS OF SERUM FREE LIGHT CHAINS VALUES ARE HIGHER IN ELDERLY PEOPLE: STUDY IN A SPANISH URBAN POPULATION

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Background. The value of determination of serum free light chains is growing in certain monoclonal gammopathies: Although some work has been focused on the reference values in the normal population, it is important that these studies are conducted in different countries, distinguishing some features of population as age or renal function. *Aims.* To determine normal ranges and reference values of serum concentrations of free kappa and lambda chain in a spanish urban population. *Material and Methods.* We analyzed 613 serum samples of participants in a spanish urban population study for screening of monoclonal gammopathies. The method of recruitment of participants in the study was through family doctors or by personal letter. Inclusion criteria: 1) to have completed 50 years on October 1 2008; 2) to live in Segovia capital. The determination of serum free light chains (serum free Kappa (sFK), serum free Lambda (sFL) and ratio Kappa/Lambda (RK/L)) was carried out by turbidimetry. We studied the possible presence of serum monoclonal component (MC) by capillary electrophoresis and serum immunofixation. Furthermore, serum immunofixation was performed in doubtful cases. Renal function of participants was estimated by glomerular filtration rate using Modification of Diet in Renal Disease (MDRD) formula. Statistical *Methods.* Descriptive statistics of central tendency and distribution. We evaluated the normality of the series with the test of asymmetry, kurtosis and Kolmogorov-Smirnov. To assess the reference ranges was performed logarithmic transformation of the values in order to achieve Gaussian distribution; concentrations were calculated limiting 95% of the determinations. Nonparametric test (Mann-Whitney U) for the comparison of independent samples. *Results.* 19 MC were detected among 613 participants, so the analysis was conducted on 594 people who had no serum MC. Descriptive statistics of the three parameter was: sFK (mean±SD: 10.44±5.83; median(range): 9.09(2.9/70); sFL (16.9±9.07; 14.8(6.11/127.36); RK/L (0.66±0.35; 0.61(0.08/6.31). Table 1 specifies the concentrations of these parameters by gender, age (cutoff point 70 years) and renal function (MDRD cutoff point 60 ml/h). There were no significant differences between the values by gender. By contrast, concentrations of sFK ($p < 0.001$), sFL ($p < 0.001$) and RK/L ($p < 0.001$) were significantly higher in patients older than 70 years. Also, concentrations of sFK ($p<0.01$) and sFL ($p<0.05$) were significantly higher in patientes with renal function worsened, although RK/L was not significantly different by MDRD. The elderly people (> 70 years) had significant worsening renal function ($p<0.01$). The test proved the non-Gaussian distribution of the series. For this reason, we applied the statistic methods described above for determining reference intervals that were: sFK: 3.97-22.19 mg/L; sFL: 6.89-34.12 mg/L; RK/L: 0.28-1.31. *Conclusion.* The reference values and intervals obtained in our population are similar to

Table 1.

	Sex	n	Median (Range)	Age	n	Median (Range)	MDRD	n	Median (Range)
Free Kappa chain (mg/L)	Men	232	9.11 (2.9-70)	<70	369	8.43 (2.9-70)	≤60	269	9.79 (3.56-36.8)
	Wom	360	9.1 (3.36-61.6)	≥70	223	10 (3.6-61.6)	>60	300	8.6 (2.9-70)
Free Lambda chain (mg/L)	Men	233	14.28 (6.9-127.3)	<70	370	14.05 (6.11-48.9)	≤60	269	15.31 (6.52-48.9)
	Wom	361	15 (6.11-92.48)	≥70	224	16 (8.18-127.3)	>60	301	14 (6.11-127.3)
Ratio K/L	Men	230	0.65 (0.08-6.31)	<70	367	0.53 (0.21-6.31)	≤60	268	0.62 (0.24-3.04)
	Wom	359	0.59 (0.22-3.04)	≥70	222	0.66 (0.08-6.31)	>60	298	0.6 (0.08-6.31

those reported in other countries. The elderly population has higher serum free light chains concentrations due to worsening renal function in this group of people.

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0864

MYELOMA CELL CONTAMINATION OF PERIPHERAL BLOOD STEM CELL HARVESTS ESTIMATED BY MULTIPARAMETRIC FLOW CYTOMETRY: POTENTIAL CORRELATION WITH COMPLETE REMISSION RATE AND DURATION

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Background. High-dose therapy and autologous stem cell transplantation (ASCT) remains an integral component of the myeloma treatment algorithm for patients considered eligible for the procedure. Recent studies suggest that the presence of malignant plasma cells in the peripheral blood stem cell harvest (PBSCH) may correlate with the clinical outcome after high dose chemotherapy followed by ASCT. *Objectives.* To estimate the presence and the proportion of plasma cells (PC) in PBSCHs in correlation with clinical data. *Methods.* PBSCHs from 30 myeloma patients (21 men and 9 women, n = 30) who underwent ASCT were studied by 6-color flow cytometry (FCM) using the following combination of fluorochrome-conjugated antibodies, CD38/CD56/CD45/CD117/CD138/CD19. Response to therapy was assessed at the time of mobilization, at day +100 and at the end of the study after 14 months median period of follow up. The 2006 International Myeloma Working Group criteria were applied as follows: complete response (CR) (n= 8), very good partial response (VGPR) (n=8), and partial response (PR) (n=14). Mobilization regimens included G-CSF (n=30), Cyclophosphamide (n=3), Plerixafor (n=2). The Chi-square and Fisher exact tests were used to compare differences between nominal variables and the Mann-Whitney U test for continuous variables. *Results.* Plasma cells bearing CD138+CD38+CD45+CD19+CD117-CD56- immunophenotype were identified in 29/30 cases. The mean PC percentage by FCM was 0,029% (0-0,27%). Plasma cell contamination in PBSCHs showed positive correlation with (1) the degree of bone marrow infiltration by morphology (Pearson correlation 0,62; p=0,000) and (2) monoclonal immunoglobulin levels (Pearson correlation 0,4; p=0,043) at mobilization. Importantly, patients who achieved CR at day 100 had ten-fold lower PC in the PBSCH than those with VGPR or PR (mean 0,005% vs. 0,046% Mann Whitney U, p = 0,02). Moreover, at latest follow-up, patients in CR showed significantly lower levels of contamination compared to the VGPR/PR group (mean 0,0046% vs. 0,03%, p=0,006). *Conclusion.* Patients with low or undetectable contaminating plasma cell levels (by FCM) in the stem cell harvest have a significantly higher probability to achieve and remain in CR following high-dose chemotherapy and ASCT.

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Myeloma and other monoclonal gammopathies - Clinical 1

0865

PATTERNS AND EFFECTIVENESS OF BORTEZOMIB USE IN ELDERLY PATIENTS: THE VESUVE COHORT

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Background. Bortezomib (BTZ) represents an important progress in the treatment of multiple myeloma (MM). BTZ combined with other agents is becoming a standard of care particularly in elderly patients not eligible for autologous stem cell transplantation. To date, no evaluation of BTZ in a real-life setting has been conducted in France. *Aims.* To describe and compare patterns and effectiveness of BTZ use in two age groups: ≤75 years (youngers) vs >75 years (olders). *Methods.* VESUVE is a national cohort conducted in 60 French centres that included patients initiating BTZ from May 2004 to April 2006 using nominative hospital pharmacy dispensations. Patients treated for MM were followed for 2 years. Data regarding treatment modalities, response and survival outcomes (overall survival, OS and progression-free survival, PFS) were collected through medical files. BTZ cycles were categorized as standard or not according to market authorisation (dose, injection and cycle rhythm). Response was assessed by an independent committee according to adapted International Myeloma Working Group criteria. *Results.* Among the 793 patients included, 82.3% were aged ≤75 years and 17.7% >75 years. Concomitant use of other agents was more frequent in youngers (73.5% vs 65.0%; p=0.04) especially regarding conventional chemotherapy (16.7% vs 1.1%; p<0.01). Mean ±SD number of BTZ cycles did not differ between groups (5.0±3.5 vs 4.6±3.0; p=0.15) but mean BTZ cumulative dose per cycle was 7.9±1.6 mg in youngers vs 7.1±1.9 mg in olders (p<0.01). The proportion of patients with at least one non-standard cycle was 68.0% in youngers vs 77.9% in olders (p=0.02). BTZ was withdrawn for safety reasons in 20.1% in youngers vs 26.4% in olders (p=0.10). Among the 588 evaluable patients for response, the overall best response rate was 57.1% in youngers vs 64.1% in olders (p=0.19). The 2-year OS rate was 44.2% (95%CI 40.2-48.0; median 20.4 months) in youngers vs 36.3% (28.2-44.4; 14.1 months) in olders (p=0.02). The 2-year PFS rate was 11.6% (9.3-14.3; 7.3 months) in youngers vs 13.8% (8.6-20.3; 6.5 months) in olders (p=0.69). *Conclusions.* Despite differences regarding use, these results show that BTZ leads to very similar survival outcomes in patients over 75 years of age as compared to younger patients.

0866

CONSOLIDATION/MAINTENANCE THERAPY AFTER INTENSIVE INDUCTION IMPROVES QUALITY OF RESPONSE AND OUTCOME IN PATIENTS WITH RELAPSED-REFRACTORY MULTIPLE MYELOMA (R-RMM)

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Background. There are some evidences that consolidation/maintenance therapy improves quality of response and remission duration in newly diagnosed MM. However, the impact of these strategies in relapsed/refractory MM (r-rMM) are still unknown. *Aims.* We assess the impact of consolidation-maintenance (C-M) therapy on quality of response and outcome, performing a post-hoc analysis of a prospective phase II study exploring an intensive combination as induction followed by consolidation and maintenance therapies in r-rMM. *Methods.* Patients

received 6 28-day cycles of oral thalidomide 100 mg/day continuously, oral dexamethasone 20 mg on day 1-2, 4-5, 8-9, 11-12, pLD 30 mg/m2 iv on day 4 and Velcade 1.3 mg/ m2 iv on day 1, 4, 8, 11 (ThaDD-V). As consolidation patients underwent to 6 28-day cycles of rotating Velcade 1.3 mg/ m2 iv on day 1, 4, 8, 11 plus dexamethasone 20 mg on day 1-2, 4-5, 8-9 (3 courses) and thalidomide 100 mg/day continuously plus dexamethasone 20 mg on day 1-4 (3 courses). Maintenance therapy included thalidomide 100 mg/day until relapse or intolerable toxicity. Due to an excess of peripheral neuropathy, protocol was amended as follow: Velcade 1.3 mg/m2 on day 1, 4, 11 and thalidomide 50 mg/day in all therapeutic phases. Response, time to progression (TTP), progression free survival (PFS) and overall survival (OS) were assessed according to International Myeloma Working Group (IMWG). To assess the role of C-M therapy we compared TTP and OS of patients who were able to continue planned therapy with those of patients not receiving C-M because of toxicity by a 6-months landmark analysis. *Results.* Median age of 46 patients was 63.5 years (range 31-85 years) and the median number of prior regimens was 1 (range 1-4). Fifty-two percent had undergone autologous stem cell transplantation, 59% thalidomide, 17.5% bortezomib and 35% were refractory to the last regimen. After induction 13% of patients achieved sCR, 21.5% CR, 32.5% VGPR and 8.5% PR. Additional 4 patients had SD while 7 progressed. Out of 46 patients undergone induction, 26 (56.5%) received consolidation therapy since 10 patients progressed or died during induction, and 10 developed severe toxicities requiring therapy interruption. Five (25%) among the 20 patients without a prior sCR further improved response. Therefore, the best response after induction and consolidation was 17.5% sCR, 19.5% CR, 34.5% VGPR and 4.5% PR. The 20 patients receiving thalidomide maintenance therapy had no improvement in response. Globally, median TTP and PFS were 18.5 and 17.5 months, respectively while median survival was 40 months. Patients achieving sCR had a significantly better 3-year TTP if compared with those obtaining CR (86% vs 46%; p= 0.035) or VGPR-PR (86% vs 11%; p= 0.003). Patients who completed the protocol had a significantly longer TTP (NR vs 7 months, p= 0.001) and OS (NR vs 28 months; p= 0.038) compared with those who did not. *Conclusions.* This retrospective analysis suggests that, in r-rMM, the possibility of receiving intensive and continuous therapy containing bortezomib and thalidomide allowed to improve quality of response that translates into a significantly better outcome.

0867

BASELINE CHARACTERISTICS AND EFFICACY OF BORTEZOMIB-BASED THERAPY IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: RESULTS AFTER COMPLETE ENROLLMENT FROM AN ELECTRONIC BORTEZOMIB OBSERVATIONAL STUDY (EVOBS)

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Background. The international, non-interventional electronic bortezomib observational study (eVOBS) is ongoing to prospectively evaluate the efficacy and clinical outcomes associated with bortezomib-based therapies for primarily relapsed/refractory multiple myeloma (MM) in the 'real-world' clinical practice setting. *Aims.* With study enrollment now complete, we report patient demographics, baseline disease characteristics, and efficacy data for the entire eVOBS study population. *Methods.* Adult patients scheduled to begin bortezomib-based therapy for the treatment of predominantly relapsed/refractory MM were eligible for enrollment into the study. Patients were enrolled at clinical practices in Belgium, Brazil, Canada, France, Greece, Russia, Spain, Sweden and Turkey, and provided written informed consent. Treatment history was retrospectively documented for 1 year prior to initiation of bortezomib-based therapy. Prospective observational data were collected for up to 3 years after initiation of bortezomib-based therapy to document efficacy data. All administered bortezomib doses and concomitant treatments were permitted, except for investigational therapies. MM disease stage was assessed at the time of initiation of bortezomib-based therapy using Durie-Salmon (DS) or International Staging System (ISS) criteria. Responses were investigator-assessed and based on European Group for Blood and Marrow Transplantation (EBMT), Southwest Oncology Group (SWOG), or M-protein criteria. Response data were censored at the start of subsequent therapy, and for deaths. *Results.* 1560 patients were enrolled between October 2006 and December 2010. Sociodemographics, chronic co-morbidities, and patient characteristics were recorded at baseline (Table). The majority of patients (97%) included in this study received bortezomib-based therapy for relapsed/refractory MM; 3% of patients received bortezomib as their first-line therapy for MM. The most common prior MM treatments documented within 1 year of starting bortezomib-based therapy were melphalan-prednisone combinations (20%), thalidomide-dexamethasone (9%), vincristine-doxorubicin-dexamethasone ± cyclophosphamide (9%) and thalidomide monotherapy (6%); 5% of patients had received an autograft and 41% had no MM treatment in the previous year. The majority of patients received bortezomib in combination with other agents including dexamethasone (44%), prednisone (9%, mostly with melphalan), thalidomide (4%, mostly with dexamethasone), and lenalidomide (2%); 30% of patients received bortezomib monotherapy. Most (84%) patients received bortezomib at an initial dose of 1.3 mg/m2. At the data cut-off of 08 January 2011, and after a median follow-up of 15.3 months, 26.9%, 16.7%, 32.5%, and 8.4% of patients achieved a best response of complete response (CR), near-CR (nCR), partial response (PR), and minimal response (MR), respectively. In responding patients, the median time to CR, nCR, PR, and MR was 3.9, 3.2, 1.9, and 1.6 months, respectively. *Conclusions.* The eVOBS database contains valuable information about the efficacy of bortezomib-based therapies for relapsed/refractory MM in 'real-world' 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.

Table 1.

Characteristic	No. of patients (N=1560)
Median age, years (range)	62 (27–95)
Male / female, %	50 / 50
Time since first MM treatment, years (range)	2.1 (0–21)
Stage III MM by DS or ISS, %	49
No. of co-morbidities at baseline, 0 / 1 / 2 / ≥3, %	27 / 26 / 20 / 27
No. of prior lines of therapy, 0 / 1 / 2 / ≥3, %	3 / 56 / 22 / 19
Baseline peripheral neuropathy, none / grade 1 / grade 2 / grade 3, %	82 / 10 / 6 / 2

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BETTER TREATMENT RESPONSE IS ASSOCIATED WITH LENALIDOMIDE 25 MG ONCE-DAILY VS. 25 MG EVERY-OTHER-DAY IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background. 25 mg once-daily Lenalidomide on days 1-21 of a 28 day cycle in combination with 40 mg dexamethasone has demon-

strated significant improvements in time to progression (TTP) and overall survival (OS) in patients with relapsed/refractory multiple myeloma who have received ≥ 1 prior therapy. A pooled analysis (Dimopoulos *et al.* Leukemia. 2009;23: 2147-2152) of two large multicenter phase III trials (MM-009 and MM-010) assessed long-term outcomes (median follow-up 48 months in surviving patients) of 25 mg once-daily lenalidomide plus 40 mg dexamethasone vs. placebo plus 40 mg dexamethasone. This analysis demonstrated significant response and survival benefit with the lenalidomide plus dexamethasone combination (overall response rate (ORR) of 60.6% in patients receiving lenalidomide plus dexamethasone compared with 21.9% in patients receiving dexamethasone plus placebo). Median OS for the combination was 38.0 months with the combination and 31.6 months with dexamethasone plus placebo. *Aims.* To evaluate treatment response (CR, VGPR, PR, MR, SD and PD), OS, and safety with lenalidomide 25 mg given once-daily or every-other-day for 21 days of a 28 day cycle in patients with relapsed (53%)/refractory (47%) multiple myeloma. *Methods.* In this clinical practice chart review, 169 evaluable patients, 58% male, median age 64.3 years, received 25 mg once-daily (n=140) or every-other-day (n=29) oral lenalidomide-based regimens [57% with glucocorticoids (87% dexamethasone,160 or 320 mg per cycle); 43% with glucocorticoids plus conventional cytostatic agents]. Patients treated with lenalidomide monotherapy were excluded from this analysis. Patients in both groups received a median of 3 previous lines of therapy, 89% and 86% with ≥2 therapies, in the once-daily and every-other-day lenalidomide groups, respectively. *Results.* VGPR was 7.9% and 0%, PR was 30.3% and 10.0%, and MR was 6.7 and 20.0% with lenalidomide 25 mg once-daily and every-other-day, respectively. Despite a short median time to follow-up of 8.4 months (range 0.3 - 26.2), ORR (≥PR) was 38.2% versus 10%. Based on the definition of response (60 days after end of treatment), 89 and 10 patients were evaluated. Median OS from the start of therapy was 16.7 and 10.3 months in the lenalidomide 25 mg once-daily and every-other-day groups (evaluated in 140 and 29 patients, respectively). Lenalidomide once daily did not result in significantly higher incidence of hematologic adverse events. Grade 3/4 adverse events included neuropathy (5.6%, 3.4%), thrombosis (8.6%, 3.4 %), infection (14.9%, 3.6%), thrombocytopenia (12.4%, 3.4%), neutropenia (34.1%, 27.6%), and anemia (14.8%, 6.9%) in the once-daily and every-other-day lenalidomide groups. There was no significant difference between the two treatment groups in grades 1-5 thrombocytopenia or neutropenia. One patient treated with 25 mg once-daily lenalidomide experienced grade 5 thrombosis and one patient experienced grade 5 infection. *Conclusions.* Lenalidomide is active and generally well-tolerated in relapsed/refractory myeloma. These results support the approved 25 mg once-daily dosing of lenalidomide on days 1-21 of a 28 day cycle in patients with relapsed/refractory multiple myeloma and indicate that every-other-day dosing compromises response with no significant difference in tolerability.

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THE DOSE OF INFUSED NKT CELLS IN THE AUTOGRAFT DIRECTLY CORRELATES WITH LYMPHOCYTE RECOVERY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN MULTIPLE MYELOMA (MM)

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Early lymphocyte recovery after ASCT in MM is an important predictor of survival. The dose of infused lymphocytes in the autograft directly correlates with early lymphocyte recovery (*Porrata LF et al. Leukemia 2004;18:1085, Hiwase DK et al. Biol Blood Marrow Transplant 2008;14:116*). However, limitations of previous studies are the lack of lymphocyte subset analyses. Thirty-two patients with MM who underwent ASCT were examined to investigate the correlation between infused cell populations and lymphocyte subsets at engraftment. The cell populations of infused autograft and lymphocyte subsets of peripheral blood at engraftment were examined by flow cytometry. Immunophenotyping was performed for the T cell panel (CD3/CD4/CD8), B cells (CD19), and natural killer (NK) cells (CD56/16). By Spearman correlation coefficients, we identified a correlation between absolute lymphocyte count (ALC) at engraftment and each lymphocyte subset at engraftment (Table 1). The cell dose of infused NKT cells ($CD3^+CD56^+CD16^+$) was significantly associated with $CD3^+$ ($r_s = 0.435$, $P = 0.013$), $CD4^+$ ($r_s = 0.455$, $P = 0.009$), $CD8^+$ ($r_s = 0.399$, $P = 0.024$), $CD19^+$ ($r_s = 0.392$, $P = 0.027$), and ALC ($r_s = 0.395$, $P = 0.025$) at engraftment. On the contrary, the dose of infused $CD34^+$ cells was not associated with changes of any lymphocyte subsets. In addition, the recovery of $CD4^+$ cells was significantly associated with the dose of infused $CD3^+$ and $CD8^+$ but not $CD4^+$ cells. Our data suggest that a certain number of NKT cells as well as the number of $CD34^+$ cells should be aimed for successful ASCT in MM. Table1. Spearman correlation coefficient between infused cell population and lymphocyte subset at engraftment** P: <0.01* P: <0.05

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RENAL FUNCTION IMPROVEMENT EVALUATED BY CYSTATIN-C SERUM LEVELS IN PATIENTS WITH SYSTEMIC AL AMYLOIDOSIS TREATED WITH MEL-DEX ASSOCIATION

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Background. Primary systemic AL amyloidosis is a clonal plasma cell disorder in which the N-terminal fragments of monoclonal light chains form fibrils that accumulate in various tissues ultimately leading to organ dysfunction and death. Renal injury is a very frequent feature in AL amyloidosis patients. Serum cystatin-C and free light chain (s-FLC) κ and λ levels were investigated to evaluate clinical severity degree of renal impairment in AL amyloidosis, also in terms of prognosis. Cystatin-C is a non-glycosilated protein with low molecular weight (13kDa) produced at a constant rate in all nucleated cells. It is freely filtered, reabsorbed for 99% in proximal tubule and not secreted so it has higher sensitivity than creatinine determination for detecting initial GFR reduction. This peculiarity may be useful for evaluation of kidney and heart dysfunction for patients with systemic diseases. *Aims and Methods.* We evaluated serum cystatin-C and s-FLC levels in 7 patients (median age 63.1 yrs) with recent diagnosis of systemic AL λ amyloidosis admitted to our Unit. Ten age-matched healthy control subjects were selected. According to age and disease risk stratification six out 7 patients were treated with upfront oral MelDex association (Melphalan 9 mg/sm, Dexamethasone 20mg day 1-4 q28). One subject started first line therapy with BorDex association (Bortezomib 1.3 mg/sm, Dexamethasone 20 mg day 1,4,8,11 q21). Three samples of peripheral blood were performed (treatment day 1, day 8 and at con-

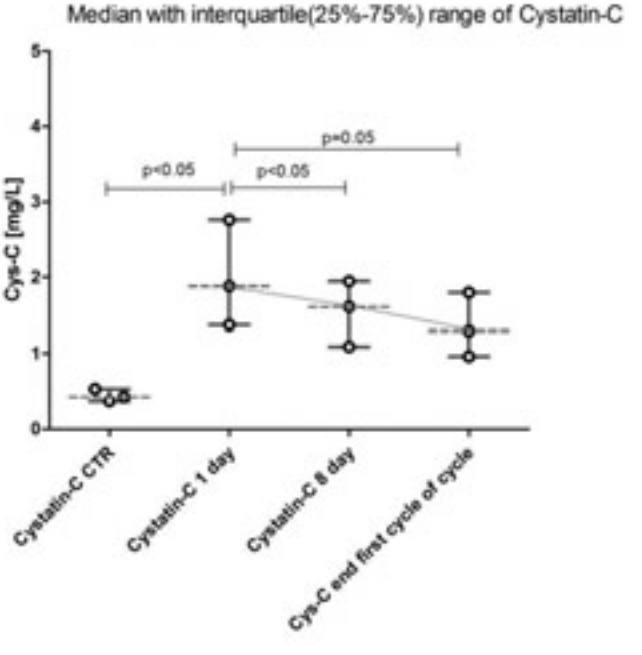

Figure 1.

clusion 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 Mann-Whitney U test, paired t test, Kruskal-Wallis one-way analysis of variance (Dunn's Method versus Control Group), Spearman rank correlation and Linear Regression were performed. P values ≤ 0.05 were considered statistically significant. *Results.* In all patients s-FLC κ/λ ratio was increased at the end of first cycle of therapy (p = 0.002). Both s-FLC λ and cystatin-C levels were significantly decreased during treatment (p < 0.05 and p = 0.05). A positive correlation between s-FLC λ and cystatin-C values was observed at all times (r2 = 0.99, p < 0.001 at day 1; r2 = 0.89, p = 0.006 at day 8 and r2 = 0.99, p <0.001 at the end of first cycle). Both treatments have been effective in terms of disease response and renal function improvement. Among 4 patients with persistent high serum levels of cystatin-C at the end of cycle, 3 had poor outcome (2 deaths with heart and renal failure respectively, 1 alive with end stage renal disease in haemodialysis). *Conclusions.* On the basis of these results we could consider cystatin-C a useful biomarker for renal function assessment with prognostic value according to treatment in patients with systemic AL amyloidosis.

0871

HIGH RESPONSE RATE IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH A COMBINATION OF CYCLOPHOSPHAMIDE, LIPOSOMAL DOXORUBICIN, DEXAMETHASONE AND BORTEZOMIB

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Background. Autologous stem cell transplantation (ASCT) and the use of new agents have significantly improved the outcome of multiple myeloma patients. The achievement of complete response after induction treatment is prerequisite for long term overall survival. Although ASCT is still considered standard of care, its necessity in first CR may be challenged in the era of the new agents. *Aims.* The aim of our study was to evaluate the efficacy and toxicity of an intensive treatment protocol in newly diagnosed patients with multiple myeloma. *Methods.* 26 patients (21 male, 5 female) of median age 60 years (45-75) were treated after informed consent with a combination of cyclophosphamide 750mg/m2 d1, liposomal doxorubicin 40mg/m2 d1, dexamethasone 40mg d 1-4 and bortesomib 1,3mg/m2 d1,4,8,11 every 28 days. All patients also received zoledronic acid 4 mg every 28 days and erythropoietin to maintain haemoglobin > 10.5 gr/dl. The median follow up was 17 months (3-40). The ISS stage was: low in 6, intermediate in 7 and high in 13 patients. The monoclonal globin type was IgG in 12 patients, IgA in 9, IgD in one, IgE in one and free light chain in 3. Renal impairment (Cr≥2) was present in 5 patients and hypercalcemia in 7 patients. Radiotherapy was applied in 7 patients. *Results.* All patients (n=26) who received at least two cycles of therapy were evaluated. The median number of cycles was 6 (range 2-8). 23/26 (88.5%) had responded. Thirteen patients (50%) had complete response (6 nCR, 7 CR IF-), 7 (27%) patients had very good partial response (VGPR), 3 (11.5%) had partial response (PR) while 3 (11.5%) patients didn't respond or had progressive disease. Three patients in CR had a sufficient collection of CD34+ cells but only one finally underwent ASCT. The toxicity of the treatment was acceptable. Haematological toxicity: neutropenia grade II 3/26, grade III 5/26, grade IV 1/26, thrombocytopenia grade II 7/26, grade III 2/26. In terms of non-haematological toxicity, one patient had deep vein thrombosis and more than half of the patients experienced gastrointestinal disturbances. Two patients were hospitalized with neutropenic fever. Maintenance treatment with thalidomide 50mg/d or lenalidomide 10-15 mg/d 1-21/mo after the achievement of CR or VGPR was introduced to 10 patients for at least 6 months. The progression free survival for the patients who achieved CR or VGPR was 14,5 months. The probability of relapse is 35% at 36 months. Four patients died, one in CR and three in relapse due to infections. The probability of survival at 36 months is 68 %. None of the analyzed factors in mono- and polyparametric analysis were significant for probability of relapse and survival. *Conclusions.* The above protocol was efficient in terms of response rate. It was safe with acceptable and manageable toxicity. Addition of bortezomib in 1st line treatment significantly improves the response rate. Larger number of patients and longer follow up is needed to define if our approach may lead to improved progression free and overall survival, without the necessity of ASCT in first CR.

0872

ANALYSIS OF THE INCIDENCE, CLINICAL AND BIOLOGICAL CHARACTERISTICS OF IGM MONOCLONAL GAMMOPATHY DETECTED IN A URBAN POPULATION STUDY

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Background. Several studies have examined the prevalence of Monoclonal Gammopathy of Undetermined Significance (MGUS) and its characteristics. However, few studies have focused on IgM Monoclonal Gammopathies (MG), especially IgM MGUS, entities with different pathophysiological significance and follow-up, opposite to non-IgM MGUS. *Aims.* To analyze the incidence, clinical and biological characteristic of the IgM MG detected in a urban population study. *Material and Methods.* Prospective cohort study in the urban population over the age of 50 years old in Segovia (Spain). The serum monoclonal component was detected by electrophoresis and confirmed by immunofixation. The selection of the participants was done through Health Centers or personal letter. *Results.* At the first 26 months, it has been contacted by 16161 people of whom 6681 agreed to take part in the above mentioned study. 147 MG was detected, which 24 were IgM and 2 biclonals (IgM + IgG) (17.7 % of the MG series, 0.36% of the population). It was 21 males and 5 females, with a median age of 71 years (54-85 years). Distribution for light chain show a clear predominance of the light chain κ (20) opposite to light chain λ (8). 5 of these cases could not be studied because lack of consent or other social/sanitary circumstances, 1 case was a transitory Gammopathy and in 3 cases the Gammopathy was secondary to other diseases. Of 17 remaining patients, 13 (76 %) was catalogued of Monoclonal Gammopathy of Undetermined Significance (MGUS) and 4 (24 %) of asymptomatic Waldenström's Macroglobulinemia (aWM). No case of symptomatic Waldenström's Macroglobulinemia. The patients with MGUS were 10 males and 3 women, with a median age of 76 years (56-85). The median of the component monoclonal by electrophoresis was 0.6 g/l (0.22-1.74). Median hemoglobin, serum creatinin and albumin levels were: 14.3 g/dl (11.6-15.4), 1.1 mg/dl (1-1.2) and 4.35 g/dl (4-4.9). From diagnoses, a patient has evolved to aWM. The patients with aWM were all males, with a median age of 71 years (56-80). The median of the component monoclonal was of 1.16 g/l (0.57-1.5). Median hemoglobin, serum creatinin and albumin levels were: 14.1 g/dl (11.9-15), 1 mg/dl (0.8-1.3) and 4.4 g/dl (4.3-4.7). *Conclusions.* In our population study, the proportion of IgM MG is similar to reported in other studies, although the incidence appears somewhat lower. These information will be updated in the meeting.

0873

PERCUTANEOUS VERTEBROPLASTY IN MULTIPLE MYELOMA: A SINGLE CENTRE EXPERIENCE.

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Background. Multiple myeloma (MM) is frequently associated with osteolytic lesions with bone pain and pathological fractures: vertebral compression fractures are present in 60% of patients at diagnosis. Percutaneous vertebroplasty (PVP) is a minimally invasive, radiologically guided procedure, in which bone cement is injected into destructed vertebrae. It has been shown to provide rapid pain relief and improved rehabilitation in acute fractures when bed rest, analgesic, bisphosphonates and radiotherapy fail (80% of cases). This study was conducted to test the efficacy and safety of PVP. *Methods.* A prospective analysis was performed on a total of 59 levels in 26 patients with vertebral pain refractory to conventional medical therapy for at least two months. Vertebral fractures were recognized when local pain coexisted with acute compression fracture findings by magnetic resonance imaging (MRI). Twenty-four out of 26 patients were on therapy with zoledronic acid an 12 of them received radiotherapy. All the procedures were performed by two experienced neu-

roradiologist, after obtaining informed consent. Under fluoroscopic guidance, the vertebral body was reached via a transpedicular route, while a posterolateral approach was usually considered when the pedicle was too small or sagitally oriented (thoracic spine). We use 13 G needles in the vast majority of cases, but in some instances we have adopted 11 G or, for passing through small pedicles, 15 G needles. Polymethilmethacrylate is usually inserted by means of a screw injection device or, in some situations, manually, utilizing small (1,5 cc) syringes. The visual analog scale (VAS) and the Eastern Cooperative Oncology Group (ECOG) scale were used to estimate patients' perception of pain and functional status, respectively, at -1, + 1, + 30, + 90, + 180, and every years after PVP. *Results.* Fifty-nine procedures were performed in 26 patients. Regarding the type of vertebral deformity, we found 2 cases of complete body crush, 14 wedge shaped failures, 17 dips both endplates, 16 dips in only one endplate, 10 cases of MRI altered signal in the absence of deformity or body height loss. Involved levels ranged from T4 and L5; L1 and L4 were the most frequently affected, with 8 procedures each. All patients but one were discharged from hospital the day after the surgical treatment. Patients were able to reduce their analgesic requirements by 55%. The mean VAS pain score decreased from 7,1 to 2,6. Patients presented a median pre-procedural ECOG scale of 3; after VTP, it improved to 1.7. Despite leakage of PMMA was detected in 38% of cases (21 out of 55), only two patients developed a transient and responsive to medical therapy neuralgia. Besides these two cases, we didn't observe any adverse event, except for one case of moderate fever. *Conclusions.* Our experience suggests that PVP is safe and effective and results in pain control and improvement of quality of life in MM patients. Furthermore it is associated with low toxicity and costs. Therefore, due to efficacy and safety, early treatment with PVP should be considered in the management of MM.

0874

BORTEZOMIB, ADRIAMYCIN AND DEXAMETHASONE (PAD) IN PRIMARY REFRACTORY MYELOMA: EFFECTIVENESS IN PATIENTS POORLY RESPONDING TO VAD

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Background. The combination of bortezomib with adriamycin & dexamethasone (PAD) is a highly effective induction agent with response rates of up to 95% (Oakervee, *et al.*, BJH 2005; 129:755-62). We have previously shown the superior efficacy of PAD chemotherapy over VAD/VAD-like regimens by comparing response to PAD given following relapse to the response previously obtained with VAD/VAD-like regimen in the same patients. *Aims.* To demonstrate the efficacy of bortezomib, adriamycin and dexamethasone combination therapy in patients with refractory myeloma. *Methods.* This was a phase 2 study with 3 cohorts, of 23 patients. Cohort 3 was for patients refractory to VAD and who proceeded directly to PAD chemotherapy. *Results.* 23 patients (6 females, 17 males, median age 62 years) achieved <PR to a median of 4 (range 2-6) courses of VAD or VAD-like therapy (C-VAD n=2, VAD n=15, Z-DEX n=6). Following PAD therapy 4 patients achieved ≥VGPR (CR n=2) based on their PAD protein level at the time of starting PAD therapy. A PR was recorded in 12 patients. When compared to the responses achieved by VAD therapy, the exact McNemar significance probability was p=0.0005. The fall in median paraprotein levels post-VAD was 23.6% and after PAD was 56.5% (using pre-PAD protein level for comparison) (p=0.0002 using the Wilcoxon signed rank test). *Conclusion.* Using a novel trial design, requiring only small numbers of patients we have shown that PAD is superior to VAD using two independent statistical methods.

0875

NO CORRELATION OF SYSTEMIC HYPOGAMMAGLOBULINEMIA AND IMMUNOSUPPRESSION OF THE SAME IMMUNOGLOBULIN CLASS WITH TIME TO FIRST TREATMENT AND OVERALL SURVIVAL IN WALDENSTRÖM'S MACROGLOBULINEMIA PATIENTS

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Background. Systemic hypogammaglobulinemia (SH) is a very common finding in Waldenström's Macroglobulinemia (WM). Low levels of IgG have been associated with disease progression.[1] Specific polyclonal antibodies, that recognise epitopes spanning the junction of the heavy and light chains of the individual immunoglobulin isotypes have been recently developed (Hevylite®). Decreased concentrations of polyclonal isotypes of the same Ig class (ISC) have been associated with shorter progression free survival in multiple myeloma.[2] *Aim.* The purpose of the study was to evaluate the effect of SH and ISC on time to first treatment (TTT) and overall survival (OVS) in a series of WM patients. *Methods.* Retrospective sera from 70 WM patients (35 male /35 female) at diagnosis were included in the study. Median age was 66yrs (range 44-91). Analysis of IgG and IgA was performed using standard antibobies, while IgMκ and IgMλ with Hevylite® antibody, nephelometrically. SH was defined as IgG<700 mg/dl, IgA<70mg/dl or both, ISC as IgMλ<10mg/dl in IgMκ patients or IgMκ<10mg/dl in IgMλ ones. *Results.* 48 out of 70 patients were or became symptomatic during follow up. Median follow up was 37 months. At diagnosis median IgG was 896 mg/dl (206-4750), median IgA 89 mg/dl (22-638), median IgMκ 2000mg/dl (7-17300) and median IgMλ 28mg/dl (1-13000). SH was present in 34/70 patients, ISC in 18/70 and both in 9/70. Sixteen patients died from disease. Neither SH nor ISC or both correlated with TTT (p=0,358, p=0,874 and p=0,718 respectively) or OVS (p=0,159, p=0,817 and p=0,854 respectively). *Conclusions.* In our experience SH and ISC are not adverse factors for TTT and OVS.

References

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0876

SEQUENTIAL HIGH-DOSE DEXAMETHASONE AND RESPONSE ADOPTED PAD OR VAD INDUCTION CHEMOTHERAPY FOLLOWED BY ASCT FOR NEWLY DIAGNOSED MULTIPLE MYELOMA; MULTICENTER PHASE 2 STUDY (KMM-94)-INTERIM ANALYSIS

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Background. Induction treatment followed by autologous stem cell transplantation (ASCT) is the standard therapy for the newly diagnosed

Table 1.

	Total N=83	VAD group N=33	PAD group N=50
Age (median)	56	55.5	57
M:F	42:41	14:18	22:28
Protocol off	19	4	9
Light chain disease	17	7	10
D-S stage IA/IIA/IIB	6/21/5	1/11/3	4/8/2
IIIA/IIIB	36/15	15/2	18/10
ISS I/II/III	15/41/27	8/15/9	7/19/16
Chromosome abN.	24/75 (32%)	13/31 (42%)	11/56 (31%)
FISH-del (13)	13/41 (32%)	7/18 (39%)	6/20 (30%)
FISH-del (17)	3/41 (7%)	3/17 (18%)	0/21 (0%)
FISH-t(4;14)	7/38 (18%)	3/17 (18%)	4/19 (21%)
FISH-t(14;16)	4/32 (13%)	2/15 (13%)	2/16 (13%)
Evaluable population	64	28	33
Response to HD dexa			
CR/near CR/PR	0/2/26	0/2/26	0/0/0
MR/NC/PD	15/15/10	0/0/0	15/6/9
Not assessable	1	0	0
Complete VAD/PAD Tx	42	20	22
Response to VAD/PAD			
CR/near CR/PR	5/6/23 (81%)	3/1/11 (75%)	2/5/12 (86%)
MR/NC/PD	3/2/2	1/2/2	2/0/0
Not assessable	1	0	1
Number of ASCT	29	16	13
Death	7	1	4

younger patients with multiple myeloma (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 and the response adopted PAD (Bortezomib, Adriamycin, Dexamethasone) or VAD (Vincristine, Adriamycin, Dexamethasone) induction chemotherapy in the newly diagnosed younger patients with MM. *Methods.* 83 newly diagnosed patients with MM from 20 institutions received 2nd cycles of high dose dexamethasone 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 83 patents enrolled this study from November 2009, 19 patients (23%) 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.* Eighty three patients (41 male, 42 female) were enrolled (median age; 56). 17 (21%) light chain disease were included. 26 (31%) patients were D-S stage II and 51 (62%) were stage III. According to the ISS, 15 (18%) patients had stage I, 41 (49%) had stage II and 27 (33%) had stage III. 24 (29%) patients had abnormal cytogenetics. There were 32% del13, 7% del17, 18% t(4;14), 13% t(14;16) and 27% t(11;14) in FISH analysis. Among the 64 evaluable patients, CR + PR rate was 44% (28/64) after 2nd cycles of high dose dexamethasone therapy. 28 patients (44%) received subsequent VAD chemotherapy and 33 patients (52%) received PAD chemotherapy. Among the 42 patients finished VAD or PAD chemotherapy, CR + PR rate was 81% (34/42). 29 patients were finished ASCT until now. 11% (7/64) treatment related deaths were observed. The cause of death was disease progression (n=3) and infections (n=4). *Conclusion.* The short course of high dose dexamethasone and the response adopted PAD or VAD induction chemotherapy followed by ASCT showed acceptable response rates and acceptable toxicities. We will report update results of this trial.

0877

EFFICACY AND SAFETY OF BORTEZOMIB IN PREVIOUSLY TREATED PATIENTS WITH MULTIPLE MYELOMA IN ROUTINE CLINICAL PRACTICE: SLOVAK EXPERIENCE

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Background. Bortezomib is the first proteasome inhibitor approved for the treatment of patients with relapsed/refractory multiple myeloma (R/R MM). Since April 2005 bortezomib is reimbursed for the treatment of patients with multiple myeloma (MM) in relapse settings in Slovakia. *Aims.* This large, retrospective, non-interventional, single-center analysis was conducted to evaluate bortezomib efficacy and safety in patients with R/R MM in routine clinical practice. *Methods.* 169 patients with MM who received bortezomib treatment after at least one prior therapy and have demonstrated disease progression at the biggest Slovakian myeloma center were evaluated in this study. The starting dose was 1,3 mg/m2 twice or once weekly, lenght

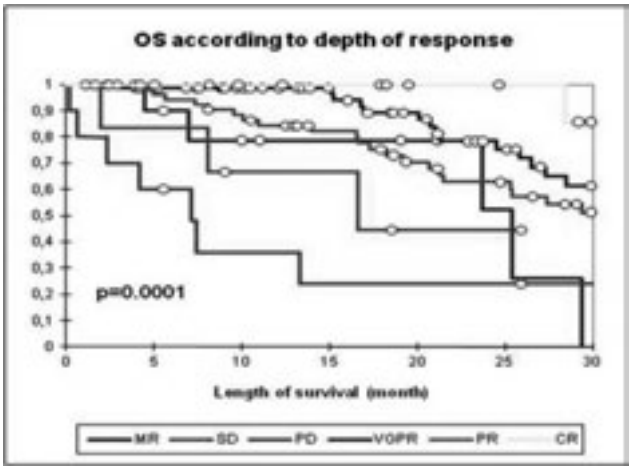

Figure 1.

of cycle 21 or 35 days, maximum 8 cycles. Bortezomib was administered as monotherapy, 2-drug (VD, bortezomib + dexamethasone) or 3-drug (BDD, bortezomib + dexamethasone + doxorubicine, VMP, bortezomib + melphalan + prednisone, VCD, bortezomib + cyclophosphamide + dexamethasone) combination. *Results.* Demographic and baseline characteristics: median age: years (range) 67 (35 - 84); male sex: n (%) 68 (40); ISS I: n (%) 18 (11); ISS II: 112 (66); ISS III: 39 (23); one prior therapy: n (%) 104 (62); two prior therapies: 48 (28); three and more prior therapies: 17 (10). Treatment exposure: median treatment cycles (range): 6 (1 - 8); bortezomib monotherapy: n (%) 19 (11); 2- drug combination: 38 (23); 3 - drug combination: 112 (66). Overall response of R/R MM patients to bortezomib: ORR (CR + VGPR + PR): n (%) 143 (85); CR: 15 (9); VGPR: 71 (42); PR: 57 (34); MR: 10 (6); SD: 6 (4); PD: 10 (6). Safety: all grades of peripheral neuropathy (PN): n (%) 79 (47); Gr 3: PN 9 (5); no Gr 4; all grades of thrombocytopenia: n (%) 20 (12); Gr 3: 3 (2); no Gr 4; GIT toxicity Gr 1,2: n (%) 20 (12), other adverse events Gr 3,4: n (%) 4 (2), for combination of all adverse events: no AE: n (%) 67 (40), Gr 1,2: 86 (51), Gr 3: 16 (9) Outcomes median (95% CI): TTP: 23,1 (19,8-25,6) months; OS: 34,7 (27,4-inf) months. Depth of response was in significant association with long-term outcome (p<0.0001), graph 1. There were no statistically significant differences in TTP and OS between monotherapy, 2 - drug and 3 - drug combinations, Long-rank test: p=0,49 (TTP) resp. p=0,80 (OS). *Conclusions.* R/R MM patients treated with bortezomib had high overall response rates and survival outcomes comparable with previously published results from phase 3 clinical studies. The safety profile was predictable, manageable and similar to experience in clinical trials. Bortezomib is the headstone of efficacy concerning combination MM treatment in routine clinical practice.

0878

KYPHOPLASTY AS FIRST LINE TREATMENT FOR VERTEBRAL LESIONS DUE TO MULTIPLE MYELOMA: A SINGLE CENTER EXPERIENCE

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Background. The complications of spinal bone disease in multiple myeloma (MM) include local pain and vertebral compression fractures (VCF). The treatment of MM vertebral lesions traditionally involves radiotherapy (RT) combined or not with bed rest, bracing and analgesics with or without chemotherapy. Recently new minimally invasive interventional methods such as Vertebroplasty and Balloon Kyphoplasty (BKP) are applying to treat the bone lesions. BKP as compared to Vertebroplasty is a more efficient method for restoration of lost vertebral height with lower incidence of cement leakage. *Aim.* To determine the safety and the efficacy of the BKP in patients with vertebral fractures secondary to MM. To verify the role of BPK as treatment of choice for vertebral bone lesions instead of the traditional treatment with local radiotherapy. *Methods.* Patients with MM and vertebral fractures or osteolytic lesions were treated with BKP the last 2 ½ years in our Department. Through minimal incisions, two special inflatable balloons were transpedicularly or extrapedicularly inserted and inflated in each treated VB. After appropriate inflation of the balloons a certain degree

Table 1. Comparison of BKP with RT.

	BKP	RT
Pain control	90%	80-85%
Biomechanical stability	+++	-
Fracture reposition	+++	-
Onset of the analgesic effect	Immediate	After 10-15 d
Osteonecrosis	-	++
Refracture (same vertebra)	-	+
Neurological complications	±	+

of reposition of the VCF was obtained. Consequently a proper amount of PMMA (polymethylmethacrylat) cement was injected in every VB. Deformity correction was evaluated using standard calculation formula based on the fractured vertical height pre and post operatively. Pain degree was estimated before and after the procedure using the VAS score. *Results.* 17 patients (4 male, 13 female, median age 67, range 45-79) with a total of 43 vertebral fractures or significant osteolytic lesions were treated. All 17 pts tolerated the procedure well. Postoperatively, all pts were admitted to the one-day clinic and all of them were discharged ambulatory the following morning. The deformity correction was 90% for the 23% of the VCF, 60-89% for the 61% and 30-59% for the rest of the reducable fractures. Six months later the correction was stable. For a median follow up time of 18 months (range 4-30) there were no early or late complications related to the technique as cement extravasation, ARDS, relapse of the MM in the same VB or even refracture. All patients experienced immediate and stable pain relief (median VAS pro-post: 6-1) and improvement in the quality of life. No patient was in need of additional local radiation therapy in the reconstructed vertebrae. The comparison of BKP in our patients vs RT (historical data) are presented on table 1. *Conclusions.* Balloon Kyphoplasty is an effective and safe minimal invasive surgical procedure. The correction of the kyphosis by BKP results in significant biomechanical advantage. Rapid and significant relief of pain is achievable with BKP. BKP could substitute local radiotherapy as treatment of choice for vertebral lesions. Absence of local relapse may reflect a possible control of the disease by mechanical or functional intervention.

0879

NEUROLOGICAL FINDINGS IN A SERIES OF PATIENTS WITH MONOCLONAL PARAPROTEINEMIA WITH OR WITHOUT ADMINISTRATION OF NEUROTOXIC DRUGS

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Background. A number of studies have demonstrated an association between monoclonal paraproteinemia and peripheral neuropathy (PN). Besides, many chemotherapeutic agents that mostly used in hematologic malignancies, especially in multiple myeloma, such as vincristine, thalidomide, and more recently bortezomib, induce peripheral neuropathy of variable severity. *Aim.* To evaluate the neurological status of patients with monoclonal paraproteinemia at diagnosis as well as after neurotoxic drug administration. *Methods.* Neurological examination was performed in 65 patients. 57 patients with relapsed/refractory multiple myeloma(MM) and 8 patients with Waldenstrom macroglobulinemia (WM). 23 MM patients were evaluated before bortezomib administration(6 patients were treatment naïve, 9 had received a prior line of chemotherapy with VAD regimen and 8 had received at least 2 prior lines of chemotherapy including thalidomide) and 34 were evaluated at different time points during bortezomib therapy while all MW pts were evaluated at the time of diagnosis. The neurological status was obtained from a questionnaire, examination by the neurologist and neurophysiologic examination at one concrete laboratory center. The method used to grade the neuropathy was the Total Neuropathy Score (TNSr). Electroneurophysiological study(ENG) included sensory nerve conduction of sural nerve and motor nerve conduction of the common peroneal nerve assessment. Motor and sensory nerve conduction studies were performed by standardized equipment and techniques. *Results.* Before Bortezomib administration, 4 out of 6 treatment naive MM patients had laboratory findings consistent with sensory neuropathy without any clinical symptoms.11 out of 17 patients who had received prior chemotherapy presented peripheral neuropathy (4 had received thalidomide and 7 the VAD regimen). Of note was the fact that 3/4 patients with prior thalidomide exposure presented sensorymotor PN. 10 out of 15 (66%) had laboratory findings of demyelination and 4/15 (27%) of axonal damage. 30 MM patients (79%) evaluated after Bortezomib initiation (median time to evaluation 3 months) presented treatment related neuropathy characterized mainly by numbness, paraesthesias, burnings and pain mainly in the lower extremities. In details 7/30 (23,3%) experienced sensory neuropathy, 1/30 (3,3%) motor neuropathy and 22/30 (73,3%) sensory motor neuropathy. 5/30 (16,6%) presented demyelinating neuropathy while 22/30 (73,3%) axonal and 3 (10%) demyelinating with secondary axonal degeneration. 3 out of 8 patients with WM(37,5%) presented peripheral neuropathy characterized by sensory symptoms, gait disorders, reduced or absent tendon reflexes, loss of vibration and pin sensibility with predominance to the lower extremities. ENG evaluation revealed findings that were consistent with a sensory motor neuropathy of demyelinating type. *Conclusions.* ENG study in MM patients is consistent with axonal degeneration neuropathy while in WM patients with primarily demyelinating sensory motor neuropathy. Bortezomib induced peripheral neuropathy is a sensory motor neuropathy of axonal type with predominance to the lower extremities, including mainly serious sensory symptoms. Preexisting peripheral neuropathy in MM patients before Bortezomib administration is common and should be taken into account. -Baseline neurological evaluation in patients with monoclonal paraproteinemia is important allowing early detection of peripheral neuropathy.

0880

LENALIDOMIDE PLUS MELPHALAN AND PREDNISONE FOLLOWED BY LENALIDOMIDE MAINTENANCE PROVIDES FAVOURABLE EFFICACY AND HEALTH-RELATED QUALITY-OF-LIFE IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS =65 YEARS

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Background. Lenalidomide maintenance following induction with melphalan, prednisone, and lenalidomide has been shown to provide sustained disease control by prolonging progression-free survival and decreasing relative risk of progression in patients with newly diagnosed multiple myeloma (NDMM) [Palumbo, 2010]. Alongside efficacy enhancements in cancer treatment, health-related quality-of-life (HRQoL) is an increasingly important determinant for choice of therapy [Osoba, 1999]. Recent findings on novel treatment of NDMM have shown efficacy of melphalan, prednisone, and bortezomib (VMP) treatment to be associated with an intermittent deterioration in patients' HRQoL [Dhawan, 2009]. *Aims.* HRQoL data were assessed from an ongoing prospective, randomized phase 3 trial designed to evaluate the efficacy and safety of continuous lenalidomide treatment (MPR-R: melphalan, prednisone, and lenalidomide induction for 9 cycles followed by lenalidomide maintenance until disease progression) vs a fixed 9 cycle duration of melphalan and prednisone (MP) or melphalan, prednisone, and lenalidomide (MPR) in transplant ineligible patients ≥65 years old with NDMM. *Methods.* Data up to Cycle 16 with the cut-off date of 11MAY2010 were analysed. Patient-reported HRQoL data from

Table 1.

Observed Minimal Important Differences (MIDs) from Baseline[a]

HRQoL Domain (scale: points from 0 -100)	Minimal Important Difference (MID)[b]	Assessment Time Points with Patients On Average Achieving MID, Cycle		
		MPR-R	MPR	MP
Global Quality-of-Life	+7	Cycles 7,10,13,16	Cycles 7,10,13	Cycle 16
Physical Functioning	+9	Cycles 10,13,16	Cycle 13	None observed
Fatigue	-10	Cycle 16	Cycle 13	None observed
Pain	-12	Cycles 4,7,10,13,16	Cycles 4,7,10,13	Cycles 4,13,16
Disease Symptoms	-10	Cycle 16	None observed	None observed
Side Effects of Treatment	-6	None observed	None observed	None observed

[a]Data from patients who discontinued from the study were carried forward to the next planned time assessment time point.
[b]MID, Minimal Important Difference; threshold for clinically meaningful score changes from baseline are based on domain-specific standard error measurements (SEM). Positive values denote improvements for Global Quality-of-Life and Physical Functioning. Negative values denote improvements for Pain, Fatigue, Disease Symptoms and Side Effects of Treatment.

EORTC QLQ-C30 and QLQ-MY20 were collected at baseline, at the beginning of every 3rd cycle, and at study discontinuation. Five HRQoL measurement time points subsequent to baseline were assessed. Six out of 19 HRQoL domains were preselected based on clinical relevance: Global Quality-of-Life, Physical Functioning, Fatigue and Pain from QLQ-C30 and Disease Symptoms and Side Effects of Treatment from QLQ-MY20. Clinically meaningful HRQoL improvements from baseline were assessed for individual treatment arms and classified as exceeding minimal important differences (MIDs). MIDs were determined through domain-specific standard error measurement (SEM) whereby positive values denote improvements in Global Quality-of-Life and Physical Functioning while negative values reflect improvements in Pain, Fatigue, Disease Symptoms and Side Effects of Treatment. Within group statistical significance tests ($p \leq 0.05$) were also conducted. HRQoL observations at discontinuation were carried forward to the next planned measurement time point; data with separate HRQoL measurements at discontinuation were also evaluated. *Results.* Data from all 459 randomized patients were evaluated. Clinically meaningful improvements from baseline were more frequently observed in patients receiving MPR-R than those receiving MP. HRQoL improvements were observed as early as Cycle 4. At Cycle 16, MID was achieved in 5 out of 6 domains (Global Quality-of-Life, Physical Functioning, Fatigue, Pain, and Disease Symptoms) in patients receiving MPR-R while only 2 out of 6 domains (Global Quality-of-Life and Pain) achieved MID in the MP arm. MID improvements were not reported in the MPR arm at Cycle 16 (Table). When compared to baseline, all 48 lenalidomide-related observations (6 induction-related measurements at cycles 4, 7 and 10 for MPR and MPR-R patients plus 2 additional measurements at cycles 13 and 16 for MPR-R patients during lenalidomide maintenance) at a minimum showed preservation of HRQoL across the six domains. *Conclusions.* Lenalidomide is generally well tolerated and when taken continuously, it prolongs progression-free survival in patients with NDMM. MPR-R elicited frequent and often sustained clinically meaningful improvements in patient reported HRQoL from baseline, thus showing a favourable balance between efficacy and HRQoL.

0881

HEALTH RELATED QUALITY OF LIFE INSTRUMENTS FOR USE IN PEOPLE WITH MULTIPLE MYELOMA: A SYSTEMATIC LITERATURE REVIEW

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Background. Recent treatment advances in multiple myeloma have improved survival, although a cure remains elusive. Alongside improved survival emerges a need to better understand health related quality of life (HRQOL) across all disease stages and into survivorship. Yet few trials measure HRQOL in myeloma, and there is no consensus regarding the best instruments to use. We conducted two parallel systematic reviews to help address these issues. *Aims.* Review 1: To identify multidimensional HRQOL instruments for use in people with multiple myeloma, and describe their measurement properties. Review 2: To determine the domains of quality of life important to people with multiple myeloma. *Methods.* We conducted systematic literatures reviews searching MEDLINE, PsycINFO, EMBASE, CINAHL, BNI and AMED databases. Search terms were Myeloma OR Haematological Cancer OR Bone Marrow Transplant OR Palliative Care AND Quality of Life OR Patient Reported Outcomes OR Psychometrics OR Test Validity (and synonyms). We ran database searches on 28/9/10 with no limits by date or language, and supplemented these with manual searching of key journals and reference / citation searching of all included articles and relevant review articles. Review 1: Inclusion criteria were 1) any study developing, validating or using a multidimensional HRQOL instrument AND reported some appraisal of that instrument; 2) any study with myeloma specified in the published sample, including mixed haematological, cancer or palliative samples; and 3) studies with all participants over 18 years old at diagnosis. We extracted any psychometric or other appraisal of the HRQOL instruments, including predictive or prognostic properties. Review 2: Inclusion criteria were 1) any study identifying the domains of quality of life important to people with myeloma using methods such as (but not limited to) surveys, focus groups or interviews; 2) studies with samples entirely of myeloma patients; and 3) studies with all participants over 18 years old at diagnosis. *Results.* The database searches indentified 10,650 references. Review 1: 26 studies met the inclusion criteria, containing 10 different HRQOL instruments. Some instruments appeared only once, with the most frequent and extensively validated being the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30. We report the measurement properties of all included instruments. Review 2: 3 additional studies met the inclusion criteria for Review 2. This highlights the relative lack of published research exploring the issues important to quality of life in this group. *Discussion*. Myeloma patients can often fall within mixed samples used to validate treatment specific instruments (e.g. for use in bone marrow transplant), or 'area' specific instruments (e.g. for use in cancer or palliative groups). Disease specific instruments may not be appropriate in settings such as palliative care, where validation of existing palliative instruments in myeloma patients may be more appropriate. *Summary/Conclusion.* There is a need for more research exploring the domains of HRQOL important to people with myeloma. The lack of such research has implications for the validity of existing HRQOL instruments in this group.

0882

MULTIPLE MYELOMA PATIENT'S PERCEPTION OF THE QUALITY OF CARE IN ITALY: AN EXPLORATORY SURVEY

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Background. Patient satisfaction is an indicator of quality of care and its assessment provides feed-back to clinicians and to services. Thus it may stimulate improvement initiatives. It is also considered as an outcome measure which allows to assess the superiority of one treatment, program of care, health care organisation or system over another. *Aims.* Deepening the knowledge on multiple myeloma patients' perception of their disease and on the quality of the treatments received. *Method.* A questionnaire, built on the results of a previous study (Pelagalli e al. 2010), contained 44 items, assessing: relationship with physicians; level of satisfaction with current treatments; quality of life; sources of information on the disease; experience of and reaction to the disease; relationship with other patients and family; life styles. The questionnaire was anonymous and self-reported by the patient (Oct-Nov 2010). The

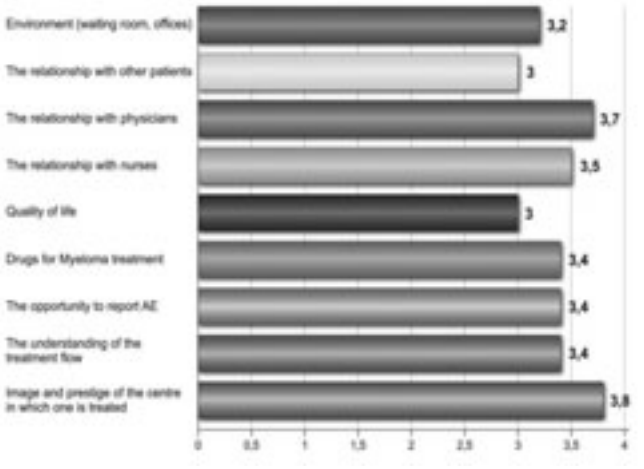

Figure 1.

sample consisted of 482 patients, aged 18"'65 years, treated in 11 Italian Centres, 49% men and 51% women, diagnosed with myeloma between 1982 and 2010. *Results.* Here, we highlight the results on satisfaction for the different components of the 'patient care' process. The questionnaire included a question on the level of satisfaction on 9 elements of the 'patient care' process (environment, relationship with other patients, with physicians, and with nurses, quality of life, drugs, possibility of reporting adverse events, understanding of the treatment flow, image and prestige of the Centre in which the patient is treated). For each item the patient could answer on a 4 point scale (from 1 "not at all satisfied"to 4 "completely satisfied"). The mean score for each item is shown in figure 1. Results highlight an excellent opinion of the Centre in which the patient is treated (mean score 3,8) and satisfaction relies on the relationship with the physician (mean score 3,7). By contrast, satisfaction is lower in the perception of the quality of life (mean score 3) and in the relationship with other patients (mean score 3). Though differences in disease phase among the various treatment groups existed, the survey reported a mean satisfaction score of 3,4 for "drugs for myeloma treatment"and suggested a higher level of satisfaction for oral drugs (thalidomide 3,3; lenalidomide 3,6) rather than intravenous drugs (bortezomib 3,1; chemotherapy 3,2). Comparing "oral"therapies (thal. e len.) vs "intravenous" therapies (bort. e chem.) mean scores, the t-test shows a higher appreciation for oral drugs (p=.0011). *Conclusions.* Patient satisfaction appears to be mainly determined by the quality of the relationship with the physician and with the Centre. Differences on perceived satisfaction between oral and intravenous treatments were also shown. Further studies are needed to confirm these preliminary results.

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0883

COST-EFFECTIVENESS OF ZOLEDRONIC ACID VERSUS CLODRONIC ACID AND PAMIDRONIC ACID IN PATIENTS WITH MULTIPLE MYELOMA FROM A CANADIAN HEALTHCARE SYSTEM PERSPECTIVE

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Background. The Medical Research Council (MRC) Myeloma IX study demonstrated that the intravenous (IV) bisphosphonate, zoledronic acid (ZOL) 4 mg q 3-4 wk improves overall survival (OS) and progression-free survival (PFS) and reduces the incidence of skeletal related events (SREs) compared with the oral bisphosphonate, clodronic acid (CLO) 1,600 mg/d PO, in patients with newly-diagnosed multiple myeloma (MM) in addition to chemotherapy (CT). Previous analyses have demonstrated the cost-effectiveness of ZOL vs. CLO in this setting. However, in many countries, pamidronic acid (PAM) is the usual alternative to ZOL. *Aims.* To evaluate the cost-effectiveness of ZOL vs. CLO and ZOL vs. PAM in patients with newly-diagnosed MM. *Methods.* An economic model was used to project PFS, OS, the incidence of SREs and adverse events (ARF, ONF, thromboembolism, and infection) as well as expected lifetime healthcare costs for patients with newly-diagnosed MM who are alternatively assumed to received ZOL 4 mg q 3-4 wk, CLO 1,600 mg/d PO, or PAM 90 mg IV q 4 wk x 9 cycles, in addition to CT. Cost-effectiveness was expressed in terms of incremental cost per quality-adjusted life-years (QALYs) gained with ZOL vs. CLO and ZOL vs. PAM. Estimates of OS, PFS, SREs, and incidence of AEs for ZOL and CLO were based on data from the MRC Myeloma IX trial. Hazard ratios (HRs) for SREs and AEs for PAM vs. ZOL were from the Phase III trial of ZOL vs. PAM. HRs for PFS and OS for PAM vs. CLO were from an adjusted indirect comparison of controlled trials of PAM and CLO vs. no bisphosphonate therapy. AEs with PAM were assumed the same as with ZOL. Costs (2009/10 Canadian dollars) and utility values were from published sources. Costs and QALYs were discounted at 5% annually. *Results.* Expected lifetime costs of bisphosphonate therapy (including administration and monitoring costs) were estimated to increase by $13,026 with ZOL vs. CLO ($15,530 vs. $2,504) and by $13,436 with ZOL vs. PAM. Expected costs of SREs were projected to be reduced by $720 with ZOL vs. CLO ($4,152 vs. $4,872) and by $324 with ZOL vs. PAM. Expected total lifetime costs were estimated to increase by $12,923 with ZOL vs. CLO ($32,088 vs. $19,165) and by $2,397 with ZOL vs. PAM. Life expectancy (undiscounted) was increased by 0.83 years with ZOL vs. CLO (6.43 vs. 5.60) and by 0.65 with ZOL vs. PAM. On a discounted basis, QALYs gained were 0.37 with ZOL vs. CLO (3.57 vs. 3.20) and 0.28 with ZOL vs. PAM. Cost per QALY gained was $34,848 with ZOL vs. CLO and $44,272 with ZOL vs. PAM. Results were sensitive to methods used to estimate PFS and OS and the estimated HR for OS with PAM vs. CLO. *Conclusions.* In patients with newly-diagnosed MM, the cost-effectiveness of ZOL vs. CLO and ZOL vs. PAM is substantially below the generally-accepted threshold of $100,000 per QALY gained in Canada. Given this threshold, ZOL should be the preferred bisphosphonate treatment for patients with newly-diagnosed MM in Canada.

0884

IMPROVED PROGRESSION-FREE AND OVERALL SURVIVAL WITH THALIDOMIDE MAINTENANCE THERAPY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA: A META-ANALYIS OF FIVE RANDOMIZED TRIALS

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Background. In two randomized studies, lenalidomide maintenance therapy after autologous stem cell transplantation (autoSCT) improved progression-free survival (PFS) but not yet overall survival (OS) in patients with multiple myeloma (MM). For thalidomide, more data are available and meta-analyses have been performed but the effect of maintenance therapy after autoSCT on OS remains unclear (Hicks *et al.* Cancer Treat Rev 2008, Hahn-Ast *et al.* EHA 2010). Recently, new studies or updates of previous data were published. *Aims.* We performed a new meta-analysis to evaluate the influence of thalidomide maintenance on OS in patients with MM after autoSCT. In addition, we also analysed PFS and toxicity. *Methods.* PubMed, the Cochrane Library and conference proceedings from ASH, ASCO, IMW, and EHA were searched using the headings "myeloma"and "thalidomide", lastly in February 2011. Studies were included if they were randomized controlled trials (RCTs) of patients with MM receiving thalidomide maintenance treatment as monotherapy or combination therapy. Data were pooled using the random effects model. Measures of treatment effect were hazard ratios (HR) for survival data and relative risk (RR) for toxicity. When not available from the articles, HRs were estimated using the methods of Parmar *et al.* (Statist Med 1998). *Results.* Five RCTs of thalidomide maintenance therapy after autoSCT reporting survival data were identified (4 published as full papers, one in abstract form). The trials included 2069 patients and compared thalidomide as monotherapy (one trial) or combination therapy (corticosteroids two trials, corticosteroids + interferon one trial, and pamidronate one trial) with interferon (one trial), interferon + dexamethasone (one trial), prednisolone (one trial) or no maintenance (two trials). Dose of thalidomide ranged between 50 and 400 mg/d, but 400mg/d were rarely tolerated.

All trials included only patients with newly diagnosed MM. In two trials thalidomide was also part of the induction regimen. PFS was significantly improved with maintenance thalidomide (HR 0.64, 95%CI 0.55-0.75, p<0.001). The effect was similar in trials with or without prior thalidomide-containing induction treatment. Interestingly, OS was also improved with maintenance thalidomide (HR 0.73, 95%CI 0.60-0.89, p=0.002). In the subgroup of trials with prior thalidomide induction, the effect also reached significance (HR 0.83, 95%CI 0.69-0.99, p=0.04). No significant heterogeneity among all RCTs existed between PFS or OS HRs (I^2=51%, p=0.09 and I^2=34%, p=0.20 respectively). Toxicity was not significantly different between thalidomide and comparators except for grade 3/4 neuropathy (reported in two trials), which was worse with thalidomide (RR 6.97, 95%CI 1.44-33.78, p=0.02). The rate of thromboembolic events (TE) grade 3/4 was reported in four trials. No significant difference was detected, although there was a trend to more thromboembolic events in the thalidomide arm (RR 2.01, 95%CI 0.96-4.23, p=0.07). *Conclusion.* In our meta-analysis we found an improved PFS and for the first time also an improved OS for thalidomide maintenance therapy after autoSCT in patients with MM. This effect was accompanied by a higher rate of grade 3/4 neuropathy whereas the rate of TE was not significantly increased.

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Myeloma and other monoclonal gammopathies - Clinical 2

0885

IG'KAPPA/ IG'LAMBDA MEASUREMENTS IMPROVE DISEASE MONITORING AND IDENTIFY MINIMAL RESIDUAL DISEASE IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Currently monoclonal protein measurements are not used to prognosticate multiple myeloma (MM) patients; but they are important tools to assess responses to therapy. Traditional electrophoresis techniques (serum protein electrophoresis (SPE), capillary zone electrophoreses (CZE) and immunofication (IFE) are commonly used but the results generated can be inaccurate depending of the co-migration of the monoclonal protein with other serum proteins. Polyclonal antibodies recognising junctional epitopes between immunoglobulin light chains and their heavy chain partners (heavy/light chain [HLC]) have been developed. *Aim.* To assess the utility of HLC measurements to identify, prognosticate and monitor MM patients. *Methods.* HLC pairs (IgA κ and IgA λ; IgG κ and IgG λ) were retrospectively assessed in serial sera samples from 103 (33 IgGκ / 18 IgGλ and 31 IgAκ / 21 IgAλ) MM patients and results compared to historic markers of disease. *Results.* Patient characteristics were: median age 67 (range 32-94), 37 stage 1, 42 stage 2, 26 stage 3, median follow up was 27 months (0-158) with a median overall survival of 46 months (range 27-66). At presentation HLC ratios (HLCr) were abnormal in all 103 patients. In 36/103 patients (4 IgG and 32 IgA) MM patients with M-protein was not accurately quantifiable by CZE or SPE. Multivariate Cox regression analysis identified β2-M (p=0.01), LDH (p=0.0004) and HLCr (p=0.049) as independent prognostic markers. A risk stratification model based on β2M>3.5mg/L and abnormal HLCr (<0.025 or >40) identified patients with 0, 1, or 2 risk factors and was associated with OS (median survival 131.2 v 53.6 v 29.2 months respectively; p=0.01). Throughout monitoring there was good correlation between percentage change in SPE and percentage change in involved HLC (Pearsons' correlation: IgA= 0.98 [p=0.00001]; IgG=0.9 [p=0.00001]). Following treatment, 13/103 patients (4 IgG /9 IgA) achieved complete response (CR). HLCr remained abnormal in 4/13 patients who achieved CR. In 2/13 patients relapse of the disease was identified by HLCr before IFE (range 71-112 days), suggesting HLCr correctly identified residual disease that is undetectable by IFE. Interestingly, different responses were seen between FLC and HLC measurements in 3/103 patients: 1/3 patients relapsed with a FLC producing clone that was not identified using either IFE or HLC; in 2/3 patients FLC levels and ratios normalised at maximum response. In both cases the patients relapse was characterised by a clone producing only intact immunoglobulin. *Conclusion.* HLCr can detect hard to quantify IgA and IgG and low levels of both IgA and IgG paraproteins, indicate persistent disease in IF negative patients, depict relapse earlier than IFE, and can provide prognostic information.

0886

THE COMBINATION OF THE PROTEASOME INHIBITOR BORTEZOMIB WITH DOXORUBICIN AND DEXAMETHASONE (PAD REGIMEN) IS EFFECTIVE IN REVERSING RENAL IMPAIRMENT IN NEWLY-DIAGNOSED HIGH-RISK MULTIPLE MYELOMA

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Renal impairment (RI) due to light-chain induced nephropathy is a common presenting feature of multiple myeloma (MM), and is associated with increased morbidity and inferior survival. Reversion of RI is therefore essential for the management of MM. Among novel agents, bortezomib has shown promising efficacy in this context due to its an-

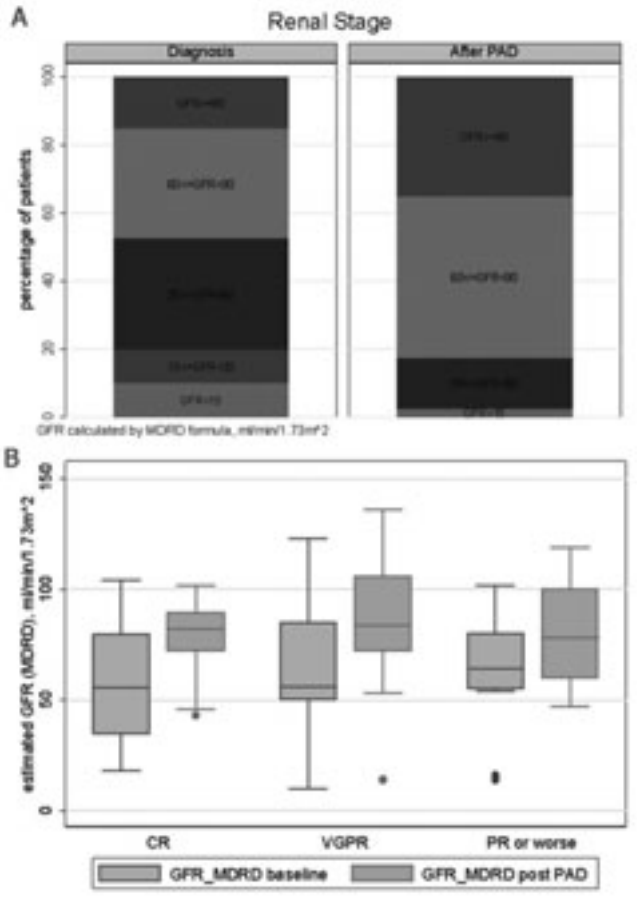

Figure 1. A. Renal stage change. B. GFR vs myeloma response.

timyeloma and anti-inflammatory effects. We examined the impact of treatment on renal function in a group of patients with high-risk MM, who received upfront therapy with a bortezomib-based regimen in a prospective phase II study. The study group consisted of 40 patients, aged 41-70 (median, 59) years, with newly diagnosed MM with high-risk features (ISS stage II/III by serum albumin and beta2-microglobulin, and/or detection of 13q deletion by karyotype or FISH). The treatment protocol included 4 cycles of the PAD regimen, i.e. the combination of bortezomib (1.3 mg/m^2 on days 1, 4, 8, and 11), doxorubicin (9 mg/m^2 on days 1-4), and dexamethasone (40 mg on days 1-4 and 8-11), administered every 21 days. Tumor response was assessed at the end of induction with PAD by the International Myeloma Working Group (IMWG) uniform response criteria (2006). Renal function was assessed at diagnosis and at the end of treatment with PAD by the estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) study equation. Stage of RI was classified from 1 to 5 for eGFR values of ≥90, 60-89, 30-59, 15-29, and <15 ml/min/1.73 m^2, respectively. For the subgroup of patients with eGFR <50 at diagnosis, renal response to antimyeloma therapy was defined as complete (CRrenal), partial (PRrenal), or minor (MRrenal) according to the IMWG criteria (2010). All patients completed the 4 cycles of PAD, with the exception of one who died of pneumonia during the 2nd cycle. The overall myeloma response rate was 95%. Complete or very good partial remission (CR+VGPR) was achieved in 27/39 (69.2%), and partial remission (PR) in 10/39 (25.5%). At diagnosis, RI was mild (eGFR, 60-89) in 13/40, moderate (eGFR, 30-59) in 13/40, severe (eGFR, 15-29) in 4/40, and end-stage (eGFR <15) in 4/40 patients. ISS stage III, lambda light chain isotype, and high serum concentration of the involved free light chain were independent risk factors for higher stage of RI (p=0.018, 0.012, and 0.028, respectively). After the 4 cycles of PAD, a significant increase in eGFR was observed in comparison with baseline eGFR (median, 83 vs. 59 ml/min/1.73 m^2, respectively; p<10^{-5}), and an improvement in the stage of RI was documented in 25/39 (64%) patients. Improvement in renal function was seen irrespective of the type of myeloma response (CR vs. VGPR vs. PR or worse, p=0.79). Among the 11 patients with pre-treatment eGFR <50, CRrenal was achieved in 4 (36%), PRrenal in 2 (18%), and MRrenal in 2 (18%). In conclusion, induction with bortezomib in combination to doxorubicin and dexamethasone resulted in overall improvement in renal function in patients with high-risk MM. Moreover, meaningful renal responses were achieved in more than half of patients with moderate to severe RI.

0887

TRENDS AND OUTCOMES OF MODERN STAGING OF SOLITARY PLASMACYTOMA OF BONE

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Background. Solitary plasmacytoma of bone (SPB) is a rare localized plasma cell dyscrasia (PCD) with no evidence of systemic disease. The majority of patients with SPB progress to multiple myeloma with a median disease free survival of five to ten years. *Aims.* Our hypothesis proposed that the low rate of cure of SPB is in part due to inadequate clinical staging, i.e. occult bone or bone marrow involvement. *Method.* A retrospective analysis of 127 Mayo Clinic patients with a diagnosis of SPB seen between 2000 and 2010 was performed. The trends in imaging techniques (magnetic resonance imaging, computed tomography, and nuclear medicine imaging), immunohistochemisty and flow cytometry were evaluated. Inclusion criteria were 1) patients with biopsy proven plasmacytoma; 2) patients with no evidence of systemic disease on skeletal survey; and 3) patients with fewer than 10% plasma cells on bone marrow biopsy. Patients with any form of systemic disease defined as anemia, hypercalcemia or renal injury related to plasma cell dyscrasias were excluded. Overall survival was calculated using the Kaplan-Meier method. *Results.* There was significant increase in the use of CT-PET (p=0.002) and flow cytometry (p=0.005) for initial staging of SPB in the latter half of the decade. Overall survival (follow up months vs. proportion surviving) was significantly improved in the latter five years, p=0.05. *Conclusion.* These data suggest that more optimistic estimates of overall survival may be appropriate for patients with SPB in the modern era when more advanced staging and selective application of the term SPB is employed.

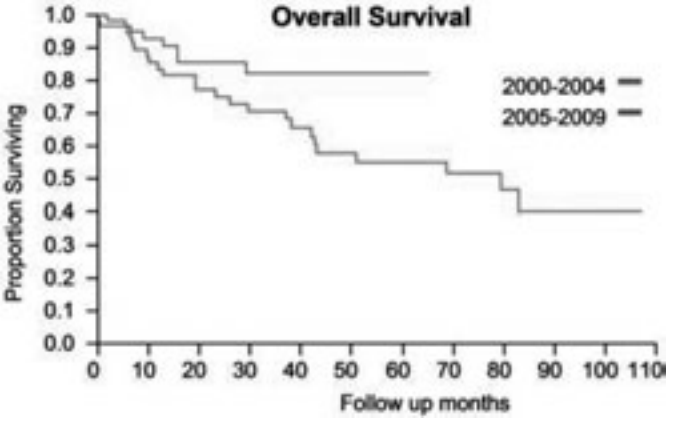

Figure 1.

0888

CIRCULATING PLASMA CELLS (CPCS) PREDICT THE OUTCOME OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RR MM)

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Background. Pretreatment detection of peripheral blood malignant CPCs by immuophenotyping has been shown to be of negative prognostic value in MM and related disorders. The number of CPCs tends to decrease in response to treatment. We hypothesized that assessment of CPC kinetics in response to one therapy cycle may be of prognostic significance and could be helpful in the early detection of MM refractoriness to treatment. *Aims.* The aims of our study were to assess the prognostic significance of pretreatment values of normal and aberrant CPC subsets as well as the prognostic significance of CPC kinetics in response to the first treatment cycle in RR

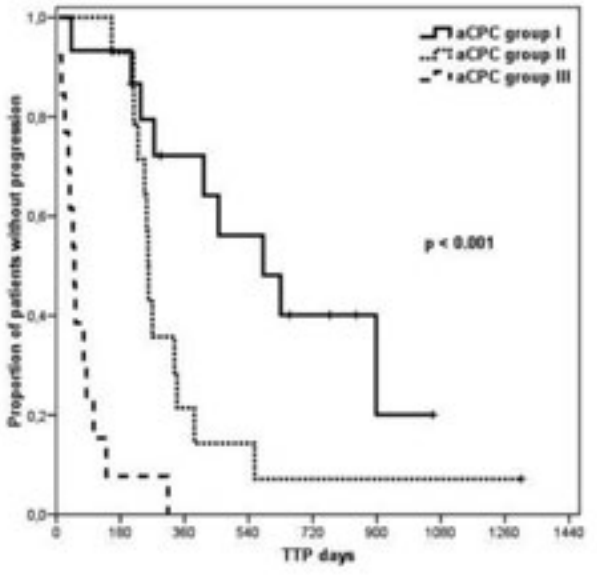

Figure 1.

MM. *Methods.* Patients were prospectively included if they had RR MM according to EBMT criteria after at least one prior line of therapy and were scheduled to receive either a Bortezomib containing regimen or VAD (vincristin, doxorubicin and dexamethasone). All patients provided informed consent. We used six-color flow cytometry to identify immunophenotypically normal (nCPC) and aberrant plasma cell (aCPC) subsets in peripheral blood (PB). Assays were performed with two tubes stained with antibody combinations: CD56/CD138/CD45/CD19/CD38/CD20 and cLambda/cKappa/CD138/CD19/CD38/CD56. Plasma cells were identified as normal (nCPCs) if they were CD138+/CD38+/CD19+/CD56-/normal kappa/lambda ratio/CD45 variable or aberrant (aCPCs) if they displayed CD138+/CD38+/CD19-/CD56+/-/abnormal kappa/lambda ratio/CD45 variable. We measured aCPC and nCPC subsets immediately before and then after one therapy cycle in RR MM patients. *Results.* 42 patients with refractory or relapsed (RR) MM were enrolled. 39 patients were treated with bortezomib containing regimen, three patients received VAD. After the median observation time of 21 month (1-49), patients with detectable pretreatment aCPCs had shorter time to progression (TTP) compared to patients with undetectable aCPCs (median 218 vs. 456 days, respectively (p=0.008)). Median TTP of 51 day and median overall survival (OS) of 308 days was shortest in patients whose aCPCs did not decrease after one therapy cycle compared to patients with decreasing (median TTP 258 days and OS 856 days) or undetectable (median TTP 581 days and OS 1006 days) aCPCs ($p < 0.001$ and $p = 0.007$ for TTP and OS, respectively). aCPC2/aCPC1 ratio of 0.8 could predict early progression with the sensitivity of 100% and the specificity of 93.8% in patients with detectable aCPC. *Conclusions.* Detection of aCPCs in PB before treatment may identify patients with more aggressive disease. Nonreduction of aCPCs in RR MM patients after the first cycle of therapy may be useful to identify resistant patients early who may be candidates for immediate switch to alternative therapy. Figure 1 Time to tumor progression in three aCPC kinetic groups: group I - patients with no detectable aCPCs in both pre and postchemotherapy samples, group II - patients with a decrease in aCPCs postchemotherapy as compared to aCPCs before chemotherapy, group III - patients with no change or increase in aCPCs postchemotherapy as compared to aCPCs before chemotherapy.

0889

PROGNOSIS OF MULTIPLE MYELOMA (MM) PATIENTS AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN THE LAST DECADE. COMPARISON OF TWO COHORTS WITH DIFFERENT INDUCTION TREATMENT APPROACHES

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Background. The incorporation of bortezomib and IMIDs to MM treatment has improved substantially improved pre-SCT overall and complete remission (CR) rates in candidates to SCT. However, post-SCT results have not dramatically improved and disease-free survival (DFS) is only marginally superior. In available randomized trials, overall survival (OS) appears to be the same regardless of the first line pre-SCT induction treatment administered. *Aims.* Compare the outcome in terms of survival of two cohorts of patients with newly diagnosed MM and treated with different induction strategies. *Patients and Methods.* Data of two cohorts of patients with a newly diagnosed MM, treated in a single centre and submitted to ASCT in first response were collected. The first cohort (C1: 1999-2005) received dexamethasone, alkylanting agents and anthracyclin-based induction before ASCT and bortezomib and/or IMIDs at relapse. The second cohort (C2: 2005-2009) received IMIDs and/or bortezomib first line and was submitted to ASCT in first response and were treated at relapse with the same or alternative drugs. All patients had at least one year follow-up after ASCT. Post-ASCT CR rates, time to progression (TTP), event-free survival (EFS), time to next treatment (TNT) and OS were determined for both cohorts to compare their outcomes. *Results.* Out of 141 potential ASCT candidates diagnosed during both periods, 88 received an ASCT after induction (N=49 in C1 and N=39 in C2). Their median age was 58 years (range 32-68). Both cohorts were comparable in terms of gender, age, type of myeloma and stage. Median time from diagnosis to ASCT was 34 weeks (range 14-80 weeks) without significant differences between cohorts. Post-SCT CR rates were 35% for C1 and 61% for C2 (p=0.025). During the first year post-ASCT 6 patients died due to toxicity or infection (12%), 3 relapsed (6%) and 1 died of unrelated causes among 49 patients at risk in C1, during the same period, 1 patient died due to progression (2%) among 39 patients at risk in C2. During the second year post-ASCT 1 patient died due to toxicity or infection (2%) and 3 relapsed (8%) among 38 patients at risk in C1, while during the same period, no events were recorded among 36 patients at risk in C2. During the first year after SCT, both the probability of death of any cause (p=0.012) and the probability of infectious or toxic death (p=0.025) were significantly higher for C1. Differences in outcome, after a median follow-up of 6 years for C1 and 3 years for C2, are reflected in the following table. The projected post-ASCT for C2 at 4 years was 83% (95%CI 68-98%). *Conclusion.* In our experience, patients with MM who received chemotherapy as first line induction presented an increased risk of death due to infections or toxicity during the first year post-ASCT compared with those receiving bortezomib or thalidomide first line. Post-ASCT CR rate and survival of patients were superior for the cohort of patients treated with bortezomib or thalidomide as pre-ASCT induction.

Table 1.

	Cohort 1		Cohort 2		
	Median (years)	95% CI	Median (years)	95% CI	p-value
TTP	2	1.5-2.7	3.4	1.8-5	0.243
EFS	1.8	1.1-2.4	3.4	1.8-5.1	0.049
TNT	1.9	1.3-2.4	3.6	2.3-4.9	0.034
OS	4.1	1.1-7	NA*	NA*	0.02
			*projected post-ASCT for C2 at 4 years was 83% (95%CI 68-98%).		

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0890

CARFILZOMIB PHARMACOKINETICS, SAFETY, AND ACTIVITY IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA AND RENAL DYSFUNCTION: FINAL RESULTS

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Background. Carfilzomib, a novel, highly selective epoxyketone proteasome inhibitor, has demonstrated single-agent activity in patients

Table 1.

Linear Regression of PK Parameters as a Function of Creatinine Clearance

Parameter	n	Slope (95% CI)	p-Value for Slope
Carfilzomib clearance (L/h)	19	-0.607 (-2.129, 0.914)	0.4114
C_{max} (ng/mL)	27	0.225 (-0.242, 0.692)	0.3299
AUC_{last} (hr·ng/mL)	27	0.008 (-0.031, 0.048)	0.6635
AUC_{inf}	19	0.012 (-0.030, 0.054)	0.5496

C_{max}, peak plasma concentration; AUC_{last}, exposure based on first and last plasma measurements; AUC_{inf}, exposure extrapolated to infinity

with relapsed and/or refractory multiple myeloma (MM) with an acceptable safety and tolerability profile. Renal insufficiency, a common and often progressive complication of MM, is known to impact the pharmacokinetic (PK) behavior of multiple drugs, potentially affecting tolerability and toxicity and necessitating dosing modification. *Aims.* This study examined the impact of renal dysfunction on the PK parameters, safety, and activity of carfilzomib in patients with relapsed and/or refractory MM. *Methods.* Patients with relapsed, refractory, and/or progressing MM, treated with ≥2 prior regimens, were eligible to participate in this phase 2 open-label, multicenter study. Creatinine clearance (CrCl) was used to categorize normal (CrCl >80 mL/min), mild (CrCl 50-80 mL/min), moderate (30-49 mL/min), and severe (<30 mL/min) renal dysfunction, including patients receiving hemodialysis. Carfilzomib was administered by slow intravenous push (≤10 min) on days 1, 2, 8, 9, 15, and 16 of a 28-day cycle (15 mg/m^2 in cycle 1, 20 mg/m^2 in cycle 2, and 27 mg/m^2 in subsequent cycles, based on safety and tolerability). Patients achieving stable disease or better (IMWG criteria) after 2 cycles could continue to receive carfilzomib for a total of ≤12 cycles. Dexamethasone 40 mg/wk could be added to the regimen after cycle 3. The primary endpoint was the influence of renal function on carfilzomib. PK parameters of peak and total carfilzomib exposure were compared between patients with varying degrees of renal dysfunction. Adverse events (AEs) and serious AEs were monitored and graded by NCI CTCAE v3.0 standards. In the event of a reversible AE, carfilzomib dose could be reduced to the previous level. Best overall response (≥PR), response duration (DOR), and time to progression by IMWG criteria were also assessed. *Results.* At baseline, 12 patients had normal renal function, 30 had renal insufficiency of varying degrees (12 mild, 10 moderate, and 8 severe), and 8 were on chronic hemodialysis. As of November 2010, patients received a median of 21.5 doses of carfilzomib (4-72); with mean doses per administration consistent across groups (14.3-25.3 mg/m^2). Renal function did not appear to affect carfilzomib clearance, peak exposure, or total exposure, even with age/weight included as covariates (Table). There were no significant differences in PK parameters between cohorts. The frequency and grade of AEs did not differ among groups. The most common AEs (≥10%, grade 3/4) were anemia, thrombocytopenia, lymphopenia, fatigue, pneumonia, and pain. Serious AEs occurred in 33 patients. Notably, in these heavily pretreated patients, ORR was 21.3%. Six patients were treated with carfilzomib for more than a year by continuing on extension protocol PX-171-010. Two patients on chronic hemodialysis improved their renal function, no longer needing dialysis treatment. *Conclusions.* Renal impairment had no observed effect on the PK and safety profiles of carfilzomib and should not necessitate a change in carfilzomib dose or treatment schedule. AEs in this population were manageable and comparable to those seen in other studies with carfilzomib in MM.

0891

EVALUATION OF EFFICACY AND SAFETY OF BORTEZOMIB, DOXORUBICIN AND DEXAMETHASONE (PAD) REGIMEN IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Background. Multiple myeloma is a malignant plasma cell disorder. It is the second most frequent haematological malignancy and characterized by malignant plasma infiltration of the bone marrow and is associated with an increased level of monoclonal protein in the blood and/or urine. The treatment of multiple myeloma (MM) has undergone significant developments in recent years. The development of new agents with potent anti-tumor activity has considerably improved the survival of MM patients. Bortezomib has been investigated as part of a number of different induction regimens. A randomized phase III study by the French Myeloma Study Group (Intergroupe Francophone du Myélome [IFM]) examined the combination of bortezomib plus dexamethasone and found this to be significantly superior to the comparator arm, which consisted of VAD, with respect to response rates postinduction and post-transplant, as well as the 2-year PFS rate. A number of bortezomib induction regimens are now available. The results of the IFM trial indicate that the combination of bortezomib and dexamethasone is an appropriate regimen that is superior to the traditional VAD regimen. *Aim.* Evaluation of the effect and safety of combination of bortezomib, doxorubicin and dexamethasone (PAD) in the treatment of relapsed/ refractory myeloma patients in the retrospective analysis. *Patients and Methods.* 101 patients were treated for median of four 28-day PAD cycles (1-8). Bortezomib was given at 1.3 mg/m2 (days 1, 4, 8,11), doxorubicin at 9 mg/m2 (days 1-4) and dexamethasone 20 mg po (days 1-4, 8-11). *Results.* 101 patients were evaluable for efficacy and safety, 63.2% had refractory disease and 36.8% were relapsed. The median age was 60 years (41-78), 45.5% were male, 54.5% female. Median time from diagnosis was 12 months (1-139) and median number of prior therapy lines was 1 (1-4): 90.9% had undergone thalidomide-base regimen, 9.1% conventional chemotherapy and 23.9% were autografted. Overall response rate of 77.4% was observed, 32.8% of patients achieved a complete response (CR), 19.8% a very good partial response(VGPR), 24.8% a partial response (PR). Stable disease (SD) was observed in 15.8%. After PAD 42.9% of patients were autografted. The median progression free survival (PFS) was 19.3 months. The probability of 2-years overall survival (OS) was 68.7% and the median of OS was not reached. The most common grade 3-4 toxic effects were neutropenia 14%, thrombocytopenia 15%, anemia 4.3%, infections 5.4%, peripheral neuropathy 4.3% and gastrointestinal disturbances 2.1%. One toxic death (1.1%) due to sepsis was noted. *Conclusion.* The combination of bortezomib, doxorubicin and dexamethasone (PAD) is well tolerated and induced clinically significant responses and prolonged remission duration in patients with relapsed and refractory MM .

0892

OVERALL SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION: RELATION WITH KINETICS OF NEUTROPHIL AND PLATELET RECOVERY

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Background. Autologous stem cell transplantation (ASCT) is the gold standard as first-line treatment in young patients with multiple myeloma (MM). Prognostic factors have been usually related to patient characteristic and disease stage. Few investigations on the impact of

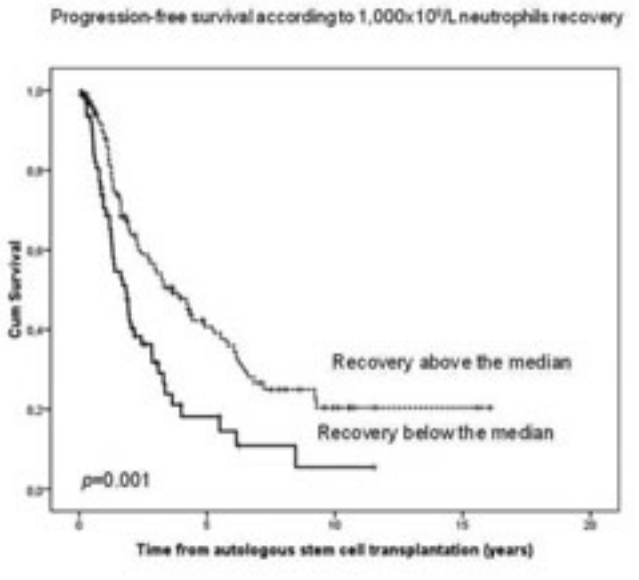

Figure 1.

platelet and neutrophil recovery after ASCT have been addressed. The *aim* of this study was to investigate the prognostic influence of the kinetics of peripheral blood recovery on progression-free survival (PFS) and overall survival (OS) after ASCT. *Patients and Methods.* One hundred and ninety one patients (109M/82F; median age 55 years) underwent melphalan-based ASCT in our institution from 1994 to 2010. The median follow-up after ASCT was 4 years (range 4 months to 17 years). Peripheral blood recovery was assessed as the day when the neutrophils reached 500 (N500) and 1000x109/L (N1000) and platelet count 20,000 (P20) and 50,000x109/L (P50) after CD34+ infusion. Patients were classified in two groups according their recovery above or below the median. *Results.* N1000 (Figure), P20 and P50 predicted for a longer OS ($p<0.05$). No significant association with the number of infused CD34 cells was observed. PFS was also correlated with N1000 (Figure) ($p=0.001$) and there was a trend for N500 ($p=0.053$), with no impact of P20 and P50. In Cox multivariate regression analysis, including age, international staging system and immunoglobulin isotype, N1000 remained at significant level for PFS ($p=0.02$), and there was a trend for OS ($p=0.09$). Finally, a stratification model showed 3 prognostic stages according to the achievement of complete remission and early N1000 after ASCT ($p<0.001$). *Conclusion.* Early neutrophil recovery was associated with significantly longer PFS and OS, while platelet recovery was only associated with OS.

0893

REAL LIFE HIGH DOSE TREATED PATIENTS BENEFITS FROM NOVEL AGENTS

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Background. Treatment results in clinical practice and in real life can be different from each other. Therefore, evaluating real life outcomes of high dose treated (HDT) Multiple Myeloma (MM) patients in order to understand what is effective treatment in clinical practice is very important. *Aim.* Evaluate real life responses, time to progression (TTP), time to next therapy (TTNT) and overall survival (OS) and prognostic power of patient variables in real life HDT MM patients. *Methods.* All HDT patients, n=214, with MM between Jan 2000 and Jul 2010 at Karolinska Huddinge and between Jan 2005 and Jul 2010 at Karolinska Solna were included. Near complete response (nCR) was defined as an immeasurable M-protein by standard electrophoresis. Very good partial response (VGPR), partial response (PR), no response (NR) and TTP were defined according to IMW criteria. Standard statistical methods were used. *Results.* The median age was 58 years with 66% male. Baseline creatinine was 89 µmol/L, albumin 34 g/L, hemoglobin 110 g/L and β2-microglobulin 2.9 mg/l. The median number of treatment lines was 2. 53% of the patients were given at least 2 treatment lines and only 36% 3 or more. The most common 1st line treatment was VAD + HDT (43%). The response distribution nCR/VGPR/PR/NR was 51/23/15/11 in 1st line, 28/17/22/33 in 2nd line. nCR in the 1st line implied a 48% probability to receive a ≥VGPR and 35% probability to receive an nCR in 2nd line. NR in the 1st line implied a 25% probability to receive a NR in the 2nd line. Logistic regression analysis shows that the patients receiving novel agents (Bortezomib, Lenalidomide, Thalidomide) in 1st line had a higher probability of achieving nCR. Baseline creatinine values seem to be of importance for the response and most likely Hb and albumin as well. The median TTP/TTNT was 538/639 days in the 1st line 210/257 in 2nd line and 186/212 in 3rd line. There was a significant trend of increasing TTP/TTNT in 1st line depending on the increased depth of the response with TTP/TTNT for nCR of 706/879 days, VGPR 511/533 days, PR 393/536 days and NR 58/63. Median OS was 6.3 years 95% CI [5.3;8.5] with 74% censored. Patients receiving an nCR in 1st line had a median OS of 6.9 years. There is a correlation between TTP in the 1st line and increased OS. *Summary.* HDT patients receiving 2nd and 3rd line treatment were declining rapidly. To get a good response in 1st line increases the likelihood of having a good response in 2nd line. Receiving an nCR in 1st line seems to be very important. Receiving a VGPR or PR seems to give similar results, less good than nCR but better the NR. Variables of importance are creatinine, albumin and hemoglobin. The use of novel agents improves response in 1st line.

0894

VORINOSTAT PLUS BORTEZOMIB COMBINATION THERAPY IN PATIENTS WITH RELAPSED OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA: VANTAGE STUDY PROGRAM UPDATE

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Background. Vorinostat is an oral multi-histone deacetylase inhibitor approved for treatment of patients with cutaneous T-cell lymphoma who have progressive, persistent, or recurrent disease following 2 prior systemic therapies. Vorinostat regulates genes and proteins involved in tumor growth and survival. The synergistic effects of vorinostat and bortezomib have been shown in preclinical studies and confirmed in phase I trials in patients with relapsed/refractory multiple myeloma (MM), producing objective response rates (ORRs) of up to 42% and overall clinical benefit of up to 90%. *Aims.* To provide an enrollment update and demographic data for patients with relapsed/refractory MM participating in the Vantage clinical trial program. *Methods.* Vantage 088 is a global, phase III, randomized, double-blind study investigating bortezomib plus vorinostat or placebo in patients with relapsed MM and progressive disease after 1-3 prior regimens. The primary objective is determination of progression-free survival; secondary objectives include assessment of safety, overall survival, time to progression, and ORR. Vantage 095 is a phase IIB open-label study of vorinostat plus bortezomib in bortezomib-refractory patients with relapsed/refractory MM who had received ≥2 prior antimyeloma regimens and were relapsed, refractory to, intolerant of, or ineligible for other MM therapies, including immunomodulatory drugs. The primary objective is to determine the ORR. In both studies, patients receive 21-day cycles of bortezomib (1.3 mg/m^2 IV; days 1, 4, 8, and 11) plus oral vorinostat 400 mg/day (or matching placebo in Vantage 088) on days 1-14. Efficacy is assessed by European Bone and Marrow Transplantation Group criteria. Adverse events, including clinical and laboratory events are assessed and recorded using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). All patients in both studies gave written informed consent prior to enrollment. *Results.* Both trials have been fully enrolled (Vantage 088 in January 2011; Vantage 095 in October 2010). As of February 10, 2011, 185 of 637 patients in Vantage 088 and 15 of 143 patients in Vantage 095 were still on active study therapy. Vantage

088: The median patient age is 62 years (range, 29-86 y), 59% of patients are male, and 56% are white. Patients had received a median of 2 prior regimens (range, 1-3), 23% had previously received bortezomib, and 35% had received a prior autologous transplant. Vantage 095: The median age of the patient population is 63 years (range, 37-81 y), 61% of patients are male, and 71% are white. Patients had received a median of 4 prior regimens (range, 2-17), all had received prior bortezomib, and 74% had received a prior autologous transplant. Both trials have passed protocol-specified futility analyses by the independent data monitoring committee. *Conclusions.* Two ongoing global, multicenter, investigational trials of vorinostat plus bortezomib in patients with relapsed/refractory MM have completed enrollment, with final results expected in 3Q2011.

0895

A PHASE I STUDY OF BHQ880, A NOVEL OSTEOBLAST ACTIVATING, ANTI-DKK1 HUMAN MAB, IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA PATIENTS TREATED WITH ZOLEDRONIC ACID AND ANTI-MYELOMA THERAPY

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Background. DKK1, a negative regulator of the Wnt signalling pathway, is overexpressed in multiple myeloma (MM) patients with osteolytic lesions. BHQ880 is a novel human mAb that neutralises DKK1, resulting in activation of the Wnt signalling pathway, leading to upregulation of osteoblasts and increased bone mass. Although both osteoclast activation and osteoblast inhibition contribute to lytic bone disease in MM, approved treatment options, such as bisphosphonates, are focused on inhibiting osteoclast function. Therefore, dual therapy with zoledronic acid (Zol) to decrease bone resorption and BHQ880 to increase new bone formation may provide an effective treatment strategy for MM bone disease. *Aims.* Determine the maximum-tolerated dose and characterize dose-limiting toxicity of escalating BHQ880 doses in combination with MM therapy and Zol. Evaluate bone-related effects of this treatment. *Methods.* Patients with relapsed/refractory MM with prior skeletal-related event/s were treated with IV BHQ880 q28 days. Patients also received Zol 4 mg and approved MM therapy (excluding bortezomib). Bone markers and serum DKK1 levels, along with BMD via DXA, were measured. Full PK profiles were obtained for the first and second cycles, after which predose samples were collected to assess accumulation. *Results.* Twenty eight patients (18:M;10:F), with a median age of 60 years (range: 38-79 years), PS 0 (n=12), 1 (n=13), or 2 (n=3) were enrolled at dose levels (mg/kg) 3 (n=5), 10 (n=10), 20 (n=7), or 40 (n=6). Sixteen patients received >6 cycles, 12 received >12 cycles, 9 patients ongoing (maximum 21 cycles). No BHQ880-related SAEs have been reported. No MTD was defined. One grade 3 neutropenia was observed in a patient with a history of pancytopenia receiving filgrastim; other toxicities, hypertension, bone pain, muscle spasm, chills, dysgeusia, alopecia, insomnia, cough, congestion and hirsutism were all grade 1. BMD data available from 21 patients showed a >6% increase from baseline in seven (33%) patients. Positive changes in BMD were noted across all dose cohorts, with positive slopes exceeding those with static or negative change. Bone marker changes with serial sampling at least until the end of cycle 2 were analysed in 24/28 patients; 10/24 patients showed a decrease in uNTx of >50%, and 3/24 patients showed a pattern suggestive of sustained increase in PINP and osteocalcin. Baseline free DKK1 levels from 28 patients ranged from ≤0.3 to 13.7 ng/mL. PK is available from 28 patients. Despite nonlinear pharmacokinetics, due to target mediated drug disposition, dose proportional increases in partial $AUC_{0\text{-}672\ h}$ and C_{max} were observed with doses of ≥10 mg/kg, suggesting saturation of DKK1. After second infusion, mean $t_{½}$ from doses 3, 10, 20 and 40 mg/kg ranged between 10.3-16.9 days. *Conclusions.* At doses up to 40 mg/kg, IV BHQ880 q28 days appears to be well tolerated in combination with Zol and chosen MM therapy. Recommended Phase II dose of 10 mg/kg is supported with current data predicting maximal saturation and suppression of DKK1. BMD changes > 6% from baseline in 33% of patients and changes in bone markers in this patient population suggest a potential anabolic effect.

0896

AN OPEN-LABEL, PHASE 1/2 TRIAL OF BENDAMUSTINE PLUS BORTEZOMIB IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background. Although significant improvements in the understanding and treatment of multiple myeloma (MM) have been realized over the last decade, MM remains an incurable disease, and novel, effective treatment combinations are needed for patients with relapsed or refractory disease. Bendamustine, approved in Europe for the treatment of MM, is an akylating agent with a multifaceted mechanism of action. Bortezomib is a proteasome inhibitor approved for the treatment of MM that has demonstrated efficacy in combination with alkylators (e.g., melphalan and cyclophosphamide). *Aims.* To assess the efficacy and safety of bendamustine plus bortezomib in patients with relapsed or refractory MM. *Methods.* This open-label, phase 1/2 study enrolled patients ≥18 years, with measurable, relapsed or refractory MM; patients were required to provide informed consent. Escalating doses of bendamustine IV at 50, 70, or 90 mg/m^2 (days 1 and 4) plus bortezomib at a fixed dose of 1.0 mg/m^2 (days 1, 4, 8, and 11) were administered for up to eight 28-day cycles. Dose-limiting toxicity (DLT) was assessed after cycle 1. A standard 3+3 approach was used to determine the maximum tolerated dose (MTD). The MTD cohort was expanded until a total enrollment of 40 patients was reached. Study endpoints included response, duration of response (DOR), time to progression (TTP), and safety. *Results.* Thirty-eight patients (median age, 67; range, 43-89) received study drug and were included in the analysis. Patients received a median of 3.5 (range, 1-21) prior therapies, including bortezomib in 71% and alkylators in 68%. A median of 3 treatment cycles (range, 1-9) were administered; study treatment remains ongoing in 14 patients (median cycles to date: 4 [range, 1-7]). No DLT was observed in phase 1 and the MTD was not reached; thus, the maximum dose (90 mg/m^2) of bendamustine plus bortezomib 1.0 mg/m^2 was studied in phase 2. Grade 3/4 adverse events that occurred in ≥10% of patients were neutropenia (13 patients [34%]), thrombocytopenia (7 [21%]), and anemia (4 [11%]). Grade 3/4 infection was reported in 3 patients (8%) and grade 3 renal failure in 2 patients (5%). No grade 3/4 peripheral neuropathy (PN) was observed; grade 1/2 PN was reported in 10 patients (26%), but 8 of these had grade 1/2 PN at baseline. Among 36 evaluable patients, the objective response rate (ORR) was 47%, including 1 very good partial response, 6 partial responses, and 10 minimal responses. An additional 17 patients had stable disease for a clinical benefit rate of 94%. In patients who received bendamustine at the 90 mg/m^2 dose (n = 27), the ORR was 52%. In patients previously treated with bortezomib (n = 27) or alkylators (n = 26), the ORR was 37% and 40%, respectively. Median DOR and TTP have not been reached. *Conclusions.* The combination of bendamustine 90 mg/m^2 on days 1 and 4 plus bortezomib 1.0 mg/m^2 on days 1, 4, 8, and 11 is well tolerated and demonstrates promising efficacy in heavily pretreated patients with MM.

0897

A SYSTEMATIC REVIEW ON THE USE OF BORTEZOMIB IN MULTIPLE MYELOMA PATIENTS WITH RENAL IMPAIRMENT: WHAT IS THE PUBLISHED EVIDENCE?

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Background and Aims. A systematic and comprehensive search of the literature was performed using MEDLINE databases from 1978 to 1st December 2010 and hand search of references. We used the following Medical Subject Headings (MESH) to identify potential studies: 'myeloma renal failure' (hits1225) and 'bortezomib' (hits 2554). An additional search performed by combining the MESH terms 'myeloma renal failure' and 'bortezomib' yielded 50 citations. Five additional case-control studies judged relevant for the purpose of study were also included. *Methods.* In total, 6 case reports, 9 cases series, and 9 case control studies were identified that reported on myeloma, renal failure and bortezomib. Only case series and case control studies were considered.We formulated some key questions dealing with reversal of renal impairment (RI), doses and association of bortezomib therapy as well as toxicity. *Results.* Overall 877 patients were considered suitable for this analysis.Median age was 65 years (range 40-88) and M/F ratio 1.02 while most patients hadrelapsed/refractory MM (597 out of 877 or 68%) and 79% were in Durie& Salmon stage III. Heterogeneous were methods used for assessing the extent of RI. Serum creatinine concentration has been used in 6 studies and CrCl in 12. Another relevant issue concerns the heterogeneity of threshold selected to define RI. A CrCl value of 30 ml/min was used in 3 studies, between 50 and 60 ml/min in 3 additional reports, while CrCl cutoff was increased to 80 ml/min in a single study.Even definition of reversal of RI shows differences across studies. In 7 reports reversal of RI meets criteria of a reduction of creatinine levels. A more accurate method to define the degree of reversal of RI based on Ludwig criteria has been used in 4 recently published studies. Overall response rate after treatment with bortezomib was relatively high and did not reflect the pre-treatment renal status.Time to reversal of RI assessed in 10 reports appears to be relatively short (median 1.4 months; range from 0,5 and 3,7 months) and led to the dialysis independence 8 out of 32 (25%) previously dialyzed patients. Almost all patients were treated according standard schedule at dose of 1.3 mg/m 2 on days 1, 4, 8, and 11. re¬peated every 21 days. Adverse events and side effects were described in all 18 studies considered. Remarkably toxicity was generally found comparable in 5 studies including MM patients with and without renal failure. *Conclusion.* Despite the paucity of data, this article represents the first systematic review of the entire body of available clinical evidence dealing with the use of bortezomib in MM patients with RI.

0898

PX-171-007: A PHASE 1B STUDY EVALUATING THE SAFETY AND EFFICACY OF A 30-MINUTE IV INFUSION OF CARFILZOMIB IN PATIENTS WITH RELAPSED AND/OR REFRACTORY (R/R) MULTIPLE MYELOMA (MM)

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Background. Carfilzomib is a novel, highly selective, epoxyketone proteasome inhibitor. In MM patients, single-agent IV carfilzomib shows significant activity at doses up to 27 mg/m^2 when given over a period of 2-10 min. In rats, 48 mg/m^2 was the LD50 when carfilzomib was administered as an IV bolus, however the same dose was nonlethal with minimal toxicity when given as a 30-min infusion. *Aims.* We conducted a dose-escalating trial of carfilzomib given as a 30-min IV infusion to patients with R/R MM. The intent of this study was to define the MTD and safety profile of carfilzomib delivered by this route. *Methods.* Carfilzomib was given as a 30-min IV infusion on days (D) 1, 2, 8, 9, 15, and 16 of a 28-day cycle (C). C1 D1-2 doses are 20 mg/m^2, with subsequent escalation to 36, 45, 56, or 70 mg/m^2. Dexamethasone (4 mg for ≤45 mg/m^2, 8 mg for >45 mg/m^2) was given prior to infusion. Responses were determined according to International Myeloma Working Group Uniform Response Criteria. Pharmacokinetic and pharmacodynamic analyses were performed on samples obtained at C1D1 and C2D1. *Results.* A total of 20 patients are enrolled (4 at 36 mg/m^2; 3 at 45 mg/m^2; 11 at 56 mg/m^2; 2 at 70 mg/m^2). The median number of prior treatment regimens for all patients is 4 (range 1-9). The current median duration of treatment is 4 cycles (range 1-13+). Dose-limiting toxicities (DLTs) were seen in both patients treated at 70 mg/m^2 (Grade 3 reversible renal failure after the first dose; Grade 3 fatigue with fevers after 4 doses). 11 patients enrolled at 56 mg/m2, with 1 DLT (Grade 3 hypoxia/fever). Responses for the 17 evaluable patients are shown in the table below. Three patients treated at 20/56 mg/m^2 were not evaluable for efficacy (patients were withdrawn due to: DLT after 3 doses; Grade 4 neutropenia after 2 doses; and Grade 4 thrombocytopenia after 3 doses). The C_{max} obtained with the 30-minute IV infusion of carfilzomib is ~4-fold lower than that achieved with the 10-minute IV push. Proteasome inhibition of >90% was observed at doses ≥36 mg/m^2. Increased immunoproteasome inhibition was seen with the 56 mg/m^2 dose compared with the 20 mg/m^2 dose. The most common adverse events (AEs), irrespective of relationship to carfilzomib, were nausea (42%), fatigue (37%), chills (26%), pyrexia (26%), and vomiting (21%). *Conclusions.* Carfilzomib administered as 30-minute IV infusion is highly active and well tolerated at 20/56 mg/m^2 (the recommended dose for R/R MM via 30-minute infusion). Enrollment will continue at 20/56 mg/m^2 for a total of 25 patients. Similar to animal studies, improved safety outcomes in MM patients can be achieved with near complete proteasome inhibition when carfilzomib is administered as a 30-minute IV infusion.

Table 1.

Best response	20/36 mg/m^2 (n=4)	20/45 mg/m^2 (n=3)	20/56 mg/m^2 (n=8)	20/70 mg/m^2 (n=2)
VGPR	1		3	
PR	1	1	5	1*
MR				1*
SD	2	2		

* Re-treated at lower doses

0899

FREE LIGHT CHAIN CLONAL ESCAPE IS USEFUL FOR EARLY DETECTION OF RELAPSE IN INTACT IMMUNOGLOBULIN MULTIPLE MYELOMA

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Background. Serum free light chains (sFLC) and ratio (sFLCR), are used for the evaluation of sCR in intact immunoglobulin (Ig) MM. Their use is not established for follow up and treatment initiation in intact Ig Multiple Myeloma (MM). *Aim.* To investigate the importance of sFLC and sFLCR elevation for the detection of clinical relapse, in the absence of any other clinical and laboratory finding. *Patients and Methods.* 51 intact immunoglobulin MM patients were studied from diagnosis to last follow up. Their sera samples (n=312) were analyzed for sFLC quantification using Freelite® immunoassay. *Results.* Median lines of therapy were 2 (range 1-11). Median follow up was 28 months (4-135). Retreatment was initiated in all patients according to standard criteria. In 8/51 patients during remission (2 in plateau,1 in MR, 1 in PR, 3 in nCR and 1 in sCR) only sFLC and sFLCR increased gradually (light chain clonal escape) and shortly after they relapsed, (2 acute renal failure,1 acute renal failure and rise of paraprotein, 2 plasmatocytomas, 1 liver plasmacytoma and rise of paraprotein, 1 rise of paraprotein and 1 plasmacytic leukemia).Median time from onset of light chain clonal

escape to clinical relapse was 6 months (2-11months). *Conclusion.* Light chain clonal escape was observed during disease course in 15% of patients with intact Ig MM; shortly after they relapsed. Measurement of sFLC during follow up is useful for early detection of relapse in a subset of patients.

0900

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. Many patients with multiple myeloma (MM) do not respond to currently available bortezomib-based therapeutic strategies and novel combinations are urgently needed. Panobinostat is an oral pan-deacetylase inhibitor (pan-DACi) with demonstrated synergistic anti-myeloma activity in combination with bortezomib through dual inhibition of the aggresome and proteasome pathways. In the dose escalation phase of a phase Ib study (B2207) of panobinostat + bortezomib, responses of ≥ minor response (MR) of 76% (36/47) were observed. Of note, a ≥ MR rate of 66% (10/15) was observed among bortezomib-refractory patients. Based on these data, a multicenter, U.S.-based, single-arm phase II trial was initiated to further evaluate panobinostat + bortezomib + dexamethasone in patients with relapsed and bortezomib-refractory MM. In B2207, panobinostat was originally dosed in all 3 weeks of the treatment cycle. In the PANORAMA 2 study, the dosing schedule of panobinostat is modified to 2 weeks out of 3; matching the dosing schedule for bortezomib. *Aims.* The aim of this study was to determine if panobinostat could sensitize patients with relapsed and bortezomib-refractory MM to a bortezomib-based therapeutic regimen. *Methods.* Adult patients with relapsed and bortezomib-refractory MM (≥ 2 prior lines of therapy including an immunomodulatory drug and who had progressed on/within 60 days of last bortezomib-based therapy) are eligible. Treatment is comprised of two phases. Phase 1 consists of 8 three-week cycles of panobinostat (20 mg days 1, 3, 5, 8, 10, 12, ie, thrice weekly (TIW) 2 wks on 1 wk off) + bortezomib (intravenous 1.3 mg/m^2 days 1, 4, 8, 11) + dexamethasone (20 mg on day of and day after each bortezomib day). Patients demonstrating clinical benefit can proceed to treatment phase 2 which consists of 4 six-week cycles of panobinostat (20 mg TIW 2 weeks on 1 week off, and repeat) + bortezomib (intravenous 1.3 mg/m^2 day 1, 8, 22, 29) + dexamethasone (20 mg on day of and day after each bortezomib day). A Simon two-stage design will be used to test for the primary endpoint of overall response rate (≥ partial response) with ≥ 4 responses required to proceed to Stage 2. Secondary endpoints include MR rate, time to and duration of response, progression-free survival, time to progression, overall survival, and safety, including assessment of peripheral neuropathy. *Results.* As of Jan 24, 2011, 24 patients were enrolled and responses have been seen in bortezomib-refractory patients, with manageable toxicity reported to date. Further assessment for response is ongoing and additional evaluation for toxicity is in process. *Summary/Conclusions.* Enrollment to Stage 1 has been completed and interim analysis is underway, with Stage 2 anticipated to start soon. Updated demographic information along with preliminary safety and efficacy data will be presented at the meeting.

0901

BORTEZOMIB PLUS DEXAMETHASONE (VD) FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) PRODUCES MOLECULAR REMISSIONS (MOLR) IN 23% OF PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (MM)

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Background. The depth of complete remission (CR) may be critical for the long-term outcome of patients with MM. Thus, MolR is proposed as the new goal of treatment. *Aim.* The aim of this Finnish study was to explore the response rates after VD induction followed by ASCT, including minimal residual disease (MRD) assessment in patients with at least near-CR response. *Methods.* Until Dec 2010, 35 symptomatic MM patients with a median age of 61 (53-65) years with informed consent have been included. Study protocol consists of induction with four cycles of VD followed by ASCT with melphalan 200mg/m^2. Patients were evaluated first time for response after two cycles of induction treatment. Those who had not attained at least partial remission (PR) were taken out of protocol treatment. In case of progressive disease patients were also out of protocol. MRD was assessed by allele specific quantitative polymerase chain reaction (PCR) using pretreatment sample with proportion of myeloma cells determined by flow cytometry as the reference. *Results.* After VD induction nine patients (26%) were in nCR/CR; two of them were PCR negative (sensitivity 0.003%-0.01%) and four PCR positive (range 0.002%-0.2%). Three patients had inadequate samples. Sixteen patients (46%) were in very good partial remission (VGPR) or in PR, and seven (20%) patients had response less than PR. Three patients are not yet evaluable. Two more patients were PCR-negative (sensitivity <0.001%) after mobilisation. Eighteen patients have underwent ASCT. Three months after ASCT, 12 patients (34%) are in nCR/CR, four in VGPR/PR, 10 patients not yet evaluable and nine patients out of study. Five patients are out of study, because their response was less than PR after two induction cycles, two patients had progressive disease, and two had severe adverse event (thromboembolic events with treatment delay). Of the 12 patients in nCR/CR, six are PCR-negative (sensitivity 0.001%-0.007%) and six PCR-positive (range 0.003%-0.09%). Two more patients have achieved PCR-negativity six months after ASCT. In all patients, except one, PCR-negativity is confirmed with two consecutive samples. *Conclusion.* Even with the relatively short median follow-up time PCR-negativity has already been achieved in 23% of the patients. Longer follow-up is needed to study if molecular remission is associated with beneficial long-term outcome.

0902

CHROMOSOMAL ABNORMALITIES AMP(1Q21) AND DEL(13Q14) PREDICT SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH CTD REGIMEN

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Background. Chromosomal abnormalities are frequently found in multiple myeloma(MM) and play a major role in patient outcome and management of the disease. The most important chromosomal aberrations associated with worse outcome are del (17p13), t(4,14), t(14,16) and t(14,20). Others that may be associated with adverse prognosis include del(13q14), del(8p21), amp(1q21) and hypodiploidy. In the era of novel agents such as thalidomide, lenalidomide, and bortezomib, risk stratification by chromosomal abnormalities may enable rational therapeutic approach in patients with MM. *Aims.* The study aimed to investigate the influence of amp(1q21) and del(13q14) on survival of MM patients treated with CTD (thalidomide, cyclophosphamide and dexamethasone) regimen. *Methods.* We analyzed the prognostic value of del(13q14) and amp(1q21) by fluorescence *in situ* hybridization (FISH) and hyper- (H-MM) or hypodiploidia (NH-MM) by conventional cyto-

genetic methods in a series of 71 patients with newly diagnosed MM treated with CTD between 2007 and 2010. *Results.* We found del(13q14) in 35 patients, amp(1q21) in 37 patients and combination of del(13q14) and amp(1q21) in 25 patients. Response rate assessed according to EBMT criteria, was 90% for NH-MM patients without additional aberrations (≥VGPR in 60%), 63% for H-MM patients without additional aberrations (≥VGPR in 45%) and 50% for patients with combination of del(13q14) and amp(1q21). The median overall survival (OS) for H-MM patients reached 31 months and was significantly longer than in NH-MM group (17 months, p<0.001). The combination of del(13q14) and amp(1q21) was adverse cytogenetic signature resulting in shortened OS in both groups (13 for H-MM and 6 months for NH-MM, p<0.01). *Summary/Conclusions.* The results of the study suggest that combination of del(13q14) and amp(1q21) is adverse prognostic factor that affects OS by patients H-MM and NH-MM. Improved therapeutic strategies including bortezomib and lenalidomid are required for these patients.

0903

HOSPITAL COSTS DURING TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA IN THE ERA OF TARGETED THERAPIES: REAL-WORLD ESTIMATES FROM THE NETHERLANDS

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Background. Targeted therapies for multiple myeloma (MM), such as thalidomide and bortezomib, promise gains in health but come at increased acquisition costs. Newer targeted therapies for treatment of MM are expected in the near future, and assessment of cost-effectiveness may be required for decision-making regarding their use. In the Netherlands, treatment with targeted therapies has until recently been confined to relapsed/refractory disease (RRMM). We conducted a cost study to estimate the total costs and drivers of increased costs during treatment for RRMM in the era of targeted therapies in the Netherlands. *Aims.* To describe the costs of treatment for RRMM and determine whether there are differences between the treatment costs of thalidomide and bortezomib. *Methods.* Patients in this cohort represented a subset of 139 patients treated in the HOVON50 study, which compared VAD (vincristine adriamycin dexamethasone) with TAD (thalidomide adriamycin dexamethasone) in first-line treatment of MM. In this subset, 65% (n=90) progressed from the VAD treatment arm compared to 35% (n=49) from the TAD treatment arm, of which 23% (n=21) and 17% (n=8) received autologous stem cell transplantation during first-line therapy, respectively. Detailed clinical data were retrospectively collected for each patient up until last known follow-up between January 2001 and May 2009. Total costs for individual patients were determined by the identification of hospital resource use and unit costs of all cost components. Monthly resource use and costs attributable to each cost component were described across all treatment lines and separately by treatment line. Results were also calculated for thalidomide- and bortezomib-based treatment regimens. *Results.* The combination of treatment regimens and sequence of administration throughout treatment of RRMM varied greatly. Total mean follow-up of the patient group equaled 24 months (range: 0.6-70.1) with 49% of patients still in follow-up at the time of data collection. In total, 87 patients were treated with thalidomide and 72 with bortezomib, with some patients receiving either therapy more than once as well as concurrently in combination during treatment of RRMM. Mean total monthly costs for treatment of MM patients in the Netherlands were approximately €4,109 and the minimum-maximum range was large (€142-€31,740). Mean total monthly costs did not differ significantly between patients still in follow-up and those with complete follow-up. The structure of the total costs incurred during treatment differed between bortezomib and thalidomide treatment lines. Total mean monthly acquisition costs for bortezomib were higher compared to thalidomide (€2,574 vs €603; unit prices were €955/3.5 mg vial of bortezomib and €0.24/mg of thalidomide). Moreover, monthly costs associated with adverse events and drug administration were higher during bortezomib-based treatment regimens compared to thalidomide-based treatment regimens. *Summary/Conclusions.* The costs of treatment during RRMM are substantial and vary depending on the order in which therapies are given. Cost-effectiveness analyses of targeted therapies for MM should take into account the impact of increased therapy-related costs on the total treatment costs. Furthermore, the costs of potentially avoidable adverse events associated with specific treatment regimens should be considered when determining the most cost-effective treatment sequence for RRMM.

0904

PROGNOSTIC FACTORS IN ELDERLY MYELOMA PATIENTS RECEIVING BORTEZOMIB, MELPHALAN, PREDNISONE AND THALIDOMIDE FOLLOWED BY BORTEZOMIB AND THALIDOMIDE (VMPT-VT) AS FIRST LINE TREATMENT

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Background. bortezomib-melphalan-prednisone (VMP) is now considered the new standard of care for newly-diagnosed myeloma (MM) patients ≥65 years. Bortezomib-melphalan-prednisone-thalidomide (VMPT) showed to be effective in relapsed/refractory setting. *Aims.* this phase III trial compared VMPT followed by maintenance with bortezomib-thalidomide (VMPT-VT) with standard VMP in newly-diagnosed elderly MM patients. The primary end point was progression-free survival (PFS). *Methods.* 511 patients were randomized to receive 9 6-week cycles of VMPT-VT (N= 254; induction: bortezomib 1.3 mg/m^2 days 1,4,8,11,22,25,29,32, cycles 1-4 and days 1,8,22,29 on cycle 5-9; melphalan 9 mg/m^2 days 1-4, prednisone 60 mg/m^2 days 1-4, thalidomide 50 mg continuously; maintenance: bortezomib 1.3 mg/m^2 every 14 days and thalidomide 50 mg/day) or VMP alone (N=257). In March 2007, the protocol was amended with weekly infusion of bortezomib in both arms. *Results.* patients characteristics were well balanced (median age: 71 years). Response rates were higher in VMP-VT with 42% of complete remission (CR) vs 24% in VMP (p< 0.0001). After a median follow-up of 32 months, the 3-year PFS was 51% in VMPT-VT and 32% in VMP (p< 0.0001). The 3-year time to next therapy (TNT) was 70% in VMPT-VT and 51% in VMP (p< 0.0001). The 3-year overall survival was 85% vs 80% (p=0.35), respectively. The achievement of CR was a strong predictive factor of longer PFS in both groups (P < 0.0001): in VMPT-VT arm, 3-year PFS was 66% in CR patients and 47% in PR patients; in VMP arm, it was 70% and 30%, respectively. In patients ≥75 years old and in those with high risk disease (presence of cytogenetic abnormalities [t(4;14) or t(14;16) or del17p] and ISS 3) VMPT do not add any significant PFS advantage to VMP (p=0.5). Grade 3-4 neutropenia (38% vs. 28%, p=0.02), cardiological events (10% vs. 5%, p=0.04) and thromboembolic events (5% vs. 2%,p=0.08) were more frequent among patients receiving VMPT-VT. Moreover, weekly infusion of bortezomib reduced the incidence of peripheral neuropathy (PN) to less than 10%. 149 VMPT-VT patients were assessable for maintenance treatment. After a median duration of maintenance of 14.4 months, the PR rate was 90%, including 45% CR. The 1-year landmark analysis of PFS, showed a 2-year PFS of 63% in the VMPT-VT group and 40% in the VMP group, with a risk reduction of disease progression of 52% (p<0.0003). This advantage was less evident in patients ≥75 years (P=0.87) and in those with high-risk of disease progression (p=0.77). Maintenance with VT had favorable safety profile: 3% of patients experienced grade 3-4 hematological toxicity, 5% grade 3-4 PN and 7% discontinued due to adverse events. *Summary.* VMPT-VT showed a higher CR rate, prolonged PFS and TNT compared to VMP; achievement of CR was a strong predictive factor of longer PFS; higher dose-intensity seemed to be less effective in patients ≥75 years and in those with high risk disease; maintenance with VT further improved PFS with a good safety profile; bortezomib weekly-infusion significantly reduced PN incidence. A longer follow up is needed to assess overall survival.

0905

UPDATED RESULTS OF A PHASE II STUDY USING LENALIDOMIDE AS POST-TRANSPLANT CONSOLIDATION-MAINTENANCE THERAPY IN ELDERLY MULTIPLE MYELOMA PATIENTS

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Background. Bortezomib-dexamethasone-doxorubicin (PAD) induction, followed by reduce-intensity autologous transplantation proved to be safe and effective in elderly newly diagnosed multiple myeloma (MM) patients. Lenalidomide is less neurotoxic than thalidomide and represents an optimal agent to include in maintenance strategies. *Aims.* These observations provided the rationale for investigating a sequential approach including bortezomib as induction and lenalidomide as consolidation-maintenance in elderly MM patients undergoing reduced intensity ASCT. In this analysis, efficacy and safety end-points of post transplant lenalidomide consolidation and maintenance were updated. *Methods.* A hundred and two newly diagnosed patients, aged 65-75 years or younger not eligible for high-dose chemotherapy, were enrolled. Patients received PAD induction, tandem melphalan 100 mg/m2 and stem-cell support followed by consolidation with four 28-day cycles of lenalidomide-prednisone (LP), and subsequent lenalidomide maintenance (L) until relapse or until tolerated. *Results.* LP-L consolidation-maintenance therapy improved post transplant responses: VGPR rate raises from 82% to 92%, complete response (CR) rate from 38% to 71%: 1 patient improved from stable disease (SD) to partial response (PR), 1 from SD to very good partial response (VGPR), 1 from PR to VGPR, 2 from PR to CR, 16 from VGPR to CR. After a median follow-up of 3 years, the 3-year progression-free survival (PFS) for all patients was 66%, the 3-year time-to-progression was 73% and the 3-year overall survival was 85%. By exploratory analysis stratified by group, PFS was not significantly different between patients older or younger than 70 years. The 3-year PFS rate was 74% in patients with International Staging System stage I disease, 71% in patients with stage II, and 30% in patients with stage III disease. Patients who achieved CR had 3-year PFS of 81%, patients who achieved VGPR had a 3-year PFS of 56% and patients who achieved PR had a 3-year PFS of 31%. Patients with high-risk cytogenetic profiles, including del17, t(4;14), or t(14;16) and patient with standard cytogenetic profiles had no significantly different 3-years PFS (61% vs 68%). In patients with standard-risk cytogenetic abnormalities who achieved CR 3-year PFS was 100%. Consolidation-maintenance treatment was well tolerated, grade 3-4 adverse events included neutropenia (31%), thrombocytopenia (15%), pneumonia (8%), cutaneous rash (7%); no treatment-related deaths were reported. *Conclusions.* This sequential approach improved depth and rate of response, was well tolerated, and can be considered a safe and effective treatment strategy for elderly MM patients eligible for reduced-intensity ASCT.

Myeloproliferative disorders - Clinical

0906

A PHASE-2 TRIAL OF POMALIDOMIDE IN MYELOFIBROSIS WITH CYTOPENIA

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Background. Safety and efficacy of pomalidomide at 0.5 and 2 mg/d with or without prednisone was reported in myeloproliferative neoplasm (MPN)-associated myelofibrosis and cytopenia (J Clin Oncol 2009; 27:4563-4569, Leukemia 2011;25:301-304). We studied (ClinicalTrials.gov No. NCT00669578) efficacy of pomalidomide 2 mg/d alone or, when no response after three months, combined with prednisone (starting dose of 30 mg/d). *Aims.* To evaluate clinical efficacy of pomalidomide alone and combined with prednisone in patients with MPN-associated myelofibrosis and cytopenia. *Methods.* Patients with primary myelofibrosis (PMF) or post-polycythemia vera/essential thrombocythemia (post-PV/ET MF) myelofibrosis were included. The main eligibility criteria were red blood cell (RBC)-transfusion-dependence or hemoglobin <10 g/dL, and/or thrombocytopenia <50/nl and/or neutropenia <1.0/nl; patients >50 years were eligible. The main exclusion criteria were history of thrombosis or pulmonary-embolism. The primary endpoint was response assessed by the International Working Group for Myeloproliferative Neoplasms Research and Treatment criteria extended by the criterion transfusion-independence (Leuk Res. 2011;35:8-11). Concurrent hydroxyurea for proliferative disease and acetylsalicylic acid 100 mg/d in patients with platelets between 50/nl and 1000/nl were included. The statistical design of the study was based on the Simon optimal two-stage design. Here we report on the first stage of the study. *Results.* Pomalidomide 2 mg/d was given to 38 patients (median age 71 years), 25 were male. Twenty-seven had PMF, 3 had post-ET and 8 post-PV MF. Disease stage at study-entry according to the Dynamic International Prognostic Scoring System was high-risk in 13 (34%), intermediate-2 risk in 22 (58%) and intermediate-1 risk in 3 (8%). Karyotype was available in 30 patients with high-risk cytogenetics in 6 (20%). JAK2 V617F mutation was present in 21 (55%) and MPL W515L mutation in 7 (18%) of 38 patients, two patients had both mutations. Twenty-six (68%) were RBC-transfusion- and 7 (18%) platelet-transfusion-dependent, 18 of 38 patients had platelets < 100/nl. Eleven stopped treatment within 3 months and additional 5 within 6 months. Prednisone was added after month 3 in 19 of 27 eligible patients. Non hematological serious adverse reactions potentially related to pomalidomide were thromboembolism (n=2), pulmonary infection (n=2), rash (n=2), worsening of general condition (n=2), hypertension (n=1) and cardiac failure (n=2). Six patients (16%) experienced transformation into blast phase disease (n=3, high-risk; n=3 intermediate-2 risk). Pomalidomide dose-reduction (n=10, 1mg/d; n=2, 0.5mg/d) was performed for fatigue (n=3), thrombo- or neutropenia (n=8) and rash (n=1). There was an anemia response in 5 patients (4 receiving concomitant prednisone) and all became RBC-transfusion independent, platelet response was evident in 6 patients (2 receiving concomitant prednisone). Responses occurred in 3 patients within 3 months in 3 within 6 months and in 5 beyond month 6 of treatment. 5 of 11 responders had pomalidomide dose reduction due to toxicity, notably before response occurred. No correlation was evi-

dent between responses and JAK2 or MPL mutational status or cytogenetics. *Conclusions.* Pomalidomide with or without prednisone may be effective in the treatment of cytopenia in patients with MPN-associated myelofibrosis. Most responses were seen during combined therapy with pomalidomide and prednisone beyond month 3 of treatment.

0907

LONG-TERM SAFETY AND EFFICACY ANALYSIS OF THE TWO PHASE 1 STUDIES OF SB1518, A NOVEL ORAL JAK2 INHIBITOR, IN PATIENTS WITH ADVANCED MYELOID MALIGNANCIES

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Background. SB1518 is a potent inhibitor of both wild-type JAK2 and JAK2V617F, implicated in the pathogenesis of myeloproliferative neoplasms. In mid-2008, we initiated two Phase 1 studies of SB1518: one in patients with advanced myeloid malignancies, and the other exclusively in patients with myelofibrosis (MF). We previously reported the study data that supported 400 mg/d as the recommended Phase 2 dose (RD). These trials are ongoing, as some patients continue to receive SB1518. *Aims.* To report the overall safety and efficacy results for the combined study populations as of January 2011. *Methods.* Qualifying patients with MF had palpable splenomegaly ≥5 cm. Patients were sequentially assigned to doses from 100-600 mg/d and were dosed continuously. Intrapatient dose escalation was allowed up to the RD once the MTD was established. *Results.* Sixty-three patients were consented and enrolled; 39 (62%) were men, and median age was 65.5 years. Fifty-six had MF, and 7 had AML. Fifty (79%) were JAK2V617F mutation-positive (46 MF, 4 AML). At baseline, hemoglobin ranged from 5.6 to 16.3 g/dL (median, 9.4). Platelet count ranged from 5 to 954 x 103/μL (median, 122 x 103/μL); with 16 patients having baseline counts <50 x 103/μL, 12, 50-100 x 103/μL, and 35; <150 x 103/μL. Median time on study is 13.3 months (1-29+). As of January 2011, 21 MF patients remain on study. The most common treatment-related AEs were gastrointestinal, which were generally low grade and manageable. GI AE's > Gr 2 included 5 patients with Gr 3 diarrhea (7.9%) and 1 patient each (1.6%) with Grade 3 nausea, Grade 3 abdominal pain, and Grade 4 vomiting. Grade 4 hematologic AE's considered possibly related to treatment were rare and comprised anemia (n=2, 100 & 200 mg), and thrombocytopenia (n=1, 300 mg). These events occurred after 5.7-18.5 months on study. Two patients were discontinued for these AE's and one continues on study at 200 mg dose. Fifteen patients had dose reductions, most within the first 6 months; of these, 11 (73%) started treatment at ≥500 mg/d. No patients discontinued study medication because of a dose-limiting toxicity. No long-term toxicities were identified. Forty-one MF patients had palpable baseline splenomegaly ≥5 cm and were evaluable for spleen response; 18 (44%) of these 41 experienced clinical improvement (CI) (IWG criteria). Three patients had CI in platelet count (IWG criteria), and one patient had CI in hemoglobin (IWG criteria). Twenty-one patients achieved stable disease (IWG criteria). Overall, 39 (70%) of the 56 MF patients experienced CI or stable disease. Among all enrolled patients, duration of progression-free survival (PFS) ranged from 1 to 875 days (median, 563 days), with an estimated 67% rate of PFS at 12 months (Kaplan-Meier). *Conclusions.* SB1518 shows promising efficacy in MF patients with splenomegaly. Once-daily dosing is well tolerated to 29 months, with manageable GI toxicity as the main AE. SB1518 does not appear to cause myelosuppression; patients with significantly impaired hematopoiesis can receive full-dose daily SB1518 without exacerbating hematocytopenias.

0908

BLEEDING IN ESSENTIAL THROMBOCYTHEMIA: ANALYSIS OF INCIDENCE AND RISK FACTORS IN A COHORT OF 565 PATIENTS

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Background. Hemorrhages are potentially severe complications of essential thrombocythemia (ET). Extreme thrombocytosis and previous bleedings are currently considered the main risk factors for bleeding; however, only few data are available. *Objectives.* We retrospectively analyzed the hemorrhagic events of 565 consecutive ET patients followed for a median time of 7.8 years, with the aim to evaluate: (1) the incidence and the type of the bleeding complications; (2) the correlation between these events and clinical/laboratory data and (3) the therapeutic implications of the study findings. *Results.* Sixty patients (10.5%) had one or more hemorrhages, for a total of 77 hemorrhagic events. Twenty-four major bleeding (grade 3-4 according to WHO criteria) occurred in 23 patients (4%) at ET diagnosis (4) or during follow-up (20). The remaining 53 hemorrhages were minor (cutaneous or mucosal). Overall, 85.6% of the patients received a cytoreductive treatment during the observation time, achieving at least a partial response in 78% of the cases. Antiplatelet drug was administered to 521 patients (92%), with no differences between patients with or without hemorrhages. The incidence rates of total and severe bleeding were 13.4 and 4.8 events per 1000 person-years, respectively. The risk of hemorrhage was higher at diagnosis (0.5%) and progressively decreased during the follow-up. Patients with hemorrhages presented more frequently splenomegaly and displayed lower hemoglobin concentration and higher leukocyte and platelet count (Student's t-test and $\chi 2$ test). By univariate (log-rank) analysis, splenomegaly baseline ($p<0.0001$), a history positive for hemorrhages ($p=0.005$), a platelet count higher than the median value ($p=0.003$) and higher than 1000x109/L ($p<0.0001$) and a leukocyte count>11x109/L ($p<0.0001$) significantly correlated with subsequent bleedings. JAK2V617F mutational status (qualitative analysis) was not found to correlate with bleeding: however, only 2 patients were homozygous. Analogously, antiplatelet treatment, which was administered to the great majority of the patients, did not appear as a risk factor. By multivariate (Cox) analysis, splenomegaly (HR 2.9, 95% CI 1.5-5.4, $p=0.002$), platelet count>1000x109/L (HR 2.3, 95% CI 1.3-3.9 $p=0.003$) and leukocyte count>11x109/L (HR 1.9, 95% CI 1.1-3.2, $p=0.023$) retained their prognostic significance. Considering these 3 parameters, patients were stratified in 3 subgroups, characterised by an increasing risk for hemorrhage: low (no risk factors, 339 patients), intermediate (1 risk factor, 160 patients) and high (2 or 3 risk factors, 66 patients). The cumulative risk for haemorrhage at 10 years was 8.2% in the low-risk, 14.4% in the intermediate risk ($p=0.22$) and 45.8% in the high risk group ($p<0.001$). When severe hemorrhages were considered separately, only splenomegaly retained its prognostic significance on bleedings. In one case, bleeding was the ultimate cause of death. *Conclusions.* During the course of ET, bleeding represents a relatively rare event, whose incidence decreases over the time. Marked thrombocytosis, leucocytosis and spleen enlargement, particularly when concomitant, identify a cohort of patients at higher hemorrhagic risk. In these cases, the use of antiplatelet treatment should be balanced between the risk of bleeding and the thrombotic risk, which may itself be increased by the same features.

0909

PEGYLATED INTERFERON ALPHA - 2A IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS (MPN): INTERNATIONAL EXPERIENCE IN 90 CASES

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Background. Pegylated interferon alpha-2a (Peg INF2a) has been demonstrated to be active therapy for high risk essential thrombocythemia (ET) and polycythemia Vera (PV), as well as treatment for early myelofibrosis (MF). We retrospectively analyzed the outcomes of Peg INF2a therapy in MPN patients treated outside the constraints of a clinical trial in the USA and EU. *Methods.* Clinical records of MPN patients treated at the participating centers, receiving Peg INF2a outside of the context of a clinical trial, were analyzed for response (ET and PV by ELN criteria; MF by EUNMET and IWG-MRT criteria), toxicity, and duration of response. *Results. Patients:* 90 patients were identified [46 PV (51%), 30 ET (33%), 14 MF (16%)] with a median age (57) and gender distribution (56% Females) typical for the disorders. The patients were a median of 96 months (2.0-324 months) after the diagnosis of the MPN and 65% harbored the JAK2-V617F mutation. 80% of patients had received at least one prior cytoreductive therapy for their disease [58 hydroxyurea, 28 anagrelide, 21 prior interferon (non pegylated)]. There were 18 patients with vascular events (15 thrombotic, 3 hemorrhagic) prior to initiating Peg INF2a, with no vascular events occurring while on therapy. *Therapy.* Median starting dose of Peg INF2a was 90 µg/week (range: 45-180) with peak doses ranging from 45 to 270 µg. A total of 75 patients (83%) remain on Peg IFN2a with median duration of treatment of 17 months (range: 2.0-58). *Toxicity.* Overall the Peg INF2a was well tolerated. Hematological toxicity was Gr 3 or lower. There were 6 cases with anemia (7%), 9 with thrombocytopenia (10%) and 9 had leukopenia (10%). Most common non-hematologic toxicities were Fatigue Gr 1-3 in 17(19%), Gr 1 LFT elevation in 6 (7%) and Gr 2-3 mood disorder in 3 (3%) patients. Only 11 (12%) discontinued therapy secondary to toxicity. *Response.* ET-PV: By ELN criteria, 20 PV patients achieved CR (43%), 21 achieved PR (46%) and NR in 5 (11%). In ET, the responses were CR in 15 (50%), PR in 10 (30%) and NR in 5 (15%). MF: the responses by IWG criteria were 1 CR (7%), 2 PR (14%), 4 CI (28%) and 7 SD (50%). By EUNMET, there were 2 CR (14%), 5 Major responses (36%), 4 moderate responses (29%), 1 minor response (7%) and 2 no response (14%). *Conclusions.* Peg INF2a used at doses consistent with published clinical trials is active and well tolerated when administered in an active clinical setting outside of the support of a clinical trial. Given the majority of patients had previously failed cytoreductive therapy these results further substantiate prior observation of Peg INFa in MPNs. Upcoming randomized clinical trials through the Myeloproliferative Disorders Research Consortium will help further define the role of Peg INFa as first line therapy in high risk MPNs.

0910

SEQUENTIAL EVALUATION OF CHROMOSOMAL ABERRATIONS USING HIGH-RESOLUTION SNP MICROARRAYS IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASM EXPERIENCING DISEASE PROGRESSION

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Background. Myeloproliferative neoplasms (MPN) include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Both PV and ET may evolve into myelofibrosis, and all conditions are also characterized by an increased risk of progression to acute myeloid leukemia (AML). The genetic mechanisms underlying these transformations are poorly defined at present. *Aims.* The aim of the present study was to establish whether disease progression is associated with development of chromosomal aberrations in MPN, and whether the acquisition of these abnormalities has an impact on overall survival. *Methods.* This study included 24 MPN patients who progressed to secondary myelofibrosis (MF) or AML and could be followed with collection of at least two sequential DNA samples in different phases of disease. In detail, 14 patients progressed from chronic-phase MPN to AML, five patients from PV to post-PV MF, and five from ET to post-ET MF. All patients provided their written informed consent before DNA collection. The Genome-Wide Human SNP 6.0 Array was used to detect chromosomal aberrations, including copy number variation (CNV) and copy-neutral loss of heterozigosity (LOH). Paired samples collected before and after disease progression were compared by means of the Wilcoxon test for paired data. *Results.* Considering the whole cohort, disease progression from chronic-phase MPN to secondary MF or AML was associated with a significant increase in the number of chromosomal aberrations (P=0.0023) without any significant change in JAK2 (V617F) mutant allele burden (P=0.189). This increase remained statistically significant even after distinguishing patients with evolution from chronic-phase MPN to AML (P 0.016) and those with evolution from PV/ET to secondary MF (P=0.043). As abnormalities involving chromosome 5, 7 and 17p have been previously shown to be associated with worse survival in de novo AML, we evaluated whether the acquisition of these specific aberrations during follow-up had an impact on survival. By applying a Cox regression analysis, the acquisition of one or more of the above aberrations was associated with reduced survival from the time of the initial diagnosis of MPN (Hazard ratio 18, 95% CI 1.9-164, P=0.011). Fisher exact test showed that the acquisition of aberrations of chromosome 5, 7 and 17p was closely associated with leukemic transformation (P=0.013). *Conclusions.* MPN patients who progress to secondary MF or AML show a significant increase in the number of chromosomal aberrations such as CNV and LOH. The acquisition of aberrations involving chromosome 5, 7 and 17p is closely associated with leukemic transformation.

0911

MANAGEMENT OF ELDERLY PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA: AN OBSERVATIONAL STUDY OF 471 PATIENTS OF 80 YEARS AND OLDER INCLUDED IN THE EXELS EUROPEAN STUDY

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Background. Essential thrombocythemia (ET) is usually diagnosed in patients aged around 60 years and its incidence increases with age. According to international guidelines, an age above 60 years is a risk factor for thrombosis and therefore, all patients aged >60 years receive cytoreductive therapy. However, there are no pharmaco-epidemiological data available in elderly ET patients. We identified patients aged ≥80 years in the cohort of ET patients included in the European observational EXELS (Evaluation of Xagrid Efficacy and Long-term Safety) study. *Aims and Methods.* To present data from elderly ET patients enrolled in this observational study. Clinical and biological data are collected every 6 months. Analyses were performed in Feb 2011 (based on data cut in Sept 2010). *Results.* Among the 3604 patients included from 13 European countries, 471 patients were ≥80 years at study enrollment. Across countries, the proportion of included patients aged ≥80 years ranged from 10 to 19%. There was no difference between patients ≥80 years (elderly) and <80 years regarding gender (66% and 60% females, respectively), history of vascular events (41% vs. 36%), proportion of treatment-naive patients (16% vs 20%), and antiaggregation therapy (both 69%). Anagrelide was the current cytoreductive treatment at enrollment in 40% of patients <60 years, 14% of patients 60-79 years, and 9% of patients ≥80 years. Evolution of blood cell counts over time showed similar efficacy with comparable decreases in platelet counts in all patients, irrespective of age. Hematological tolerance to cytoreductive therapy was also comparable between elderly and younger patients. Switch from anagrelide to another cytoreductive

therapy was recorded in 10% of patients <80 years, and in 26% of those ≥80 years. The proportion of patients switching from hydroxyurea (HU) to another drug was similar in both age groups (10% and 12%, respectively). Median time to switch from anagrelide was 239 days in elderly patients, and 266 days in younger patients. Reasons for switch from anagrelide in patients ≥80 years was intolerance in 7/18 cases (vs 30/97 in patients <60 years), and no elderly patient changed because of inefficacy (vs 18/97 for <60 years). Reasons for switch from HU in patients ≥80 years was intolerance in 17/57 (vs 30/105 in patients <60) and inefficacy in 10/57 (vs 26/105 in patients <60). The number of patients with predefined events recorded (including vascular events and transformation to leukemia) was numerically higher in patients ≥80 years (19% of patients had one event vs. 7% in patients <60, and 12% in patients 60-79 years), but numbers are too low to allow subgroup analyses. *Conclusion.* This cohort of 471 elderly patients (age ≥80 years) with ET enrolled in a prospective observational study shows that elderly patients' characteristics do not differ substantially from those of younger patients at presentation. These elderly patients received similar management across EU countries, and the use of anagrelide decreased along with increasing patient age. Anagrelide tolerability and efficacy appeared comparable in elderly and younger patients, and no new safety concerns arose in this patient population.

0912

RESULTS USING THE MODIFIED MYELOFIBROSIS SYMPTOM ASSESSMENT FORM (MFSAF V2.0) IN COMFORT-I: A RANDOMIZED, DOUBLE-BLIND PHASE III TRIAL OF JAK1/2 INHIBITOR RUXOLITINIB VS PLACEBO IN MYELOFIBROSIS (MF)

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Background. Symptom burden is a major component of MF. *Aims.* The MFSAF (Mesa *et al. Leuk Res*. 2009) was developed to measure MF-associated symptoms. The modified MFSAF v2.0 diary (a refined version of the instrument) was implemented to evaluate effects of the JAK1 and JAK2 inhibitor ruxolitinib (INCB018424) on MF symptoms over a 24-week period compared with placebo in a randomized trial. *Methods. Ruxolitinib Trial:* 309 patients with ≥ intermediate-2 risk MF provided informed consent and were randomized to start placebo or ruxolitinib at doses of 15 or 20 mg BID depending on baseline platelet count, with dose modifications allowed for efficacy and safety. *Symptomatic Change Assessments:* The modified MFSAF v2.0 diary is a daily e-diary comprising 7 items, each scored on an 11-point scale from 0 (absent) to 10 (worst imaginable) in the 24 hours prior to assessment. The instrument measures patient-reported symptoms and impacts: abdominal discomfort (AD), abdominal fullness/early satiety (ES), bone/muscle pain (BMP), pain under the left side of ribs (P), night sweats (NS), itching (I), and MF-related inactivity (first 6 items are pooled to create the total symptom score [TSS]). Patients completed the modified MFSAF v2.0 diary daily for 1 week prior to starting therapy (baseline, BL) and for 24 weeks on therapy. A Patient Global Impression of Change (PGIC) scale was used for patient self-evaluation of treatment benefit. PGIC was measured monthly from BL through week 24 using a 7-point scale (1-very much improved to 7-very much worse). Change from BL for individual symptom scores and the TSS were anchored to PGIC responses at week 24. *Statistics:* The proportion of patients achieving ≥50% improvement (ie, treatment responders) in individual symptom scores and TSS were compared (ruxolitinib vs placebo) using chi-square tests. *Results.* 148 patients receiving ruxolitinib and 152 receiving placebo completed diary entries. No significant difference existed in individual BL mean symptom scores between the two groups. Mean change for TSS from BL to week 24 for ruxolitinib was -8.6 (18.0 to 9.4) vs 3.2 (16.5 to 19.7) for placebo (p<0.0001). The responder rate for TSS was 45.9% vs 5.3% for patients treated with ruxolitinib vs placebo, respectively (p<0.0001). Using a moving 7-day average of TSS, the median time to response was 4.4 weeks for patients receiving ruxolitinib but could not be estimated for placebo because of insufficient responders over the 24-week period. At week 24, treatment responders for individual symptoms for ruxolitinib vs placebo were: AD, 48.3% vs 9.4%; ES, 48.3% vs 10.9%; BMP, 39.6% vs 9.1%; P, 52.9% vs 14.8%; NS, 49.6% vs 11.2%; I, 58.5% vs 11.5%; and inactivity, 33.1% vs 10.9% (all p<0.0001). 91.2% of ruxolitinib patients who were TSS responders had PGIC scores of "much"or "very much improved,"while 73.6% of placebo nonresponders had PGIC scores of "unchanged"or "worsened". *Conclusions.* Serial administration of the modified MFSAF v2.0 diary demonstrated that treatment with ruxolitinib provided rapid, significant, and sustained improvements in MF symptoms vs placebo over the 24-week treatment period.

0913

SIDE EFFECTS OF HYDROXYUREA IN CLASSIC CHRONIC MYELOPROLIFERATIVE NEOPLASMS. A RETROSPECTIVE STUDY OF 3,411 PATIENTS

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Backgroud. Hydroxyurea (HU) is the drug of choice for the treatment of patients with high risk myeloproliferative neoplasms (MPN). Its more frequent side effects are represented by gastrointestinal toxicity, cutaneous and mucosal toxicity, pulmonary toxicity and fever. A number of anecdotal cases have been reported in the literature, but epidemiological information on large series of patients are not yet available. *Aims.* To collect information on the rate and characteristics of HU-related side effects, we examined data from a retrospective survey of ten hematological centers in Italy. To ensure consistency of data, we only included clinically relevant HU-related manifestations such cutaneous and mucosal lesions, fever and pneumonitis. *Methods.* This study was performed within the GIMEMA (Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto) MPN Working Party, under the auspices of AGIMM (AIRC-Gruppo Italiano Malattie Mileoproliferative). Centers were asked to identify, in their own data base, all subjects with a diagnosis of MPN who had received hydroxyurea and developed one of the side effects considered above. Diagnosis of PV, ET or PMF was made according to either the WHO2008 or PVSG/WHO2001 criteria, while diagnosis of post-PV or post-ET MF was made according to the IWG-MRT plus the histological WHO criteria. Furthermore, for comparison populations, each participating centre provided information on the total number of referring patients with matched diagnosis who had been in treatment with HU and had not developed side effects in the same period of time. *Results.* The whole study population was comprised of 3,411 MPN patients, of whom 963 were PV, 1912 ET, 357 PMF, 93 PPV and 86 PET. A total of 184 patients (5%) who developed HU-related side effects were identified: 16 of them developed fever, 167 muco-cutaneous lesions and 1 pneumonitis. Pulmonary toxicity attributed to HU was diagnosed in a 68-year-old male with JAK2V617F negative PMF who was being treated since ten years with 1 g/ daily. High degree (>39.°C) fever developed in 16 patients: 4 PV, 11 ET and 1 MF. Eight males and 8 females, median age 64 yrs (range 50-79 yrs), 86% were JAK2V617F mutated. Fever was reported after a median period of 31

Table 1.

	Mucosal lesions	Cutaneous ulcers	Other cutaneous lesions
PTS n	28	118	21
M/F	4/24	46/72	13/8
Age (yrs) median	60 (19-76)	66 (23-85)	63 (38-81)
Disease	PV= 11 ET= 17 MF = 0	PV= 35 ET= 61 MF = 22	PV= 11 ET= 8 MF = 2
% JAK2V617F pos	83%	72%	65%
Previous therapy	Interferon =1 Anagrelide = 2	Pipobroman = 7 Interferon= 2 Busulfan= 3 Others = 3	No
Site	Oral =27 Genital =1	Footankle = 15 Malleolus = 58 Leg = 38 Other = 7	Keratosis=7 Dyschromias 4 Dermatitis=4 Basalioma =3
HU median daily dose (gr)	1 (0.5-5)	1 (0.25-2)	1 (0.15-1)
HU total dose for patient (gr)	1157 (41-6948)	1947 (12-9483)	1356 (54-4368)
Median time HU treatment (months)	41 (1-231)	78 (2-262)	60 (5-221)

days (range, 1-109) of treatment at median dosage of 0.5 g daily (range, 0.15-1 g), for a total HU median dose of 15 g (range, 0.5-52.5 g) for patient. Muco-cutaneous lesions were referred by 167 patients; 28 patients developed mucosal lesions, 118 patients presented cutaneous ulcers, while other cutaneous lesions including keratosis, dyschromia, basalioma and dermatitis developed in 21 patients, as detailed in the table. Two patients reported both mucosal and cutaneous lesions. *Conclusions.* With the intrinsic limitations of the retrospective design, this study provides, for the first time, an estimate of the rate of HU-related side effects (5%) overall strenghtening the good tolerability of the drug.

0914

VALIDATION OF THE MYELOPROLIFERATIVE NEOPLASM SYMPTOM ASSESSMENT FORM (MPN-SAF) IN FRENCH, SPANISH, GERMAN, AND ENGLISH (UK)

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Background. We previously validated the MPN-SAF, a unique instrument for assessment of MPN symptoms based on patient reported outcomes, among 402 MPN patients including independent translations in English (USA), Italian and Swedish (Blood 2010). *Aims.* We sought to further validate MPN-SAF using prospective translations in French, Spanish, German, and Dutch and a new comparison cohort for English (UK). *Methods.* The MPN-SAF was translated using previously reported methodology involving four collaborating translators. MPN patients completed a symptom packet during a physician visit consisting of the MPN-SAF, EORTC-QLQC30, and survey-related feedback. Concurrently, physicians provided assessment of patient symptom burden and pertinent demographic and disease information. *Results.* Patients: 1,031 MPN-SAF surveys were administered (English (UK) (N=57), Dutch (N=236), French (N=482), German (N=59), and Spanish (N=197, including Argentina (n=22), Uruguay (n=8), Puerto Rico (n=10), and Spain (n=157)) in 433 ET (42%), 393 PV (38%), and 197 MF patients (19%) (8 missing). Participants were of typical age (mean 61.0, range 20 - 94 years) and gender (54% female) of disease. Prior hemorrhage (5%) and thrombosis (24%) were frequent, as were hematological abnormalities of anemia, thrombocytopenia, or leukopenia (21%). Validation of New Translations of the MPN-SAF: Patients were symptomatic (>50% prevalence) in the majority of MPN-SAF items (Table 1). MF in general had the highest severity and prevalence of symptoms, followed by PV and then ET. Fatigue was the most prevalent item (89%), with moderate or severe rating present in 60% of patients. Similarly, 75% of patients had reduced QOL and this reduction was moderate-to-severe in 34%. Patients rated the MPN-SAF as "easy to understand"(1.7/10) and having "addressed most symptoms"(2.0/10). Comparison to EORTC-QLQC30: Strong correlations existed between individual symptoms represented on both the MPN-SAF and the EORTC-QLQC30 including fatigue, inactivity, insomnia, and bone pain (r=0.51 to 0.82, all p<0.001). Additionally EORTC-QLQC30 subscales were highly correlated with corresponding items of fatigue, inactivity, concentration, and sad mood (r=-0.50 to -0.73, all p<0.001). Comparison to Physician Perceptions: Physician's blinded opinions of patients' symptoms displayed strong correlations for fatigue and weight loss (r>0.5, p<0.001 for both), with moderate correlations for fevers, night sweats, bone pain, and pruritus (r=0.34 to 0.48, p<0.001). Comparison Across Languages: After adjusting for age and MPN type, there were no significant differences across languages for specific MPN-SAF items of abdominal pain, abdominal discomfort, headache, numbness, insomnia, fever and weight loss. There were no significant differences between UK and USA cohorts for any MPN-SAF item. Comparison with prior MPN-SAF: After adjusting for age and MPN type, there were no significant differences between current data and prior English (USA), Swedish, and Italian cohorts, except for early satiety, inactivity, headache, and QOL (p<0.05). *Conclusions.* The MPN-SAF is a valid PRO assessment of symptom burden for MPN patients worldwide. Strong correlations were seen between the translations and co-validation measures. Minor language-specific variations in MPN-SAF severity exist which likely represent cultural influences, language nuances, or variations in patient cohorts. Further use of the MPN-SAF in clinical trials is recommended as a tailored assessment of MPN symptom burden internationally.

Table 1.

Symptomatic burden of MPNs as assessed by the MPN-SAF in a prospective international survey of 1,031 MPN patients (results are reported as mean [prevalence]). All items measured on a 0 (Absent) to 10 (worst imaginable) scale.

	English (UK) (N=57)	Dutch (N=236)	French (N=482)	German (N=59)	Spanish (N=197)	Total (N=1,031)
Fatigue (BFI Score)	3.4 (70%*)	4.3 (75%*)	2.8 (52%*)	2.8 (78%*)	3.2 (55%*)	3.2 (69%*)
Sexuality Problems	2.8 (33%)	4.2 (72%)	2.6 (58%)	3.3 (65%)	3.3 (80%)	3.2 (67%)
Insomnia	3.1 (49%)	3.4 (72%)	2.8 (68%)	3.3 (72%)	2.9 (44%)	3.0 (58%)
Quality of Life	2.9 (33%**)	4.0 (54%**)	3.9 (21%**)	3.8 (33%**)	3.1 (40%**)	3.7 (34%**)
Early Satiety	2.7 (67%)	3.2 (72%)	2.7 (60%)	2.3 (58%)	2.7 (65%)	2.8 (65%)
Concentration Problem	2.9 (59%)	4.1 (79%)	3.7 (57%)	2.5 (78%)	2.5 (62%)	2.5 (64%)
Numbness	1.9 (50%)	2.7 (64%)	2.6 (66%)	2.1 (57%)	2.7 (63%)	2.5 (63%)
Inactivity	3.4 (65%)	3.1 (72%)	2.3 (61%)	3.1 (77%)	2.0 (50%)	2.4 (62%)
Sad Mood	2.8 (67%)	2.7 (66%)	2.1 (60%)	2.2 (57%)	2.9 (65%)	2.4 (62%)
Itching	2.2 (33%)	3.0 (64%)	1.9 (50%)	2.2 (59%)	2.1 (49%)	2.2 (53%)
Night Sweats	2.3 (47%)	2.9 (64%)	1.7 (48%)	1.4 (41%)	2.1 (59%)	2.1 (57%)
Dizziness	2.8 (54%)	3.0 (73%)	1.6 (52%)	1.8 (58%)	1.6 (47%)	2.0 (59%)
Headache	2.3 (33%)	2.2 (58%)	1.8 (57%)	1.8 (51%)	1.7 (59%)	1.9 (55%)
Bone Pain	1.8 (44%)	2.1 (59%)	1.5 (45%)	1.4 (41%)	2.7 (66%)	1.9 (49%)
Abdominal Discomfort	1.8 (31%)	2.1 (57%)	1.7 (55%)	1.8 (44%)	1.7 (57%)	1.8 (54%)
Abdominal Pain	1.3 (42%)	1.8 (49%)	1.4 (49%)	1.2 (34%)	1.4 (42%)	1.5 (47%)
Cough	1.3 (49%)	2.0 (50%)	1.2 (44%)	0.9 (32%)	1.7 (47%)	1.5 (45%)
Weight Loss	0.9 (24%)	1.2 (38%)	0.9 (38%)	0.8 (24%)	1.2 (31%)	1.0 (29%)
Fever	0.2 (8%)	0.5 (20%)	0.4 (21%)	0.1 (7%)	0.3 (13%)	0.4 (18%)

*Indicates % with moderate (4-6) or severe (7-10) ranking of worst fatigue item.
**Indicates % clinically deficient (>4/10) QOL score.

0915

SYMPTOMATIC BURDEN OF MYELOPROLIFERATIVE NEOPLASMS (MPN) IN FRANCE: A PROSPECTIVE TRIAL OF 482 PATIENTS

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Background. Myeloproliferative neoplasms (MPNs) are a subset of hematological malignancies with significant symptomatic burden and a rapidly evolving treatment options. We have previously reported on the disease burden and impact on Quality of Life (QoL) among afflicted patients, but no prospective trials existed among a sizable European cohort. *Aims.* We prospectively sought to gauge the cultural relevance of a measure of symptom burden amongst a large cohort of MPN patients in France. *Methods.* Patient completed a symptom assessment packet including a French translation of the Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF: French) and the French EORTC-QLQ-C30. Physicians concurrently completed a form detailing patient demographics, perception of the pt's symptomatic burden, and MPN diagnosis, treatment and clinical course. *Results.* Patients: 482 patients (ET (N=227; 48%), PV (N=175; 37%) and MF (N=75; 16%)) were prospectively enrolled from university and private hospitals in France. Patients were of a median age (62; range 22-91 years) and gender (56% females) typical of the disease with a median of 7 years (range:1-35) from their diagnosis. Patients frequently had a history of either thrombotic events (27%) and/or hemorrhagic events (4%). Anemia (13%), polycythemia (5%), leukopenia (5%), thrombocytopenia (8%), and thrombocytosis (9%) were common. Symptomatic Burden: The MPN-SAF: French indicated that insomnia, fatigue, problems with sexuality, numbness, and inactivity were most severe among French patients (see Table 1). Symptoms with highest prevalence included insomnia, numbness, early satiety, inactivity and sad mood. Similar to our prior studies, symptomatic burden was most severe and prevalent in MF, except for headache which was least severe in MF. Interestingly, pruritus was not most burdensome in PV patients. Validation Analysis: EORTC-QLQ-C30: Consistent with our experience findings, Pearson correlations between MPN-SAF: French individual symptom scores and the French EORTC-QLQ C30 showed excellent correlations with co-validation questions (>0.5 Pearson correlation). EORTC assessment of fatigue correlated significantly with overall BFI, worst BFI, inactivity and insomnia score ($p<0.001$). Additionally, excellent correlations were demonstrated between EORTC-QLQ-C30 subscales and corresponding MPN-SAF measurements, particularly for items of BFI, inactivity, concentration, and sad mood ($p<0.001$). Physicians and Patient's perceptions: Correlations between physician's blinded perceptions of disease burden and patient-reported symptom severity in six categories of disease symptoms (night sweats, fevers, fatigue, weight loss, bone pain, and pruritus) were excellent for all items ($p<0.001$). Patients indicated that they felt that the survey was comprehensive of their disease symptoms (mean score 2.2/10) and easy to understand (mean score 1.7/10). *Conclusions.* The MPN-SAF: French is an easily administered, clear 27-item inventory of patient-reported outcomes that is specific to MPNs and validated by 1) comparison to compiled international data on MPN-SAF scores and 2) the correlation with the EORTC-French. Utilization of the instrument in French MPN clinical trials will serve as a valuable and specific clinical marker of disease symptom severity among this population.

Table 1.

Symptomatic burden of MPNs as assessed by the MPN-SAF in 482 French patients based on disease type (results are reported as mean (% prevalence). All items measured on a 0 (Absent) to 10 (worst imaginable) scale.

	ET (N=227)	PV (N=175)	MF (N=75)	French (N=482)
Insomnia	2.8 (69%)	2.8 (66%)	3.0 (69%)	2.8 (68%)
Fatigue (BFI Score)	2.5 (33%*)	2.7 (41%*)	3.8 (61%*)	2.7 (53%*)
Sexuality Problems	2.3 (55%)	2.6 (56%)	3.6 (75%)	2.6 (58%)
Numbness	2.2 (64%)	2.4 (66%)	3.1 (73%)	2.4 (65%)
Inactivity	1.8 (58%)	2.3 (60%)	3.7 (81%)	2.3 (61%)
Early Satiety	2.1 (61%)	2.0 (60%)	3.1 (78%)	2.2 (63%)
Sad Mood	1.9 (57%)	2.1 (59%)	2.7 (72%)	2.1 (59%)
Itching	1.5 (46%)	2.2 (53%)	2.4 (61%)	1.9 (50%)
Quality of Life	1.7 (18%**)	1.9 (21%**)	2.4 (31%**)	1.9 (21%**)
Headache	1.8 (57%)	1.8 (57%)	1.6 (58%)	1.8 (57%)
Abdominal Discomfort	1.7 (56%)	1.4 (49%)	2.5 (70%)	1.7 (55%)
Concentration Problem	1.7 (56%)	1.6 (53%)	2.2 (70%)	1.7 (57%)
Night Sweats	1.6 (49%)	1.5 (43%)	2.3 (64%)	1.7 (49%)
Dizziness	1.6 (50%)	1.4 (53%)	2.2 (58%)	1.6 (53%)
Bone Pain	1.5 (42%)	1.4 (46%)	2.2 (54%)	1.5 (45%)
Abdominal Pain	1.4 (49%)	1.2 (47%)	2.0 (58%)	1.4 (50%)
Cough	1.1 (43%)	1.1 (42%)	1.9 (56%)	1.2 (44%)
Fever	0.4 (19%)	0.4 (21%)	0.6 (32%)	0.4 (21%)
Weight Loss	0.8 (25%)	0.7 (30%)	1.8 (48%)	0.9 (30%)

**Indicates % with moderate (4-6) or severe (7-10) ranking of worst fatigue score.*
***Indicates % clinically deficient (≥4/10) QOL score due to MPN*

0916

THE ITALIAN MASTOCYTOSIS REGISTRY: AN UPDATE FOCUSED ON HISTOPATHOLOGICAL FINDINGS

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Background. Mastocytosis is a rare disease characterized by an abnormal proliferation and accumulation of mast cells (MC) in several organs and tissues such as skin, bone marrow, liver, gastrointestinal tract and lymphnodes. It is a clonal disease associated to a somatic mutation of the proto-oncogene c-kit (KIT). Clinical signs and symptoms of mastocytosis mainly depend on the liberation of chemical mediators produced by the mast cells. While the diagnosis of systemic mastocytosis requires the presence of multifocal dense mast cell infiltrates in one or

multiple extra-cutaneous organs (mostly bone marrow, due to the origin of MCs), mastocytosis encompasses a wide range of clinical entities, extremely heterogeneous for clinical course and prognosis. Due to its heterogeneity, mastocytosis is a multidisciplinary pathology involving different specialists. *Aims.* The Italian Mastocytosis Registry was constituted in 2009 with the aim of collecting data about patients diagnosed with mastocytosis at a national level. *Methods.* Anagraphical, anamnestic, clinical, hystopathological, biological, treatment and follow-up data of patients are being routinely collected after written informed consent in 17 Italian centers. An on-line database (www.registroitalianomastocitosi.it) has been set up for this purpose. *Results.* At present, data on 293 patients have been collected. One-hundred-forty-two (48.5%) are females; 151 (51.5%) are males. Ninety-five (32.4%) were diagnosed when younger than 18 years old. One-hundred-thirty patients (44.4%) have been diagnosed with systemic mastocytosis and 73 (56%) of them progressed from a cutaneous disease. Two-hundred-thirty-six patients (80.55%) have cutaneous symptoms. Among the 98 adult patients who were assessed at the time of diagnosis, 88 (89.8%) were symptomatic. One-hundred-and-one (34.5%) patients reported allergies. Of 207 reported lines of therapy, 28% were symptomatic, 43% anti-HH1, 17% anti-HH2, 24% corticosteroids, 16% phototherapy, 4% alpha-interpheron and 13% chemotherapy (total exceeds 100% because therapy combination is allowed). As to histological findings, 134 (46%) patients have data on bone marrow biopsy: 98 (73%) had a positive finding, with a median cellularity of 25% (range 3%-90%). Among 208 patients tested for tryptase, 152 (73%) were positive (median 41.3 µg/L, range 11-746). Among the 128 patients who have been evaluated for both bone marrow biopsy and tryptase, there is a positive association between the two parameters. When considering both variables as dichotomous (positive vs. negative), the two tailed Fisher exact test shows a highly significant association (p=0.00003). When analyzing the 81 patients for which the percentage of bone marrow substitution was reported, there was a significantly positive correlation with the level of tryptase (Spearman rank correlation 0.59; p<0.000001). *Conclusions.* This is the first spontaneous observational study on mastocytosis in Italy. The on-line database is a useful tool for data collection at a national level. The Registry is an opportunity to carry out epidemiological studies aimed at estimating prevalence, incidence and geographical distribution of the disease. It will also provide a standardization of the histopathology criteria to improve the understanding of this disease, also in comparison to other diseases. It will finally allow specialists to investigate possible prognostic factors and provide a starting point for the research into ad hoc therapies.

0917

VALIDATED AND CANDIDATE THROMBOTIC RISK FACTORS IN ESSENTIAL THROMBOCYTHEMIA: PRELIMINARY ANALYSIS OF THE REGISTRO ITALIANO TROMBOCITEMIA (RIT)

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Background. In essential thrombocythemia (ET), the validated risk factors for thrombosis at onset and during the follow-up are represented by age over 60 y, and history of thrombosis. The thrombocytosis, constitutive abnormality in ET, is associated with both thrombotic and hemorrhagic complications. JAK2 mutation and leukocytosis have been reported as associated with high rate of thrombosis. *Aims.* To evaluate in a large cohort of ET patients the potential thrombotic risk factors as JAK2 mutation, leukocytosis, and other clinical and biological parameters. *Methods.* A cohort of ET patients (PVSG or WHO criteria) of the Registro Italiano Trombocitemia (RIT), with information also on the bone marrow biopsy at diagnosis, were considered for this retrospective analysis. *Results.* A total of 977 patients, 387 males and 590 females, presented at diagnosis: median age 55 y, median PLT count 783 x 10^9/L, median WBC count 8.8 x 10^9/L, median Hb 14.1 g/dL, history of thrombosis in 189 cases (19.3%), history of hemorrhage in 49 cases (5.0%). The patients at high risk (age over 60 y and/or history of thrombosis) were 511 (52 %). During the follow up (4088 pt-y), thrombotic events were reported in 35 patients (3.6%). The thrombotic events at onset of disease were significantly related to: age over 60 (p 0.001), male gender (p <0.05), lower grade of thrombocytosis (PLT < 783 x 10^9/L, p 0.001), higher grade of leukocytosis (WBC > 8.8 x 10^9/L, p 0.01), and JAK2 V617F mutation (p 0.06). No relationship was found with bone marrow fibrosis grade. The 977 patients were subdivided in four groups: lower thrombocytosis and higher leukocytosis (group 1: 202 pts); lower thrombocytosis and lower leukocytosis (group 2: 270 pts); higher thrombocytosis and higher leukocytosis (group 3: 272 pts); higher thrombocytosis and lower leukocytosis (group 4: 197 pts). In those patients, the rate of thrombosis at onset was: 26.7% in the group 1(both risk factors); 24.1% in the group 2 (PLT risk factor); 20.2% in the group 3 (WBC risk factor); 7.6% in the group 4 (no risk factors). The rate of thrombosis in patients with one or two of these risk factors was significantly (p 0.001) higher than in patients with no risk factors. The thrombotic events during the follow up are still object of analysis. *Conclusions.* In this cohort of ET patients the rate of thrombosis at onset of disease has been confirmed to be related to age over 60 y. Moreover, a significant relationship has been found with male gender, JAK2 mutation, WBC count over the median value (8.8 x 10^9/L), and PLT count below the median value (783 x 10^9/L).

0918

INFLUENCE OF 46/1 JAK2 HAPLOTYPE IN THE NATURAL EVOLUTION OF JAK2V617F ALLELE BURDEN IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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Background. The 46/1 JAK2 haplotype predisposes to the development of JAKV617F-associated myeloproliferative neoplasms (MPN) but its clinical relevance after diagnosis is unknown. Objetive. To assess the influence of 46/1 JAK2 haplotype on the natural evolution of JAK2V617F allele burden in patients diagnosed with JAKV617F-associated MPN. *Methods.* JAK2V617F allele burden was prospectively measured in 62 patients with newly diagnosed JAKV617F-associated MPN, corresponding to polycythemia vera (PV) and essential thrombocythemia (ET) in 26 and 36 cases, respectively. Molecular monitoring was performed at diagnosis and every 6-12 months while patients remained free of cytoreductive therapy. SNPs rs12340895 and rs12343867 were used to determine 46/1 JAK2 haplotype status. JAK2V617F allele burden at diagnosis and during follow-up was compared according to 46/1 JAK2 haplotype status (negative, heterozygous, homozygous). The study was approved by the local Ethics Committee and informed consent was obtained according to the Declaration of Helsinki. *Results.* Twenty cases (ET 13, PV 7) were negative for the 46/1 JAK2 haplotype whereas 42 patients carried the 46/1 JAK2 haplotype in heterozygosis (n=29: ET 16, PV 13) or homozygosis (n=13: ET 7, PV 6). Mean JAK2V617F allele burden at diagnosis was 27%, 34% and 41% in patients negative, heterozygous and homozygous for the 46/1 JAK2 haplotype, respectively (p=0.06). Median molecular follow-up was 50 months (range: 12-92) with mean JAK2V617F allele burden at last follow-up being 30%, 38% and 55% in patients negative, heterozygous and homozygous for the 46/1 JAK2 haplotype, respectively (p=0.01). JAK2V617F allele burden remained stable during follow-up in 44 patients whereas 18 patients showed a JAK2V617F increase higher than 10%. The mean increase in JAK2V617F allele burden from diagnosis to last sample was 4.5%, 6% and 15% in patients negative, heterozygous and homozygous for the 46/1 JAK2 haplotype, respectively (p=0.02). There was no significant difference in the mean percentage increase of JAK2V617F allele burden according to age, gender and type of MPN diagnosis. *Conclusion.* The natural evolution of JAK2V617F-associated MPN in patients carrying the 46/1 JAK2 haplotype in homozygosis is associated with a progressive increase in the JAK2V617F allele burden.

0919

THE IMPACT OF CLINICO-HEMATOLOGICAL FEATURES AND MOLECULAR BIOMARKERS ON OUTCOME OF PATIENTS WITH PRIMARY MYELOFIBROSIS

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Background. Primary myelofibrosis (PMF) is the rarest subtype of the Philadelphia-negative myeloproliferative neoplasms (MPN). Several

biomarkers, including Endogenous Erythroid Colony (EEC), PRV-1 mRNA expression, X-chromosome inactivation patterns (XCIPs) in females, JAK2^{V617F} and *MPL* gene mutations have been described as useful tools to characterize MPN. The implication of these biomarkers and their correlations to the clinico-hematological features and outcome remained to be determined in patients with PMF. *Aims.* We aimed to determine (1) the frequencies of the biomarkers, and (2) the correlations among the biomarkers, clinico-hematological data and outcome of PMF patients. *Methods.* Granulocytes were isolated from 115 patients with PMF diagnosed by WHO criteria. Allele-specific PCR assay was used to detect JAK2^{V617F}, PCR followed by direct sequencing for *MPL* mutation. EEC assay was performed in a serum-free culture system. PRV-1 mRNA expression in granulocytes was measured by RQ-PCR TaqMan assay, and HUMARA-PCR assay for XCIPs was performed in female patients. *Results.* Of the 115 patients, the median age was 63.8 years, 59 were males. The median level of Hb was 9.6 g/dL, WBC was 11.6 x 10^9/L and PLT count was 365 x 10^9/L. JAK2^{V617F} was detected in 58 of 115 (50.4%) with homozygous pattern in 9; *MPL* mutations in 3 of 93 (3.2%). EEC formation was present in 42 of 85 (49.4%), and PRV-1 overexpression in 32 of 59 (54.2%). Clonal XCIPs was detected in 19 of 22 female examined (86.4%). JAK2^{V617F} mutation was strongly associated with the presence of EEC formation and PRV-1 overexpression ($p<0.0001$). EEC formation was highly associated with PRV-1 overexpression ($p=0.0007$) and lower grade bone marrow fibrosis ($p=0.0245$). Both JAK2^{V617F} and EEC formation were associated with WBC >25.0 x 10^9/L and less circulating blast cells (<1%). PRV-1 overexpression was associated with a high WBC count only. Median overall survival (OS) was 70.2±11.3 months. Fourteen patients (12.2%) developed acute leukemia transformation. Adverse prognostic factors of OS were older age (> 65), Hb <10 g/dL, PLT count < 100 x 10^9/L and absence of EEC formation in a univariate analysis; older age and lower Hb level in the multivariate analysis. Patients who had EEC formation and less circulating blast cells (<1%) at diagnosis had a longer time to leukemia transformation. JAK2^{V617F} mutation and PRV-1 overexpression did not influence OS or time to leukemia transformation. Splenic radiation and ≥1% of circulating blast cells at diagnosis increased the risk of acute leukemia transformation, but other clinico-hematological data, the marrow fibrosis grade or the status of biomarkers did not. *Conclusions.* Our data showed that JAK2^{V617F} was highly associated with EEC growth and PRV-1 overexpression. Factors that adversely affected OS included older age, lower Hb, lower PLT count and absence of EEC formation. Splenic radiation and higher circulating blast cells increased the risk of acute leukemia transformation.

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0920

NEUROLOGICAL SYMPTOMS IN ESSENTIAL THROMBOCYTHEMIA (ET): INCIDENCE, ROLE OF BRAIN IMAGING, INFLUENCE OF JAK2V617F MUTATION, AND OUTCOME

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Background. ET is characterized by thrombotic and ischemic complications in various vascular territories, including the brain. Such neurological complications include transient ischemic attacks (TIA), strokes or cerebral venous thromboses (CVT). In addition, patients may present with a broad range of transient neurological symptoms such as blurred vision, headache, tinnitus, dizziness. Those subjective symptoms are usually considered to be due to cerebral ischemia and highly sensitive to low-dose aspirin (ASA). They were frequently reported in retrospective series (in 20 to 55% of pts), but their incidence in newly diagnosed patients was not clearly assessed prospectively. *Patients and Methods.* From January 08 to June 09, all consecutive newly diagnosed ET patients (WHO criteria) from our center presenting with neurological symptoms were systematically referred to a neurologist for evaluation. Specific explorations included brain MRI with perfusion sequences, cardiac and supra-aortic echography in case of thrombotic symptoms. *Results.* 37 patients were newly diagnosed with ET during the study period. 11/37 (30%) pts complained of possibly ET-related neurological symptoms (several symptoms could be present in the same pt): TIA in 3, CVT in 1, cephalalgia in 5, dizziness / tinnitus in 5, visual disturbances in 2, loss of consciousness in 1 patient, respectively. In the majority of patients (8/11, 72%), those symptoms were subjective, chronic cephalalgia being the most frequent (5/8, 62%), and were always either transient (from few seconds to few minutes) or fluctuating. Neurological examination found no objective abnormality in any patient. Brain MRI (performed in 9/11 pts) failed to detect any objective substratum for these symptoms, even with perfusion sequences (performed in 7/9 pts). Clinical and hematological characteristics (including sex, age, cardiovascular risk factors, platelet counts, WBC, hemoglobin) were not statistically different between the 11 patients with, and the 26 patients without neurological symptoms. JAK2V617F mutation was found in 9/11 (82%) patients with neurological symptoms vs. 14/26 (54%) patients without symptoms. Response to ASA was heterogeneous (1/11 pts already on anticoagulant excluded): complete resolution of neurologic symptoms was observed in 3/10 (30%), improvement with persisting transient episodes in 2/10 (20%), and resistance to ASA in 2/10 (20%) of patients, respectively. In the 2 last patients, complete resolution of symptoms was only observed after initiation of cytoreductive therapy with hydroxyurea. *Conclusion.* In this prospective study of 37 consecutive, unselected newly diagnosed ET patients with specialized neurological evaluation, we found that: 1) incidence of neurological symptoms was 30%, mainly composed of subjective symptoms; 2) brain MRI was always normal in patients with subjective symptoms, suggesting that MRI is not useful in the baseline evaluation of ET patients with atypical neurological symptoms after careful exclusion of focal neurological deficits; 3) JAK2V617F mutation could be a risk factor for developing neurological symptoms in ET, if confirmed in a larger cohort; 4) in contrast to previous reports, low-dose aspirin successfully resolved neurological symptoms in only 30% of cases, the addition of cytoreductive therapy being necessary in some patients.

0921

PRELIMINARY DATA ON CLINICAL ASPIRIN RESISTANCE IN A LARGE COHORT OF PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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Background. Thrombotic complications are major cause of morbidity and mortality in patients with myeloproliferative neoplasm (MPN). Low-dose aspirin significantly reduce the risk of thrombotic complications in polycythemia vera (PV) patients, and is commonly used also in essential thrombocythaemia (ET). In general population, however, some patients taking aspirin (ASA) have thrombotic event in spite of treatment: this is defined as "clinical resistance to ASA". The aim of our study was to retrospectively evaluate clinical ASA resistance in patients with MPN. *Patients and Methods.* The study comprehends 290 MPN patients diagnosed between 1970 and 2009 in accordance with the criteria in use at the time of the first observation and treated in agreement with the current treatment guide-lines. All major thrombotic events occurred during follow up were recorded. Among the 290 patients enrolled, 231 (group A; 79.6%) used ASA (100mg/day) and 59 (group B; 20.4%) did not. The main features of our patients are summarized in table 1. *Results.* 16 patients (4 males, 12 females; 8 ET and 8 PV) of group A (6.9%) had a thrombotic event after a median ASA therapy of 6.04 years: 8 arterial thromboses (3.4% of ASA-treated patients) (4 coronary disease, 1 stroke, 2 TIA and 1 intestinal infarct) and 8 venous thromboses (5 deep and 3 splanchnic vein thrombosis). 62.5% of patients had thrombosis after more than 5 years of treatment. All but 13 patients were JAK2V617F. 10 females (6 ET and 4 PV, all carrying JAK2V617F mutation) of group B (16.9%), had a thrombotic event after a median disease duration of 4.19 years: 3 arterial (1 coronary disease, 1 stroke and 1 TIA) e 7 venous (3 deep, 3 splanchnic vein e 1 cerebral sinus thrombosis). The overall occurrence of thrombotic complications was significantly less ($p = 0.03$) in group A than in group B. *Discussion.* The concept of therapeutic resistance emerged in general population with the clinical obser-

Table 1.

	TOTAL	GROUP A	GROUP B
Total	290	231	59
M/F	120/170	93/138	27/32
ET/PV/MF	154/132/4	127/101/3	27/31/1
Mean age at diagnosis (y)	53 ± 17	55 ± 16	51 ± 18
Median follow-up (y)	9.01	8.76	7.91
JAK2 V617F/WT	193/77	152/63	41/14

vation that aspirin-takers are not invariably protected from acute cardiovascular events. Moreover, loss of therapeutic advantage has been found in individuals on long-term treatment. Our study shows that also in MPN patients ASA-resistance occurs, mainly in long-time treated patients. While it is not surprising that vein thrombosis are not prevented by aspirin, the occurrence of arterial thrombosis in 3.4% of MPN treated with ASA suggest that clinical ASA-resistance is not absent in this particular set of patients. No relation with JAK2 mutational status was observed. Our results need to be confirmed by specific laboratory tests.

0922

HAEMATOLOGICAL ABNORMALITIES ARE COMMON IN NEONATES WITH DOWN SYNDROME AND MAY REVEAL CLINICALLY 'SILENT' LEUKAEMIA

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Background. Neonates with Down syndrome (DS) are uniquely predisposed to Transient Abnormal Myelopoiesis (TAM), a leukaemic disorder characterised by acquired mutations in the GATA1 gene, which transforms to a clonally-linked myeloid leukaemia (ML-DS) in 20-30% of patients. Children with ML-DS often have no history of TAM, suggesting TAM can be 'silent' since full blood counts (FBC) and blood smears are not routinely performed despite reports of frequent haematological abnormalities in DS neonates. *Aim and Methods.* The prospective Oxford Down Syndrome Cohort Study (ODSCS) systematically determined haematological indices and blood cell morphology in the first week of life in neonates with DS (n=170) compared to normal neonates (n=123). Mutational analysis of GATA1 was performed on leucocyte DNA by PCR. *Results.* Compared to gestation-matched controls, neonates with DS had higher Hb concentrations (20.1+0.2 v 16.6+0.2 g/dL; p<0.0001), increased erythroblastosis (23.5+5 v 3.2+0.5/ 100 leucocytes; p=0.0001) and macrocytosis (MCV 107.6 v 101.5fl; p<0.0001). Neonates with DS had lower platelet counts (165+7 v 252+5x109/L; p<0.001), 98.0% had abnormal platelet morphology and almost half (71/170, 41.8%) were thrombocytopenic (platelets <150 x 109/L). Leucocytes (p=0.0012), neutrophils (p=0.0005), monocytes (p<0.0001) and basophils (p<0.0001) were higher in DS neonates and dysplasic features, rare in controls, were seen in all DS neonates. Fourteen neonates (7.5%) had a final diagnosis of TAM confirmed by the presence of exon 2/3 GATA1 mutations. Neonates with TAM had lower Hb concentrations (17.7+0.8 v 20.1+0.2 g/dL; p=0.0012) and higher MCV (113.3+2 fl; p=0.0088) than DS neonates without TAM and a significantly higher mean platelet count (229.8+88.3 x 109/L; p=0.0042) with a very wide range (36-1208 x 109/L). Total leucocyte counts (38.3+5.4 v 15.2+0.6; p<0.0001) and neutrophils (16.2+2.2 v 9.7+0.5; p=0.0003) were higher in neonates with TAM compared to DS neonates without TAM but there was considerable overlap in values. Blast cells were present on blood smears from all neonates with TAM (33.3+4.5%, range 15-77%) and 98.1% of DS neonates without TAM (4.5+0.3%, range 0.2-18%) although only 7 neonates without TAM (4.4%) had >10% blasts and none of these neonates had GATA1 mutations. Automated blast cell counts were unreliable in nearly all cases. TAM was clinically 'silent' in 2/14 cases (14.3%) and there was no difference between the haematological features of these 2 neonates compared to the other 12 neonates with TAM. *Conclusions.* All neonates with DS have haematological abnormalities usually affecting red cell, platelet and myeloid cell lineages. The most serious complication, TAM, may be clinically silent. These data suggest that FBC and blood smears should be part of the assessment of all newborn babies with DS in the first week of life and screening for GATA1 gene mutations performed where blasts exceed 10%.

0923

RISK OF EVOLUTION TO MYELOFIBROSIS AND ACUTE LEUKEMIA IN PATIENTS WITH ET OR PV.WITHOUT CLINICAL HEMATOLOGICAL RESPONSE. ACCORDING EUROPEAN LEUKEMIA NET CRITERIA

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Background. Chronic myeloproliferative diseases, Polycythemia vera (PV) and Esential Thrombocytemia (ET) have the risk of evolution to Myelofibrosis (MF) and Acute Leukemia.(AL). European Leukemia Net (ELN) party have defined new criteria to asses clinical, histological and molecular response in order to evaluate prognosis and new therapeutic agents. *Aims.* We performed a retrospective analysis of evolution of PV and ET patients in order to analyzed incidence of transformation to MF or LA in relation to ELN clinical response criteria. *Material and Methods.* Date base with clinical and laboratory data from 147 patients diagnosed of ET or PV and followed in a teaching hospital of Madrid during the last 20 years were analyzed. *Results.* 108 patients present ET at diagnosis and 38 PV. Mean age at diagnosis was 61 and 67 years respectively. Incidence of ET was more often on females (57%F%/42%M) and PV was slightly more often on males (46%W/54%M). A total of 6% develop MF or LA under evolution. ET develop MF in 6 patients (5%) and acute leukaemia (AL) in 2 patient (2,8%). 3 patients with PV develop AL (5,1%). Evolution appear at diferent time intervals: AL appear in patients between 6 and 17 years from diagnosis. MF was developed between 3 and 20 years (mean 14 years). Other 12 patients (8%) died from additional complications (2 solid neoplasia, 2 sepsis, 5 ETE 1 Hemorrhagic, 2 unknown). All were patients of high risk and none of those patients showed Complete Clinical Response according to ELN during follow up. 21 present mayor complications (thrombotic or hemorrhagic) at diagnosis /or under evolution. Three of 6 patients with MF are still alive but present clinical resistance to HU before transformation. *Conclusion.* Resistance to treatment appears as a bad prognosis indicator in patients with ET and PV. Treatment oriented to achieve complete clinical response according to ELN seen to be mandatory in these patient population.

0924

PROSPECTIVE VALIDATION OF THE DUTCH MYELOPROLIFERATIVE NEOPLASM SYMPTOM ASSESSMENT FORM (MPN-SAF: DUTCH) IN 236 MPN PATIENTS

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Background. Myeloproliferative Neoplasma (MPN) give rise to specific symptoms generally not assessed by validated Quality-of-Life (QoL) Questionnaires. In view of the reported high impact of these specific symptoms on QoL and social participation, detailed information on the effect of new developed therapies is of importance. The MPN-Symptom Assessment Form (MPN-SAF) is a concise instrument of patient reported outcomes designed to assess the unique spectrum of symptoms present in patients with MPN. *Aims.* We sought to validate this instrument and to assess symptoms in a representative Dutch population of patients with myelofibrosis (MF), essential thrombocythemia (ET) and polycythemia vera (PV). *Methods.* The MPN-SAF was translated in Dutch by one of the authors fluent in both languages, confirmed by one member of the Dutch patient MPN Foundation and sent to all members of the Dutch MPN Foundation. The Dutch EORTC-QLQ-C30 was co-administered for validation purposes. In addition, data were compared to a cohort of 102 USA MPN patients completing the MPN-SAF English. *Results.* The questionnaires were sent to 874 patients. The response rate was 39%. A total of 236 patients were included in the analysis (ET [n=72; 30.5%)], PV [n=119; 50,4%] and MF [n=45; 19,1%]), as 104 questionnaires (34%) had not been completely filled out. The distribution of the type of MPN (19.6%, 22.5% and 57.8% respectively, p<0.001) and median age (mean 58.7 ± 11.5 versus 65.2 ± 12.5, p<0.001) were significantly different as compared to the USA cohort. The time between diagnosis and response was 7 years (median, range: 0-39). The majority of patients had received non-aspirin medical therapy 62% (n=147) and 56% (n=131) was on therapy at the time of completing the questionnaire. The frequency of thrombotic events was 19% (66% reported no events, 15% unknown) and that of hemorrhagic events was 8,5% (89% reported no events, 2,5% unknown). The MPN-SAF measured 19 items in the enrolled patients on a 0 to 10 scale (Table 1). *Validation analysis.* Consistent with our experience with the MPN-SAF English Pearson correlations between MPN-SAF Dutch individual symptom scores and the Dutch EORTC-QLQ C30 showed excellent correlations with co-validation questions concerning fatigue, pain, insomnia, early satiety, and sad mood (all p<0.001). *Comparison with MPN-SAF English.* Comparison with the USA cohort (when adjusting for age and MPN subtype) showed no sta-

Table 1.

	ET (n=72)	PV (n=119)	MF (n=45)	Total (n=236)
Fatigue (BFI score)	4,2 (3,7-4,8)	4,5 (4,1-4,9)	4,1 (3,3-4,8)	4,3 (4,0-4,6)
Early satiety	2,5 (1,9-3,2)	3,2 (2,7-3,7)	4,1 (3,2-5,0)	3,2 (2,8-3,6)
Abdominal pain	1,6 (1,0-2,1)	1,8 (1,4-2,3)	2,3 (1,4-3,1)	1,8 (1,5-2,2)
Abdominal discomfort	1,7 (1,1-2,2)	2,2 (1,7-2,6)	2,5 (1,6-3,4)	2,1 (1,7-2,4)
Inactivity	2,9 (2,2-3,5)	3,2 (2,6-3,7)	3,4 (2,5-4,2)	3,1 (2,7-3,5)
Headache	2,5 (1,9-3,1)	2,2 (1,8-2,7)	1,7 (0,9-2,4)	2,2 (1,9-2,5)
Concentration	4,4 (3,7-5,0)	4,4 (3,8-5,0)	2,8 (1,9-3,6)	4,1 (3,7-4,5)
Dizziness	3,0 (2,4-3,7)	3,3 (2,7-3,8)	2,4 (1,7-3,2)	3,0 (2,7-3,5)
Numbness	2,3 (1,7-2,9)	2,9 (2,3-3,4)	2,8 (1,9-3,7)	2,7 (2,3-3,0)
Insomnia	3,2 (2,5-3,9)	3,8 (3,1-4,4)	2,9 (2,0-3,8)	3,4 (3,0-3,8)
Sad mood	2,5 (1,9-3,0)	2,9 (2,4-3,5)	2,3 (1,6-3,1)	2,7 (2,3-3,0)
Sexuality problems	3,3 (2,6-4,1)	4,6 (4,0-5,3)	4,3 (3,2-5,5)	4,2 (3,7-4,6)
Cough	1,7 (1,1-2,2)	2,1 (1,6-2,6)	2,3 (1,4-3,2)	2,0 (1,7-2,3)
Night sweats	2,7 (2,0-3,4)	2,7 (2,2-3,3)	3,4 (2,4-4,3)	2,9 (2,5-3,2)
Itching	2,0 (1,4-2,7)	3,7 (3,2-4,3)	2,5 (1,5-3,5)	3,0 (2,6-3,4)
Bone pain	1,6 (1,0-2,2)	2,2 (1,7-2,7)	2,6 (1,6-3,6)	2,1 (1,7-2,4)
Fever	0,2 (0,1-0,4)	0,5 (0,3-0,8)	0,7 (0,1-1,3)	0,5 (0,3-0,6)
Weight loss	1,0 (0,5-1,5)	1,1 (0,7-1,5)	1,5 (0,7-2,3)	1,2 (0,9-1,5)
Quality of life	3,6 (3,0-4,1)	4,4 (3,9-4,9)	3,5 (2,8-4,3)	4,0 (3,6-4,3)

tistically significances between cohorts in all but 3 items, in which the severity was higher in the Dutch cohort (dizziness [p=0.04], effect on sexuality [p=0.005], and night sweats [p=0.03]). *Conclusions.* The MPN-SAF Dutch is a 19 item inventory of patient reported outcomes that is specific to MPNs. Additionally, the instrument is validated by 1) comparison to the Dutch EORTC-QLQ C30 2) the correlation with the MPN-SAF English. Utilization of the MPN-SAF Dutch will provide information on the effect of new treatment modalities on MPN-related symptoms. It will allow for useful comparison to patients in other countries and can be used in future international clinical trials. 34% of patients did not totally complete the questionnaire, however more detailed instructions will probably improve the use in general practice.

0925

LONG-TERM USE OF ANAGRELIDE IN CHILDREN AND ADOLESCENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE DISEASES

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Background. The efficacy and safety of anagrelide (ANA) have been demonstrated in adults with essential thrombocytemia (ET). The number of children treated with ANA is very low and data on the long-term use of this drug are sporadic. *Aim.* This single-center study was aimed at evaluating the efficacy of ANA in children and adolescents with Philadelphia-negative myeloproliferative diseases (Ph- MPD), previously untreated or resistant/intolerant to other cytoreductive drugs. *Methods.* Patients (pts) aged <20 years (yrs) at the time of a Ph- MPD diagnosis carried out according to the PVSG criteria and, from 2002 onwards, to the WHO criteria were treated with ANA at a dosage ranging between 0.5-1.5 mg/day. An informed consent was obtained in all cases. All pts were also investigated for biological markers (JAK2 mutations, PRV-1 expression, thrombopoietin and its receptor (c-MPL) mutations, clonality on female pts). *Results.* From April 1997 to January 2007, 12 pts with ET (11 pts) or familial thrombocytosis (FT = 1 pt) (8 female, 4 male; median age at diagnosis: 10 yrs [range:5-19]; median age at the ANA treatment: $15^{6/12}$ yrs [range: 5-31]) were treated with ANA alone (5 pts) or in combination with hydroxyurea (HU) when the platelet (plt) count was >1000 x 10^9/L and symptoms were present (7 pts). Ten pts had previously received HU + pipobroman (2 pts) + interferon-α (1 pt) for a median time of $5^{4/12}$ yrs; 2 pts were untreated. The median time from initial diagnosis was $5^{4/12}$ yrs (range: 1 month - 11 yrs); the median plt count prior to treatment was 1062 x 10^9/L (range 322-3338 x 10^9/L). A complete response (plt <450 x 10^9/L for >1 month) was recorded in 6/12 pts (50%) after a median time of 6 weeks (range 3-24 weeks) and a partial response (plt 450 - 600 x 10^9/L for >1 month) was observed in 3 pts after 12 months, so that 9/12 (75%) pts achieved a response at a median time of 2 months after starting treatment. Interestingly, the dose of ANA was tapered and finally stopped in 2 complete responders after 32 and 72 months, respectively, of therapy and they show a normal plt count after 26 and 10 months, respectively, without no further treatment. Six of the 9 responders are still being treated after a median of $7^{6/12}$ yrs. Ten pts experienced early isolated or combined side effects, as headache (n=7), abdominal pain (n=3), vomiting (n=1), diarrhea (n=1), that disappeared over time in all but 3 pts. In these cases, persistent headache resulted in discontinuation of ANA after 5 weeks, 11 and 28 months, respectively, of treatment. Over the long term, mild self-limiting anemia has been recorded in 2 females. None of the pts developed leukemia or myelofibrosis or thrombotic complications during the long-term treatment with ANA. *Conclusions.* In our experience, ANA proved effective in controlling the plt number in thrombocytemic pts. A decrease of side effects was observed during long-term treatment with ANA; although 25 % of the total cohort stopped ANA.

Non-Hodgkin lymphoma - Clinical 2

0926

A PHASE II TRIAL ADDRESSING THE ROLE OF HELICOBACTER PYLORI-ERADICATING ANTIBIOTIC THERAPY AS EXCLUSIVE TREATMENT FOR STAGE I-II1 DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) OF THE STOMACH (HGL-1 TRIAL)

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Background. Helicobacter pylori (Hp) infection is associated with both marginal zone lymphoma of Mucosa-Associated Lymphoid Tissue (MALT)-type and DLBCL of the stomach. Hp-eradicating therapy is the standard treatment for limited-stage Hp-associated MALT lymphoma of the stomach, whereas the role of this strategy in gastric Hp-associated DLBCL is controversial, with successful reports in a few, small monoinstitutional retrospective studies. On this background, we conducted a multicentre phase II trial addressing the role of Hp-eradicating therapy as exclusive treatment in gastric DLBCL. *Aims.* To assess feasibility, activity and efficacy of Hp-eradicating therapy as exclusive treatment for limited-stage DLBCL of the stomach. *Methods.* Inclusion criteria were histopathologic diagnosis of DLBCL, with or without concomitant MALT-type areas; Hp-infection assessed by breath test or on multiple gastric biopsies; stage I-II1 of disease; hemoglobin >9 g/dl; perigastric lymph nodes diameter <1.5 cm; normal LDH serum level. Registered patients received clarithromycin 500 mg bid, tinidazole 500 mg bid and omeprazole 20 mg bid, for 7 days as exclusive treatment. Objective response and bacterial eradication were assessed by gastric endoscopy-ultrasonography, biopsies and breath test after one and two months from antibiotics. Responsive patients were referred to observation; patients with stable (SD) or progressive (PD) disease received conventional treatment. *Results.* From 2003 to 2008, 15 patients (median age 70; range 40-87; 10 males) were registered. Five patients presented concomitants MALT areas, while 10 patients had de novo DLBCL. Five patients had stage IE, 10 patients had stage II1 disease. Five patients presented anemia; two patients had concomitant HCV infection. Eradicating therapy was completed in all patients without relevant toxicity. Eradication was documented at one month in 14 patients and achieved after second-line antibiotic-therapy in the remainder patient. According to the WHO criteria, lymphoma regression was complete in 7 (47%) patients, partial in 3 (ORR= 67%), with one SD and 4 PD. Objective response was not associated with stage or concomitant MALT areas. At a median follow-up of 37 months, six of the 7 CRs are relapse-free, the remainder experienced relapse at 10 months, with a median DFS of 31+ months. Two of the three patients in PR achieve CR after rituximab. Patients with SD/PD after antibiotics achieved CR with salvage R-CHOP ± radiotherapy; none of them experienced relapse after 6-93 months. No patient died of lymphoma; two patients died of cardiac failure and gallbladder cancer, respectively; the remaining 13 patients are alive (12 patients are disease-free), with a 5-yr OS of 92%. *Conclusions.* This is the first prospective trial addressing the role of Hp-eradicating therapy as exclusive treatment in patients with gastric DLBCL diagnosed in Western countries. Patients with stage I-II1 DLBCL can be safely managed with this strategy. Half of treated patients will achieve long-term remission without chemotherapy, a critical issue considering that two-thirds of patients are >65 years old. Unresponsive patients can be safely salvaged with conventional treatment. Analysis of correlation between treatment efficacy and biological features of these lymphomas are advisable in order to select the best candidates for this ultraconservative approach.

0927

TEMSIROLIMUS FOR RELAPSED PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA: EFFICACY AND PHARMAKOKINETIC STUDY

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Background. Salvage treatment is poorly defined in primary CNS lymphoma (PCNSL). Temsirolimus is a selective inhibitor of cell proliferation promoting intracellular protein mTOR with proven activity in lymphatic malignancies. *Aims.* We designed a phase II study (NCT00942747) to evaluate activity and toxicity of a temisrolimus (TEM) monotherapy in relapsed PCNSL and to study its penetration into cerebrospinal fluid (CSF). *Methods.* Included were immunocompetent patients with histologically confirmed PCNSL relapsing or progressive after HDMTX-based chemotherapy, age ≥18 years, and ECOG ≤ 2. Primary endpoint was overall response rate, secondary endpoints were toxicity, time to progression and CSF penetration of TEM and its metabolite sirolimus (SIR). For CSF studies blood and CSF samples were simultaneously collected 30-60min after first, second and fourth infusion, and concentrations of TEM and SIR were measured by HPLC with mass spectrometry detection. *Results.* Twelve patients (median age 64 years, median ECOG PS 2) were included. Six patients each received 25mg TEM or 75mg weekly with a median of 7 (range, 2-14) infusions. Median number of previous therapy regimens was 3 including whole brain irradiation in 3 patients and high-dose chemotherapy followed by autologous stem cell transplantation in 2. Best responses were CR in two and PR in 4 patients. 6 patients did not respond or progressed. Of all objective responses, 5 (including all patients with CR) were observed in the 75mg cohort. Responding patients have a PFS of 0.7+, 1+, 2+, 2,7, 3+ and 3.1 months each (3 patients still on treatment). CTC ≥grade III toxicity was leukopenia and thrombocytopenia in 3 patients each, fatigue and pneumonia in 2 patients each and anemia in one patient,. Five patients required dose reduction, four of them in the 75mg cohort. Fourteen blood/CSF pairs were collected in 9 patients (25mg cohort: 10 pairs in 5 patients; 75mg cohort 4 pairs in 4 patients). Mean maximum blood concentration was 292ng/ml for TEM and 37.2ng/ml for SIR in the 25mg cohort; 484 ng/ml (TEM) and 91,1 (SIR) in the 75mg cohort. No drug was detected in CSF (lower detection level 1ng/ml) in either cohort. *Conclusions.* Temsirolimus at a dose of 75mg weekly showed promising activity with tolerable toxicity in the treatment of relapsed/refractory PCNSL. Neither TEM nor SIR could be detected in the CSF.

0928

HOW TO PREDICT THE OUTCOME IN MATURE T- AND NK-CELL LYMPHOMA BY CURRENTLY-USED PROGNOSTIC MODELS?

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Background. The prognosis of mature T- and natural killer (NK) -cell lymphoma (PTCL and NKTCL) is poor. Therefore, to select an appropriate prognostic model in treatment of mature T- and natural killer (NK) -cell lymphoma (PTCL and NKTCL) is crucial. *Aims.* This study investigated the usefulness of Ann Arbor staging classification 'International Prognostic Index(IPI)' Prognostic Index for T-cell lymphoma (PIT) and International peripheral T-cell lymphoma Project score(IPTCLP). *Methods.* Between 2000 and 2009, 176 patients (122 males) with PTCL and NKTCL were diagnosed and treated from a single institute in Taiwan. The correlation between complete response(CR) rate '3-year overall survival(OS)' early mortality rate and 4 prognostic models was analyzed. Thirty-one patients received hematopoietic stem cell transplantation (HSCT) and were analyzed separately. *Results.* Three-year OS rate was 34.7%, and ALCL harbored better outcome than others. IPI score had the lowest AIC value and was the best score in predicting OS. Ann Arbor stage classification can predict CR rate more precisely.

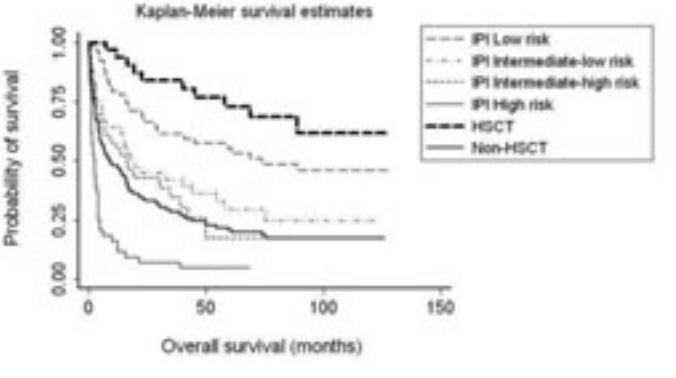

Figure 1. OS by Kaplan Meier method of HSCT group and IPI.

OS were significantly better in patients who received HSCT, even in patients with unfavorable features compared with chemotherapy alone. *Summary/Conclusion.* All prognostic models were useful to evaluate the outcome of patients with PTCL and NKTCL but IPI score did best in predicting OS.

This study also supported the role of HSCT in patients with high-risk or refractory PTCL or NKTCL.

0929

CLINICAL AND BIOLOGICAL DIFFERENCES BETWEEN PATIENTS WITH CHILDHOOD T-CELL LYMPHOBLASTIC LYMPHOMA (T-LBL) WITH AND WITHOUT MEDIASTINAL TUMORS

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Background. T-cell lymphoblastic lymphoma (T-LBL) is immature T-cell malignancies with similar morphological features and optimal treatment strategies with T-cell acute lymphoblastic leukemia (T-ALL). In adults, T-LBL is a rare form of non-Hodgkin lymphoma (NHL) with an incidence of < 1.7%. The incidence is the highest in children and adolescents, where T-LBL constitutes about 25-30% of all NHL [Swerdlow SH, *et al.*, 2008]. Results of treatment with intensive ALL-like regimens are fine and significant adverse prognostic factors could not be identified due to the small number of patients (pts) with this rare disease. Aim of this study was to investigate the effect of individual clinical and biological features on the disease outcome. *Methods.* From May 1991 to October 2008, 58 pts (m-40, f-18) with de novo LBL were treated with NHL-BFM 90 or 95 protocols for non-B-NHL (ALL-type) and 6 (10%) - NHL-BFM 90 (NHL-type). In an analysis of prognostic factors in 43 pts with T-LBL treated with ALL-type therapy were included. *Results.* Median age at time of presentation was 11.0 (range 1.5-21.6) years. 45 (90%) patients have a T-cell immunophenotype. 53 (91%) had advanced (III, IV) stage. The presenting sites of T-LBL included mediastinal mass - 35 (78%) and bone marrow (BM) involvement - 13 (29%). The complete response (CR) rate was 94 and 83% for non-B-NHL and B-NHL treatment respectively. 5-years event free survival (5y-EFS) was 0.80 ± 0.06 (median of observation 4.1 years) and 0.67 ± 0.19 (5.1 years) respectively (p>0.05). 5-years overall survival (5y-OS) was 0.85 ± 0.05 and 0.80 ± 0.06 respectively (p>0.05). The clinical features and outcome the T-LBL pts treated with ALL-type therapy was demonstrated in table 1. In a situation without mediastinal tumors the prognosis of T-LBL was unfavorable compared to without it (5y-EFS 0.56 ± 0.17 vs. 0.90 ± 0.05, p=0.036). Cases without mediastinal tumors were represented by early (pro-T/pre-T) immunological subtypes (100% vs. 36%, p=0.041) while those with mediastinal tumors by late (cortical/medullar T) subtypes. Sex, age, increased LDH, slow or fast therapy response, involvement of the central nervous system (CNS) or BM did not affect on the prognosis (p>0.05). *Conclusions.* Our data demonstrate that situation without the mediastinal tumors are a factor adverse prognosis for childhood T-LBL treated by ALL-BFM-type therapy. Based on the characteristics of a normal T-cell lymphopoiesis suggests the existence of significant biological differences between the variants of T-LBL with and without mediastinal tumors.

Table 1.

Clinical features and outcome in childhood T-LBL

Characteristic	Mediastinal tumor		P-value
	No (n=10)	Yes (n=33)	
Median age (range), years	13.5 (1.5-16.8)	10.5 (2.0-21.6)	0.41
Male sex, %	70	70	0.65
Immunophenotype, % – Pro-T/Pre-T – Cortical/Medullar T	 100 0	 36 64	0.041
CNS, %	10	10	0.70
BM involvement, %	40	24	0.27
I/II Ann-Arbor stages	40	0	0.002
5-years EFS, %	56 ± 17	90 ± 5	0.036

0930

IMPACT OF RITUXIMAB MAINTENANCE TYPE AND FCGRIIA AND FCGRIIIA GENOMIC PROFILE IN PATIENTS WITH PREVIOUSLY UNTREATED FOLLICULAR LYMPHOMA

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Background. The PRIMA study has demonstrated that maintenance with Rituximab (R) during two years significantly improve progression free survival in patients with previously untreated follicular lymphoma (FL) responding to immunochemotherapy. Polymorphisms in the IgG Fc receptor FcγRIIIa and FcγRIIa genes have been associated with response in several lymphoma types. The aim was to retrospectively analyse our experience with R maintenance (RM) in pts with previously untreated follicular lymphoma (FL) responding to rituximab or immunochemotherapy and, also to analyse the impact of polymorphisms regarding progression free survival, incidence of hypogammaglobulinemia and Ig levels. *Patients and Methods.* patients with FL in CR or PR after first-line R o R-Chemo received RM: type 1) R 375 mg/m2/week x 4 consecutive weeks every 6 months during 2 years or type 2) R 375 mg/m2 every 3 or 2 months during 2 years. FcγRIIa and FcγRIIIa genotypes were determined following a PCR-restriction fragment length polymorphism method. *Results.* Thirty-nine consecutive pts were included. Clinical characteristics at diagnosis: median age 66 years (range, 27-85); 17 (44%) male; Ann Arbor III-IV: 33 (85%); B-symptoms: 5 (11%); FLIPI ≥3: 48%. Treatment previous maintenance: 6 (15%) chemo without R, 3 (8%) R and 30 (77%) R-chemo. Twenty-two (56%) received antracyclin-containing chemo. Status previous starting RM: CR in 80% and PR in 20%. Type of RM: RM1 in 16 (41%) and RM2 23 (59%). The distribution of polymorphisms was: FcγRIIa HH 15 (22%), HR 2 (5%) and RR 22 (56%), and FcγRIIIa VV 11 (28%), VF 21 (54%) and FF 7 (18%). At a median follow-up since first R maintenance infusion of 40 months (3-106), 9 pts (23%) have relapsed and 4 (10%) have died. Overall and progression free survival at 4 years were 95% and 79%, respectively. Antracyclin-containing chemotherapy was significantly associated with a different probability of progression (HR 5.2; 95% CI 1.1-25.1, p=0.022), but not the following variables: status prior to maintenance, type of maintenance and FcγRIIa or FcγRIIIa genotype. Hipogammaglobulinemia was present in 41% and 49 % of pts before and after R maintenance, respectively. Levels of IgM diminished at the end of maintenance in pts with FcγRIIa HR-RR (p=0.019) and in those with FcγRIIIa VF-FF (p=0.017). IgG and IgA levels did not significantly change during maintenance. *Conclusions.* R maintenance for two years in patients with previously untreated follicular lymphoma (FL) responding to immunochemotherapy is a very active therapeutic strategy, but those pts receiving antracyclin-containing treatment had lower probability of progression. The two schedules of R maintenance were effective and all pts benefited independently of FcγRIIa and FcγRIIIa genomic profile. Levels of IgM were significantly influenced according to FcγRIIa and FcγRIIIa genotypes.

0931

AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) AS A CONSOLIDATION OFFERS A DURABLE SURVIVAL BENEFIT IN PATIENTS WITH PERIPHERAL T-CELL LYMPHOMAS (PTCL)

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Background. Peripheral T-cell lymphomas comprise a heterogeneous group of malignancies which are characterized by an aggressive disease course and a poor clinical outcome after conventional chemotherapy with 20-25% of patients being alived after 5 years from treatment initiation. ASCT for relapsed and refractory patients with PTCL seems to be an effective salvage regimen with event-free survival exceeding 35%, however the risk of disease progression or relapse remains high. *Material and Methods.* In a single centre retrospective study, we analyzed the results of ASCT in 29 patients with advanced stage PTCL. Patients were proceeded to transplant after achieving first or subsequent complete remission (CR) or partial response (PR) after conventional chemotherapy. *Results*. Twenty nine patients (15 male and 14 female) at a median age at diagnosis of 45 years (range 20-66 years) were analyzed. The study cohort included 16 patients with ALK-negative or unknown anaplastic large T-cell lymphoma (ALCL) and 13 with peripheral T-cell lymphomas unspecified (PTCL-U). There were no significant difference in

demographic and clinical features between both cohorts except of tendency for higher platelet count in ALCL subgroup (p=0,06). The majority of patients in both subsets had advanced disease at diagnosis (III and IV clinical stages; 73%) and B symptoms (76%). International prognostic index (IPI) ≥2 was demonstrated in 10 PTCL-U and 6 ALCL patients. Induction chemotherapy consisted of a median of six CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) cycles (range 1-10). The median number of all given cycles before ASCT for PTCL-U and ALCL was 8 (range 3-20) and 7 (3-18), respectively. There was no difference in median time from diagnosis to ASCT between subgroups. The disease status at diagnosis was as follows: eight CR and 5 PR for PTCL-U and 8 CR and 8 PR for ALCL. Conditioning regimen before ASCT consisted of CBV (cyclophosphamide, BCNU, etoposide) for 15 and BEAM (BCNU, cytarabine, etoposide, melphalan) for 4 patients. The engraftment was observed in all transplanted patients, there were no significant difference in hospitalization time and number of infections between compared subgroups. Among 29 transplanted patients, 7 (24%) died due to disease progression. Twenty two patients remain in CR. The median follow-up time was 4 years. The 4-year probability of the overall survival for whole group was 57% (58% for PTCL-U and 48% for ALCL). The probability of disease-free survival (DFS) after 4 years was 55% for whole cohort (56% for PTCL-U and 44% for ALCL). In an univariate analysis older age, the higher number of prior lines of chemotherapy and disease status at transplant were found to be associated with the lower probability of survival, but only age >45 years was found to influence the outcome independently in multivariate analysis. *Conclusions.* We have confirmed that ASCT as consolidation therapy for PTCL is a safe and efficient procedure for PTCL patients who are chemosensitive after induction chemotherapy. OS and DFS were comparable between PTCL-U and ALCL and older age at transplant was associated with inferior clinical outcome.

0932

ANAPLASTIC LARGE CELL LYMPHOMA ALK POSITIVE AND ALK NEGATIVE: CHARACTERISTICS AND OUTCOME OF 98 CONSECUTIVE PATIENTS FROM A SINGLE INSTITUTION OVER 25 YEARS

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Background. Anaplastic large-cell lymphoma (ALCL) is a T-cell lymphoma characterized by expression of CD 30, often associated with t(2;5) (p23;q35) involving anaplastic lymphoma kinase (ALK). ALK positive and ALK negative systemic ALCL are defined as distinct entities in the WHO 2008 classification. The first-line of treatment in adults patients is evaluated in clinical trials, especially in ALK negative cases. *Purpose.* Retrospective evaluation of outcome in 98 patients with ALCL ALK+ and ALK- according to clinical features and treatment regimen. *Patients and Methods.* Between 1985 and 2004 majority of patients received CHOP or CHOP-like chemotherapy (Group 1, n=75). Since 2004 most of patients were treated with intensive protocol of the German Multicentre ALL Study Group B-ALL/NHL 2002 (Group 2, n=23). ALK protein expression was tested in 82 pts. Kaplan-Meier method was used to estimate overall survival time and progression free survival time. Cox proportional hazards model was used in multivariate analysis of clinical variables. *Results.* Median age (range) was 40 (15-83), 59% were male, 45% were in clinical stage (CS) IV, 61% had B symptoms, 46% had bulky disease, 50% had LDH > N, and 56% had IPI score >1. Group 1 and Group 2 were significantly different in age: 42 vs 33 (p=0.003), ALK positivity: 41% vs 82% (p=0.001), HGB>12 g/dl: 59% vs 26% (p=0.008), and CS IV: 39% vs 65% (p=0.032), respectively. Median overall (OS) and progression free survival (PFS) was 63,5 months, 95% C.I.=[0.0,130,9] and 31,2 months, 95% C.I.=[0,00,72,40]. Five years OS and PFS was 50%, 95% C.I. = [40%, 61%] and 45%, 95% C.I. = [34%, 56%].On multivariate analysis, CS IV (p=0.001), B symptoms (p=0.001), bulky disease (p=0.001) and LDH>N (p=0.005) were independent adverse factors for OS. CS IV (p= 0.002), B symptoms (p=0.024), PS>1 (p=0.053) and group 2 (p=0.011) were significant factors for PFS. IPI score > 1 was significant adverse factor for OS (p=0.000) and PFS (p=0.000). Risk of progression was three-fold Iess in Group 2 than in Group 1. *Conclusions.* CS IV, B symptoms, bulky disease and high IPI were independent adverse factors for OS and PFS. Outcome of patients in Group 2, in majority treated with intensive chemotherapy, appears encouraging with emerging plateau on PFS, although there were marked differences in clinical characteristics between the groups.

0933

ASSOCIATION BETWEEN THE IMMUNOHISTOHEMICAL GCB/NON GCB CLASSIFICATION AND THE OUTCOME OF THE PATIENT WITH DIFFUSE LARGE B CELL LYMPHOMA TREATED WITH RITUXIMAB-CHOP REGIMEN: SINGLE CENTRE EXPERIENCE

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Immunohistohemical analyses classify diffuse large B cell lymphoma according to the origin of the tumor cells into two subcategories, as germinal center B-cell Like (GCB) and non-GCB DLBL. Many studies indicated that prognosis of GCB DLBL is better that of non-GCB DLBL after receiving CHOP regimen as initial therapy. However, recently the results from one large prospective study indicated that immunoblastic morphology and not immunohistohemical features predict the outcome of the DLBL. In order to investigate the prediction value of the immunohistohemical GCB/non GCB classification of DLBL of the outcome of our DLBL patient treated with the Rituximab (R)-CHOP regimen we conducted a retrospective study. Our study enrolled 132 DLBL patients diagnosed and treated at the University Clinic of Hematology in the period between February 2002 and December 2007. They were all treated with R-CHOP regimen and the median follow-up of the patient was 36 months. We analyzed the biopsy samples immunohistochemically for markers of germinal center (Bcl6), post-germinal center (MUM1) and apoptosis (Bcl-2).The patients were categorized as GCB subtype (68;51,6%) or non-GCB subtype (64; 48,4%). The median overall survival time (OS) were 65,25 months in GCB group and 61,1 months in non-GCB group, and median duration of the remmision (DoR) were 60,85 and 57,75months respectively for the both groups. The groups were statistically comparable regarding the both parameters. They were also comparable regarding the age, gender distributions and all others already established prognostic parameters as performance status, advanced IPI, albumin level except for the low IPI 0-2 which was statistically associated with the non-GCB group (p=.024). Our results did not show any statistical survival advantage and better outcome for the patient classified as GCB DLBL when treated with R-CHOP and indicate that immunohistohemical markers do not really reflect the molecular diversity of the tumor. They also support the studies that suggests that Rituximab eliminates or modulates the significance of some already established prognostic markers for DLBL and indicate that previously recognized markers should be re-evaluated in the context of the modern therapy and that new prognostic indicators for DLBL has to be identified.

0934

FEBRILE NEUTROPENIA RISK ASSESSMENT AND GRANULOCYTE-COLONY STIMULATING FACTOR SUPPORT IN PATIENTS WITH FOLLICULAR LYMPHOMA RECEIVING R-CHOP-21

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Background. EORTC guidelines (2010) recommend granulocyte-colony stimulating factor (G-CSF) primary prophylaxis (PP) for patients receiving chemotherapy at overall risk of febrile neutropenia (FN) ≥20%. CHOP ± rituximab (R) is frequently used in patients with high-risk follicular lymphoma both at presentation and relapse. R-CHOP-21 regimen in combination with older age and other individual risk factors can lead to high FN risk, where G-CSF PP is recommended. *Aims.* Describe current clinical practice in FN risk-assessment and G-CSF use in follicular lymphoma patients receiving R-CHOP-21 chemotherapy. *Methods.* IMPACT NHL is a multicentre, international observational study conducted in Europe and Australia. Eligible patients were age ≥18 years and planned to receive ≥3 cycles of CHOP±R. Patients provided written informed consent where required. This analysis reports data from follicular lymphoma patients who received R-CHOP-21. The pri-

mary outcome measure was the proportion of patients with an investigator-assessed individual-patient risk of FN ≥20% per guidelines who received PP G-CSF (pegfilgrastim or daily G-CSF initiated within days 1-7 of cycle 1). *Results.* Of 1829 NHL patients enrolled between 2005 and 2008, 345 had follicular lymphoma. Of these, the great majority (n=310, 90%) received R-CHOP-21 and are the focus of this analysis. The mean age was 58 (SD 12) years, almost half (43%) had poor FLIPI score, 76% had stage III-IV disease, 47% had a history of comorbidities, and 85% were previously untreated. FN risk was assessed by investigators as ≥20% in 57% (n=177) of patients, but only 37% of these (n=66) received PP G-CSF and 16% (n=28) experienced FN. Of 132 patients with <20% FN risk, 9% (n=12) received PP and 8% (n=11) experienced FN. In total, 39 patients (13%) experienced 48 separate FN events. FN was most commonly managed by hospitalization 33/48 events), followed by home care (7/48 events), and outpatient visits and no action taken (both 3/48 events). Furthermore, unplanned hospitalizations occurred in 53 (17%) patients; the most common reasons were neutropenia/FN (25/73 hospitalizations, 34%) and non-haematologic adverse events (24/73 hospitalizations, 33%); the median length of stay was 4 nights (interquartile range 3 - 8). The planned dose of chemotherapy matched the standard dose for each agent in R-CHOP (excluding vincristine and prednisone) in the large majority of patients. Most patients achieved a clinical response at the end of treatment, irrespective of G-CSF use (62% complete response, 30% partial response). Pegfilgrastim PP was received by 17% of patients (n=52). Among patients receiving daily G-CSF PP (n=26, 8%), the mean (±SD) number of doses per cycle was 4.9 (1.3). Patients who received PP G-CSF were older (mean 63 vs 56 years), more likely to have a history of comorbidities (56% vs 44%) and a poor FLIPI score (53% vs 40%) than those who did not. *Summary/Conclusions.* Follicular lymphoma patients treated with R-CHOP-21 may be at risk of FN and related complications. Older patients with a high-risk profile were more likely to receive PP G-CSF; however, inconsistency was observed between investigator FN risk-assessment and G-CSF administration, with only 37% of high-risk patients administered G-CSF PP per guideline recommendations.

0935

DOSE SELECTION FOR PHASE III STUDIES OF THE MONOCLONAL ANTI-CD20 ANTIBODY OBINUTUZUMAB (GA101) - A RATIONAL APPROACH

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Background. Obinutuzumab (GA101) is the first type II, glycoengineered, humanized monoclonal anti-CD20 antibody to enter clinical trials. *Phase I results.* Two phase I studies, BO20999 and BO21003 (Salles, ASH 2008, 2009; Sehn, ASH 2009), in patients with CD20+ NHL showed that GA101 at doses of 50-2000mg was well tolerated, with no dose limiting toxicities and promising efficacy. Pharmacokinetic (PK) data from both studies indicated increasing plasma levels of GA101 following doses ranging from 400/800mg to 1200/2000mg, consistent with modeling and simulation target saturation levels. In contrast to the pre-clinical models, no clear dose-response relationship could be established, possibly due to patient variability including various tumor sizes, histologies and number of prior therapies. However, GA101 PK data, similarly to published rituximab data, indicated that patients with higher disease burden may have faster clearance of GA101 and consequently these patients may require higher doses to achieve target saturation. *Phase II results.* To determine the phase III dose, NHL patients in the phase II part of study BO20999, (indolent [n=40] and aggressive (n=40 [25 DLBCL/15 MCL]), were randomised to receive GA101 at either a dose of 400mg for all infusions or 1600mg on Days 1 and 8 and 800mg for subsequent infusions. Results showed that GA101 was well tolerated at both dose levels and favored the 1600/800mg dose level, in indolent NHL patients with an end of treatment response (EOR) of 55% (including 50% EOR in refractory patients), compared to the 400mg dose group (EOR 17%), (Salles EHA, ASH 2010). Higher GA101 plasma concentrations were observed in the 1600/800mg group compared to the 400mg group with both mean Cmax and Cmin values rapidly reaching steady state after cycle 2 at the 1600/800mg dose level. Steady state PK at cycle 2 was observed for the 1600/800mg dose only, in indolent and aggressive NHL patients (Mean Cmax and Cmin values at steady state were 500- 600ug/mL for indolent and 300- 400ug/mL for aggressive patients), with target saturation incomplete at the 400mg dose level. Thus, early target concentrations could be best achieved using a 1600mg loading dose on d1 and d8. Modeling and simulation indicated that the same might be achieved with a more practical schedule of 1000mg used throughout a treatment course and one additional dose given at d15 of the first cycle. Moreover, comparison of the PK data obtained from study BO20999 with that from study BO21003 (where GA101 was administered weekly over 4 doses), indicates that higher GA101 plasma concentrations are achieved earlier with the more intensive regimen, indicative of target saturation. *Summary.* Based upon all available clinical data, a dose of 1000mg (flat dose) was selected for phase III studies in lymphoma, with GA101 to be administered on days 1, 8 and 15 of the first cycle to rapidly achieve and maintain adequate exposure levels. *Conclusions.* GA101 monotherapy shows promising efficacy in heavily pretreated NHL patients, including refractory patients. The phase III dose of 1000mg is currently being investigated in combination with various chemotherapeutic agents in first line, relapsed and refractory NHL.

0936

TOLERANCE OF R-CVP FOR FOLLICULAR LYMPHOMA IN AN UNSELECTED POPULATION AND IMPACT OF DOSE REDUCTION ON OUTCOME

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Background. 6-8 cycles of R-CVP is widely regarded as standard initial therapy for advanced follicular lymphoma (FCL) with studies demonstrating high response rates and durable remissions. However, there is very little published data on tolerance in unselected patients or the impact of dose reduction on outcome. *Aims.* To establish the proportion of unselected patients in routine practice able to receive full dose R-CVP for advanced FCL and the impact of dose reduction on response rate and duration. *Methods.* All patients with stage 3 or 4 FCL (excluding grade 3b) commencing therapy between 1st Sept 2005 and 31st March 2010 were identified at four centres with similar patient populations and supportive care protocols. Patients with prior chemo- or radiotherapy for a haematological disorder or HIV were excluded. Indications for treatment were bulky disease, B symptoms, symptomatic splenic or nodal enlargement or cytopenia due to marrow involvement. Progression free survival (PFS) was calculated from date of first chemotherapy. Receiving fewer than 6 cycles was considered multiple drug dose reduction unless treatment was abandoned for progressive disease (1 patient). *Results.* 88 patients were identified. Two died during treatment, one was lost to follow up, these patients were excluded from analysis. Of the remainder, 46 (52%) completed 6 cycles of full dose R-CVP (Rituximab 375mg/m2, Cyclophosphamide 750mg/m2, Vincristine 1.4mg/m2, (maximum 2mg), and prednisolone 100mg for 5 days) and 39 had some dose and/ or cycle number reduction. 2 patients received maintenance rituximab and were excluded from progression free survival analysis. Cyclophosphamide dose was reduced in 21 (median 80% of full 6 cycle dose), vincristine in 28 (median 50% full dose), rituximab in 15 (median 83% full dose),10 patients had ≤5 cycles (median 4). 18 patients had 6 cycles with reduction in only a single agent per cycle (2 rituximab, 6 cyclophosphamide and 10 vincristine), the other 21 had fewer than 6 cycles and/or reductions in dose of multiple agents. The commonest reasons for dose reduction were age (55%), neuropathy (20%) and poor tolerance (9%).There was no difference in FLIPI (median 2), ECOG performance status (median 1) or median age (64 vs 65years) between those receiving full dose or not or between those receiving reductions in single agents or in multiple drugs. Median follow up was 27 months. PFS, complete and partial response rates were determined for full dose (695 days, 37% and 63%), any dose reduction (705 days, 36%, 61%), single agent (760 days, 28%, 67%) and multiple agent (705 days, 42%, 52%) dose reduction. There were no statistically significant differences between these groups (figure 1 shows full dose vs any dose reduction). *Summary/Conclusions.* Outside clinical trials, a substantial proportion (45%) of patients with stage 3 or 4 FCL are deemed unable to receive R-CVP at full dose. However, moderate reduction in the doses of its components has no significant impact

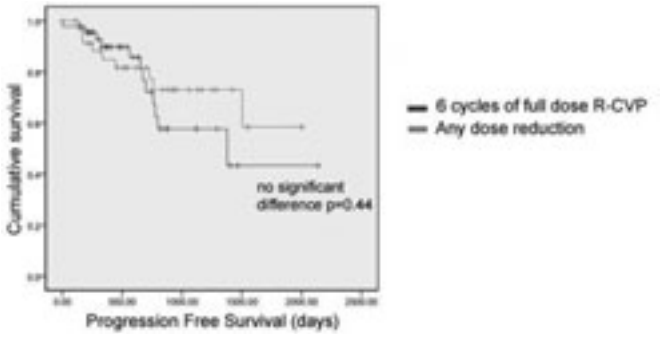

Figure 1. Kaplan Meier PFS curves full dose vs reduced.

on response rates or duration, suggesting that current standard doses may be higher than necessary to achieve useful and durable responses, especially in older or frailer patients.

0937

PET-SCAN FOR RESPONSE ASSESSEMENT AFTER RITUXIMAB-CHOP (R-CHOP) IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA (PMLBCL): PROGNOSTIC SIGNIFICANCE AND IMPLICATIONS FOR SUBSEQUENT RADIOTHERAPY (RT)

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Background. R-CHOP is superior to CHOP and is probably the current standard of care for the treatment of PMLBCL. Given the rarity of the disease and the <20% failure rate after R-CHOP, the identification of prognostic factors is difficult. PET-scan is a potent method to detect the presence of active disease in diffuse large B- cell lymphomas after R-CHOP, as well as in Hodgkin lymphoma. PET-scan has not been evaluated separately in PMLBCL, where there is also a question regarding consolidation RT. In fact, the use of PET-Scan in PMLBCL is based on the projection of data derived from Hodgkin and aggressive B-cell lymphomas. *Aims.* The evaluation of PET findings in patients with PMLBCL who have responded to R-CHOP, the assessment of their prognostic significance and their impact on the decision for subsequent RT. *Patients and Methods.* Among 52 consecutive patients with PMLBCL, who were treated in 10 Greek Centers, 40 underwent PET-scan after having responded to R-CHOP (CR, CRu, PR). The remaining 12 patients were not included in the study for the following reasons: documented progressive disease (n=7), PET done after RT (n=3), no PET availability (n=2). The endpoint was Progression Free Survival (PFS), measured from the time of PET examination. *Results.* The median post-PET follow-up was 16 months (up to 60). Among 40 evaluable patients, 24 were PET-neg (60%) and 16 (40%) PET-pos. Among 24 PET-neg patients, 13 (54%) did not receive RT and 11 (46%) were irradiated at a median dose of 3480 cGy. Two (2/13) non irradiated patients relapsed (mediastinum and isolated CNS relapse respectively) vs. 0/11 irradiated patients. Among 16 PET-pos patients, 14 (88%) were irradiated at a median dose of 4000 cGy, one was not irradiated and one was forwarded to high dose therapy and autologous transplant: 3/16 patients relapsed (all irradiated). The 2-year PFS was not significantly different between PET-neg and PET-pos patients (91% vs. 76%, p=0.41). However, among PET-pos patients, SUVmax appeared to discriminate a minority of them with adverse outcome: The 2-year PFS was 100% vs. 44% (p=0.02) for patients with SUVmax<5 (0/10 relapsed) and SUVmax≥5 (3/6 relapsed) respectively. So far 6 PET-pos patients had persistent positive findings at repeated examination after RT, but only 2 of them indeed had active disease or relapsed later. *Conclusions/Discussion.* PET-scan remains positive in a substantial proportion of PMLBCL patients who achieve a radiographic response with R-CHOP. In the majority of them, however, 18-FDG uptake is relatively low. Persistence of a positive PET was not clearly associated with inferior outcome, when additional RT was administered, although higher SUVmax values might predict a higher risk of relapse. Among 13 non-irradiated, PET-neg patients, only 1 relapse would be preventable by RT. According to these data, patients with PMLBCL should not be forwarded to autologous transplant simply based on a positive post R-CHOP PET-scan, if radiographic response is adequate.

0938

EFFICACY AND TOXICITY OF A NEW SHORT-TERM HIGH INTENSIVE PROTOCOL BL-M-04 FOR ADULT PATIENTS WITH BURKITT LYMPHOMA

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Background. Burkitt lymphoma (BL) is the most aggressive B-cell lymphoid neoplasm, whose growth fraction approximates 100%, with specific chromosomal abnormalities (t(8;14)(q24;q32), rarely - t(2;8)(p12;q32), t(8;22)(q24;q11)). Despite the rapid proliferative rate, BL is one of the most chemosensitive lymphoid neoplasm.Though it was clear, that high intensive short-term alternating multiagent chemotherapy regimens are most effective in patients with BL, adults have a less favorable outcome than pediatric patients with BL. *Aims.* to evaluate the efficacy and toxicity of the protocol BL-M-04 for adult patients with BL. *Methods.* 46 previously untreated patients with BL were eligible for our study (they had specific c-myc rearrangement. All the patients (32 males and 14 females, mean age 29 years (from 15 to 62 years)) participated in the study performed in the Russian Hematological Research Center between August 2003 and December 2010. The treatment was based on experimental high intensive protocol BL-M-04. BL staging criteria developed by S. B. Murphy were used to stage the patients. Stage I, II, III, IV were diagnosed in 3, 5, 16, 6 patients respectively. B-acute lymphoblastic leukemia (L3) was diagnosed in 16 (35%) patients. The main aim of the new treatment regimen was greater efficacy of therapy due to intensification and shorter treatment duration. The new treatment protocol is based on the modified NHL-BFM protocol for high risk patients with a reduced dose of methotrexate from 5000 mg/m2 to 1500 mg/m2. As BL, is a chemosensitive tumor that often regresses after 1-2 courses of chemotherapy, we decided to treat patients with BL in 4 courses of chemotherapy (2 induction and 2 consolidation) irrespective of the initial tumor mass. As BL is most sensitive to high dose methotrexate and cytarabine, we used these drugs in the induction phase to achieve to maximize the cytoreductive effect. Courses A and C were used to achieve remission. Doxorubicin was added to course A, and methotrexate to course C. Consolidation courses were similar to induction courses. Hence, we used A and C courses (without course B), intensified with course B drugs (doxorubicin and methotrexate), the interval between the courses being 21 days. *Results.* 41 patients (89%) achieved a complete remission (CR) after 1-2 courses (18 patients - after the 1st course, 23 - after the 2d). 39 are alive in the first CR during 50 months (median 5-86 months). Five patients died. The cause of death in four patietns were chemotherapy related complication, in one - disease progression. Two patients died due to early relapse. The 5-year disease-free survival (DFS) was 95% with an overall survival (OS) of 85%. BL-M-04 therapy resulted in higher CR rates and longer DFS in adult patients with BL and confirmed high efficacy of short-term intensive therapy. Treatment duration was 3 months. *Summary/Conclusions.* BL-M-04 is a highly effective protocol. The 5-year DFS was 95% with 5-year OS of 85%. The use of

this protocol can achieve rapid tumor regression with a short treatment duration due to chemotherapy intensification and acceptable toxicity.

0939

IMPROVED OVERALL SURVIVAL FOR VERY ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA AFTER THE INTRODUCTION OF IMMUNOCHEMOTHERAPY

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Background. Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma subtype and, as in most other malignant diseases, the incidence is strongly related to increasing age. Population-based studies have reported that the median age for DLBCL patients is 70 to 72 years and as high as about 20 % of all patients are above the age of 80. The prognosis of DLBCL patients has improved considerably during the last decade, mainly due to the introduction of the monoclonal anti-CD20-antibody rituximab combined with the standard CHOP-regimen. However, very old DLBCL patients (above the age of 80 years) are seldom included in clinical trials and, so far, there is very little information available on the survival impact of immunochemotherapy in such patients. *Aim.* To evaluate if the introduction of immunochemotherapy (R-CHOP) have influenced survival for very elderly patients. *Methods.* We have retrospectively analysed all DLBCL patients over the age of 80, who were diagnosed in the Gothenburg region during two time-periods (1997-2000; 'pre-R' and 2006-2009; 'post-R'). Patients with previously known indolent lymphomas or with primary CNS lymphoma were excluded from the study. *Results.* There were 30 and 40 patients ≥ 80 years in each time period, corresponding to 20.5 % and 23 %, respectively, of the entire DLBCL population diagnosed during these two periods. Performance status ≥ 2 was 48 and 47 % in each period and no difference in aaIPI was found. Fifty-three % in the post-R period were treated with a curative intent compared to 37 % in the pre-R period ($p<0.05$). The estimated 3-year PFS was 42 % in the post-R period and 20 % in the pre-R period ($p=0.045$). Similarly, the estimated 3-year OS in the post-R group was 40 % and 19 % in the pre-R group ($P=0.01$). *Conclusions.* After the introduction of R-CHOP, both PFS and OS was improved for a population of very elderly DLBCL patients. Based on our data, high age by itself should not be a reason to exclude the patient from an effective treatment with immunochemotherapy.

0940

CONCURRENT CHROMOSOMAL BCL2 AND MYC TRANSLOCATIONS IN LARGE B CELL LYMPHOMA

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Background. Concurrent chromosomal BCL2 and MYC translocations involving the BCL2 and MYC protooncogenes in Large B Cell Lymphoma (NHL) (double-hit, DH) recently, have received increased attention (2008 WHO classification, 'B cell lymphoma unclassifiable with features intermediate between DLBCL and BL). Patients with DH lymphomas often present with poor prognostic parameters, including elevated LDH, bone marrow and CNS involvement, and a high IPI score. All studies on larger series of patients suggest a poor prognosis, also if treated with RCHOP or high-intensity treatment modalities. *Aims.* We conducted a retrospective study of DLBCL to evaluate the frequency of double-hit B translocations in DLBCL and to analyse pathologic and/or clinical features correlated with the presence of a double-hit translocations. *Methods.* DLBCL samples, diagnosed according to the WHO criteria of 2008 and derived from 93 patients treated with R-CHOP or HD, have been subjected FISH using commercial break-apart probes for BCL2 and MYC. Clinical data were collected from patient files. *Result.* Double-hit BCL2/MYC translocations were detected in 9 of 93 cases (10%); 7 DLBCL, 1 BCLU, 1 unclassified. All double-hit DLBCL were GCB immunophenotype and showed varying morphology. Ki-67 index ranged from 15-95%. Characteristic clinical parameters included a high IPI score, a high stage an extranodal presentation with CNS involvement(4/9). Furthermore DH was also associated with an inferior OS. *Conclusions.* Our results suggest that DH is more frequent than previously estimated but could not be identified only by morphology or proliferation rate.

0941

HISTORY OF MYELOID MALIGNANCIES IN RELATIVES OF PATIENTS WITH WALDENSTRÖM MACROGLOBULINEMIA AND IGM-MONOCLONAL GAMMOPATHIES

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Background. The etiology of Waldenström macroglobulinemia (WM) is unknown. A possible role for genetic factors has been suggested by reports of familial clustering of WM. Familial cases reported so far show two patterns of aggregation: multiple cases of WM only, or mixed B-cell disorders. *Aims.* The aim of this study was to evaluate the incidence and characteristics of hematologic disorders among family members of 212 unrelated patients with IgM-monoclonal gammopathies diagnosed and followed at our Institution from 1986 to 2010. *Methods.* An interview-based investigation of family history was performed to identify a history of hematologic diseases among relatives. Diagnosis of WM, IgM-monoclonal gammopathies of undetermined significance (MGUS), and IgM-related disorders (IgM-RD) was made according to the Consensus Panel Recommendations from the Second International Workshop on WM (Owen *et al.*, 2003) for patients diagnosed from 2003 onward. The same criteria were retrospectively applied to patients diagnosed before. *Results.* We analyzed 212 patients with IgM-monoclonal gammopathies, further classified as WM (=66 patients) or IgM-MGUS/IgM-RD (=146 patients). The median age was 63 years (range: 19-92), 125 were males and 87 females. A familial history of hematologic disorders in one or more relatives was reported by 31 patients (15%), totalling 40 affected relatives. Hematologic disorders were diagnosed in 28 first-degree and 12 second- or third-degree relatives. The median number of relatives affected per family was 1 (range: 1-3). Patients with familial IgM-monoclonal gammopathies were significantly younger as compared to those with sporadic disease (53 versus 64 years, $p<0.0001$). The proportion of familial cases was higher among patients with WM as compared to those with MGUS/IgM-RD (23% versus 11%, $p= 0.02$). Specific information regarding the type of hematologic disease in the family members was not available in one case. Among the remaining 39 cases, the diagnoses were as follows: WM (=4), MGUS (=9), non-Hodgkin's lymphoma (=8), chronic lymphocytic leukaemia (=3), Hodgkin's lymphoma (=1), hairy cell leukaemia (=1), multiple myeloma (=1), acute lymphoblastic leukaemia (=1), acute myeloid leukaemia (=7), chronic myeloid leukemia (=1), polycythemia vera (=2), essential thrombocythemia (=1). According to the previously reported patterns of aggregation, we observed multiple cases of WM only in 3 families (10%) and mixed B-cell disorders in 18 (60%). In the remaining 9 families (30%) we found clustering of IgM-monoclonal gammopathies with hematologic myeloid malignancies. *Conclusions.* In this study we found that 15% of patients with IgM-monoclonal gammopathies have a familial history of hematologic disorders. Familial cases are more common among WM patients than in other IgM-monoclonal gammopathies and are characterized by younger age at diagnosis. In addition to the two patterns of aggregation described so far, we identified a subset of IgM monoclonal gammopathy patients with a familial history of myeloid malignancies. This suggests a thorough investigation of family history in patients with IgM-monoclonal gammopathies, encompassing also myeloid malignancies. Further investigations might clarify whether familial clustering reflects a genetic predisposition rather than exposition of family members to the same environmental risk factors.

0942

COMPARATIVE STUDY OF SPLENIC MARGINAL ZONE LYMPHOMA (SMZL) AND PRIMARY BONE MARROW MZL (PBMMZL)

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Background. Recently we have described a new category of MZL, the PBMMZL, characterized by bone marrow infiltration and blood in-

Table 1. Pts characteristics with SMZL and PBMMZL.

Characteristics	PBMMZL (%)	SMZL (%)
Median age (range)	64 (38-77)	64 (48-78)
Male sex	43	48
B symptoms	0	4
Palpable splenomegaly Enlarged spleen by CT	0 0	100 100
BM infiltration	100	100
LDH elevated	9	40
HCV (+)	0	0
IPI Low+low intermediate	96	95
Anemia (Hb<12g/dl)	17	52
Thrombocytopenia (<100x109/l)	0	28
Lymphocytosis (>4x109/l)	70	55
M-component	30	25
Autoimmune phenomena	0	5
CD5/ CD23/ CD38/ CD11c/ CD25/ sIgκ	0/48/4/43/22/70	10/7217/61/33/68

volvement without any other disease localization. PBMMZL harbour many similarities with SMZL besides splenomegaly. *Aim.* To compare SMZL and PBMMZL on the basis of clinical and laboratory characteristics, morphology and immunophenotypic data. *Patients and Methods.* 23 SMZL and 21 PBMMZL patients were included in the present study. Clinical and laboratory features were recorded. Blood and bone marrow mononuclear cells were studied by flow cytometry for the mAbs CD5, CD23,CD10,CD25,CD38,CD11c on CD19+ cells, while bone marrow (BM) biopsies were evaluated using morphologic and immunohistochemical methods. *Results.* Clinical and laboratory features were very similar between the two groups (table 1), except that cytopenias and elevated LDH were more common in SMZL patients. Immunophenotypic features of blood and bone marrow mononuclear cells were also similar. The percentage of BM infiltration was 40 (15-85) and 25 for SMZL and PBMMZL respectively. The histological pattern of bone marrow infiltration was variable, with the nodular infiltrates being the most frequent and typical pattern in both groups (78% for the SMZL and 62% for PBMMZL). Intrasinusoidal infiltration was observed in both groups usually in association with other patterns of infiltrates (48% for SMZL and 38% for PBMMZL). Cytological aspects were very heterogeneous in both groups with several types usually associated in varying proportions: small lymphocytes, centrocyte-like cells, small cells with plasmacytoid differentiation, monocytoid cells and variable content of medium to large cells. Well-formed germinal centres were identified in 48% of SMZL cases in contrast to only 1 case with PBMMZL. In all the cases the immunophenotypic profile of BM neoplastic population (CD20+ strong, CD79a+, CD3-, CD5-, CD10-, cyclin-D1-, BCL-6-) was consistent with a marginal zone-derived neoplasm. PBMMZL patients presented a very indolent clinical course. Only 3 patients have required so far therapy while all SMZL patients were in need of therapy at diagnosis. No death was recorded in the PBMMZL group at a median follow up time of 36 months (12-105), while 5 SMZL patients have died (3 disease related) at a median follow up time of 72 months (16-181). *Conclusions.* PBMMZL presents many similarities with SMZL, although the former presents a more indolent clinical course and is not complicated by splenomegaly.

0943

NEW MODIFIED PROPHYLACTIC SCHEME AGAINST LIPOSOMAL CYTARABINE (DEPOCYTE®)-INDUCED ARACNOIDITIS IN ADULT LYMPHOMA PATIENTS

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Background. Liposomal cytarabine (DepoCyte®) is a slow-release formulation of cytarabine designed for intrathecal administration, ensuring prolonged drug exposure. In all clinical studies, chemical arachnoiditis, a syndrome manifested primarily by nausea, vomiting, headache and fever, was a common adverse event. To reduce the incidence and severity of chemical arachnoiditis, all patients must be treated with dexamethasone 4 mg bid orally or intravenously on days 1 to 5 of each cycle beginning concurrently with administration of DepoCyte. *Aim.* We designed a new prophylactic scheme against Liposomal Cytarabine -induced aracnoiditis in patients with lymphoma to improve adherence to concomitant oral dexamethasone. *Patients and Methods.* Patients were consecutively recruited from 7 Spanish institutions. Thirty-three adult patients with lymphoma, median age of 57 years (27-79). Type of lymphoma: diffuse large B-cell lymphoma 24 pts, mantle cell lymphoma 3 pts, Burkitt 2 pts and other types 4 pts. Type of concomitant systemic chemotherapy: CHOP±R 13 pts, MegaCHOP±R 5 pts, HyperCVAD+R 3 pts, ESHAP±R 3 pts and other types 9 pts. Liposomal cytarabine (50 mg) was administered by lumbar puncture. Prophylactic scheme against Depocyte-induced aracnoiditis was: Dexamethasone 4 mg by lumbar puncture immediately after the injection of Depocyte and dexamethasone 4 mg bid orally on days 1 and 2. Toxicity was graded according to Common Terminology Criteria for Adverse Events, version 3.0. *Results.* Reasons for Liposomal Cytarabine administration were: therapeutic in 12 pts (36%) and prophylactic in 21 (64%). A total number of 78 injections of Depocyte were administered. The median number of doses per patient was 2 (range, 1-6). Liposomal cytarabine was generally well tolerated. Overall, 15 pts had some type of adverse event (all but one in prophylaxis and 9 of them with the first injection). No toxicity was observed in 56 administrations. In 22 administrations (28%) some type of toxicity was recorded for a total of 34 adverse events. Most of these events were transient and resolved spontaneously. Adverse events are listed in the Table. Only 2 events were considered clinically relevant: 1 patient with headache grade 3 with diplopia grade 2 and other patient with aracnoiditis grade 2. Interestingly, the patient who had aracnoiditis fully recovered and received 3 additional administrations of Depocyte with a dose reduction of 25 mg. He was given the same prophylactic scheme against Depocyte-induced aracnoiditis showing excellent tolerance and without any relevant symptoms. Regarding efficacy, all evaluated patients cleared cerebrospinal fluid and/or improved clinical symptoms. At last follow-up, nine patients have died, being the main causes of death: 5 systemic lymphoma progression, 1 systemic plus neurologic progression, 1 isolated neurologic progression, 1 concomitant solid tumour and 1 neutropenic sepsis. *Conclusion.* Administration of concomitant inthratecal dexamethasone and oral dexamethasone only for two days appears to be safe and well tolerated prophylactic scheme against Liposomal Cytarabine (Depocyte®)-induced aracnoiditis in adult patients with lymphoma. This strategy reduces the number of days with oral dexamethasone, while also might improve adherence allowing to administer the scheduled number of Depocyte injections.

Table 1.

	Total number	Grade 1	Grade 2	Grade 3
Headache	13	8	4	1
Nausea	6	4	2	
Vomiting	4	2	2	
Vertigo	4	1	3	
Diplopia	1		1	
Fever	1	1		
Paresthesia	3	2	1	
Hypotension	1		1	
Aracnoiditis	1		1	

0944

SPLENIC MARGINAL ZONE LYMPHOMA: CHARACTERISTICS AND TREATMENT

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Splenic marginal zone lymphoma (SMZL) characterized with primary spleen involvement and indolent course. SMZL is rare and found in less than 1% of all Non-Hodgkin`s lymphomas. *The aim* of our study was to

find stricter definition for SMZL and to determine the most effective treatment strategy. *Materials and Methods.* Eighty six patients (33 males (38%) and 53 females (62%)) between January 2001 and February 2011 were included in the study. The median age was 59.6 years (ranging from 16 to 86 years). Splenomegaly was noted in all cases. Visceral lymphadenopathy was detected in 44 (50%) patients and was shown as an increase of liver size and splenic hilar lymph nodes. In the laboratory data anemia was found in 31 patients (36%), thrombocytopenia - in 66 (76,7%), leukopenia - in 30 (35%), leukocytosis - in 39 (45,3%). Absolute lymphocytosis was detected in 2/3 of patients. High LDH was observed in 46 patients (53,5%). M-component detected in 35 patients (40,7%). Viral hepatitis markers were positive in 12 cases (14%). Aberrant expression of CD5 was detected in 4 (5.8%) patients. Expression of DBA44 were found in 45 patients. Ki-67 was below 10%. Forty six (53,6%) cases had various chromosomal abnormalities, including deletion of chromosome 7, trisomy 3, trisomy 12, trisomy 18 and in 3 cases t(14;19)(q12;q32) sequence was revealed. In order to determine the role of surgical treatment in patients with SMZL, we compared two groups of patients selected on the basis of the first-line therapy. The first group included 17 patients who completed chemotherapy in the first line, and the second group consisted of 47 patients in whom splenectomy was first-line treatment. *Results and Discussion.* All patients who received chemotherapy as a first line treatment demonstrated little clinical and hematologic effects, but some patients had even longer recurrent-free period than those in the second group. On average, progression of disease occurred during 6-8 months in all patients and manifested with spleen size increase and progression of cytopenia. One patient from the first group died due to infectious complications. Splenectomy was performed to all patients in this group with good clinical results obtained. In patients who underwent splenectomy as first-line therapy was obtained a good clinical response. In the second group 1 patient died from disease progression. Overall survival rate in both groups about 98% with 9 years follow-up. However, we obtained significant differences in the progression-free survival: in the first group (chemotherapy in first line) relapse rate during 9 years was about 90%, meanwhile the second group (splenectomy in the first line) it was 30% (p = 0,01). *Conclusion.* SMZL heterogeneous disease and characterized by different clinical manifestations, chromosomal abnormalities, secretion of M-component, often aberrant immunophenotype, but in all cases, splenomegaly played a leading role in the treatment strategy. Effectiveness of splenectomy was noted in the first-line therapy compared with chemotherapy. It was demonstrated with statistical significance that splenectomy as a first-line therapy in these patients was three times more clinically effective than chemotherapy with subsequent surgery.

0945

ADDITION OF RITUXIMAB TO REDUCED DOSE CHOP CHEMOTHERAPY IS FEASIBLE IN ELDERLY PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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Background. Recently, rituximab plus dose intensified CHOP chemotherapeutic regimens have been shown to be effective in elderly patients with diffuse large B cell lymphoma (DLBL). However, hematological toxicities and treatment related mortality were hard to be neglected in terms of relatively poor performance in elderly patients. Rituximab is effective immunotherapeutic drug in DLBL, if that, addition of rituximab to reduced dose CHOP chemotherapy may be appropriate for elderly patients with DLBL. *Aims.* To investigate outcomes and toxicity profiles of addition of rituximab to reduced dose CHOP chemotherapy in elderly patients with DLBL. *Methods.* Patients aged 60 years and over have been enrolled consecutively between January 2005 and December 2009. Reduced dose CHOP chemotherapy consisted cyclophosphamide (600 mg/m2 intravenously), doxorubicin (30 mg/m2 intravenously), and vincristine (1 mg intravenously) on day 1, and prednisone (40 mg orally) given on day 1 to 5. Patients were treated every 3 weeks for 6 to 8 cycles. Rituximab was administered at a dose of 375 mg/m2 on day 1 for each cycle. *Results.* Total 84 cases were analyzed in this study. Median age was 69 years old (range, 61-85 years old) and males were 44 (52.4%). 65 patients received at least 6 cycles of chemotherapy and 19 patients dropped out from these regimens early because of treatment-related death (4 patients), loss of follow up (4 patients), progression of disease (4 patients) and refusal of further treatment (7 patients). Mean cycles of modified R-CHOP chemotherapy was 5.96 (range, 1-8). Overall response rate was 89.3% (CR rate, 66.7%; PR rate, 22.6%). Estimated 5-year event-free survival rate was 61.5% ± 6.2% and overall survival rate was 78.3% ± 5.2%. Main hematological adverse events were 21.4% in grade 3/4 neutropenia and 3.6% in grade 3/4 anemia. Among non-hematological toxicities, grade 3/4 asthenia was reported 3.6% and other toxicities were tolerable. *Conclusions.* Addition of rituximab to reduced dose CHOP chemotherapy is an effective and tolerable treatment regimen in elderly patients with DLBL.

0946

ROUTINE METAPHASE CYTOGENETICS MAY AUGMENT THE PROGNOSTIC CAPABILITY OF THE MANTLE CELL LYMPHOMA INTERNATIONAL PROGNOSTIC INDEX (MIPI) FOR STAGE IV MANTLE CELL LYMPHOMA

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Background. Mantle cell lymphoma (MCL) is a rare and distinct subtype of lymphoma with a highly variable clinical course. The majority of patients (pts) present in advance stage with bone marrow involvement (Stage IV). While the majority of cases are clinically aggressive, it is increasingly recognized that a subset of MCL characterized by a rather indolent course and typically presenting with stage IV disease, and associated with a longer survival exists. The upfront identification of such patients however, remains a challenge. Although the MIPI is a robust tool for predicting the prognosis, the lack of genetic factors in the model may be its limitation. *Aim.* We evaluated the survival outcomes of pts with biopsy proven MCL in a single institution and sought to examine the prognostic capability of the MIPI, as well as bone marrow cytogenetics for pts with MCL. *Methods*. Cases of MCL diagnosed from 2000 to 2010 were identified from our lymphoma database, yielding unique 37 pts. The baseline patient and disease characteristics, together with the treatment history were retrospectively evaluated with respect to overall survival (OS) outcomes. Outcomes were compared using log-rank analysis of Kaplan-Meier survival analyses, and MIPI was calculated in accordance with the initial publication. *Results*. Pt characteristics at diagnosis were: median age 62 (range 41-85), 81% male and 68% stage IV disease. Five pts had the blastoid variant of MCL, while 8 pts had leukemic presentation. MIPI scores at diagnosis were: low (24%) / intermediate (38%) / high (33%). Median follow-up is 2.5 years and the median OS is 6.4 years. Although the MIPI was discriminating of 3 separate risk-groups, with OS of 7.5 yrs, 6.8 yrs and 2.2 yrs (p=0.001) for low, intermediate and high MIPI respectively (Figure 1), the distinction between intermediate and low -risk MIPI was less apparent. Bone marrow cytogenetics data were able to further fine-tune the prognostication. Among pts with high-risk MIPI, 2 pts with del17p detected on metaphase cytogenetics had the worst OS (0.13 vs. 2.2 yrs, P=0.1). Among pts with intermediate/low risk MIPI, the presence of any cytogenetic abnormality predicted for a worse outcome (1.8 vs. 12.8 yrs, p=0.03). For pts with stage IV MCL, there was a trend for a higher Ki67 (>40%) among those with detectable abnormalities on metaphase cytogenetics. *Conclusion*. Based on the follow-up of 37 pts, we are able to validate the accuracy and ease of application of the MIPI for prognostication. However, the prognostic capability can be further augmented with the inclusion of simple bone marrow metaphase cytogenetic results. Presence of metaphase cytogenetic abnormalities may reflect a higher proliferation index which portends a more inferior prognosis. We propose that similar to multiple myeloma, MCLs can be further risk-stratified with routine cytogenetic analysis. The routine use of more sensitive interphase-FISH analysis with probes for TP53, ATM gene mutation and SOX 11 to further complement metaphase cytogenetics ought to be further explored.

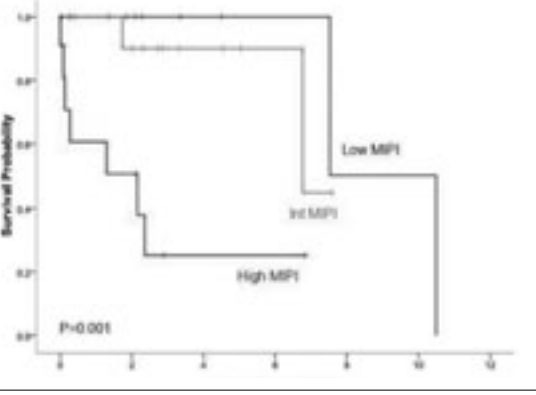

Figure 1. Overall survival by MIPI risk groups.

Non-Hodgkin lymphoma - Clinical 3

0947

SWITCHING BCL6 POSITIVE TO NEGATIVITY IN RELAPSING DIFFUSE LARGE B-CELL LYMPHOMA

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Background. Diffuse large B-cell lymphoma (DLBCL) is the most complex and heterogeneous lymphoma in adulthood presented as a biologically and clinically distinct subtypes including germinal centre B-cell-like (GCB) and activated B-cell-like (ABC). In DLBCL BCL-6 is associated with the germinal centre subtype and has a good response to modern chemotherapy. It is known that BCL-6 expression is a favorable prognostic factor in DLBCL. However, the effect of BCL-6 expression on relapse of the DLBCL was not studied. *Aim.* to show Bcl-6 gene expression changes and biological response in relapsed DLBCL and their patterns which include: LDH, b2M, CRP, Fe, ferritin, TIBC, hemoglobin, uric acid and International Prognostic Index (IPI). *Methods.* We investigated 43 (21F/20M) patients with relapsed DLBCL and role of immunohistochemically determined Bcl6 tested in 22 patients. All patients were treated with R-CHOP protocol. We also investigated biological parameters such as LDH, b2M, CRP, Fe, ferritin, TIBC, hemoglobin, uric acid level as well as IPI at the presentation of DLBCL and at the time of relapse. *Results.* Mean age of female patients was 53.1±12.7 vs. male 52.8±12.1 and overall age of patients was 53.0±12.3SD years with no significant difference. Investigated relapsed group of 41 patients with DLBCL had own control at the beginning of diseases. From 22 immunohistochemically (IHC) investigated patients for Bcl6 protein expression, 19/22 was positive and 3/22 was negative at the beginning. DLBCL was diagnosed in gastrointestinal tract (13), liver and spleen (7) lungs (5), CNS (4), ovarian (2), vertebral (1), and lymph nodes (9). Patients with Bcl6 negative relapses of DLBCL had very aggressive lymphoma, malignant in nature with the biological responses including significant higher levels of the LDH (1832.5±443.8 SD in relapsed vs. 457.3±88.3 SD U/L at presentation) ($p<0.0001$), b2M (8.4±0.8 vs. 2.5±0.8 mg/L) ($p<0.0001$), CRP (35.9±11.4 vs. 2.5±0.7 mg/L) ($p<0.0001$), uric acid (287.2±65.7 vs. 153.9±33.1 µmol/L) ($p<0.0001$), and significantly low ($p<0.01$) Fe (11.2±4.4 vs. 21.8±7.6 µmol/L) ($p<0.008$), TIBC (35.5±9.2 vs. 46.8±7.0 µmol/L) ($p<0.009$), hemoglobin (91.7±13.2 vs. 126.5±8.6 g/L) ($p<0.0001$), and significantly higher ferritin (2735.6± 628.2 vs. 88.5±20.2 µg/L) ($p<0.0007$) at the time of relapse DLBCL. At diagnosis all 19/22 patients were Bcl6 positive (IHC) and switched to Bcl6 negative at the time of relapse. IPI index as biological indicator was initially vs. relapse 2.01±0.8 vs. 4.4±0.6 respectively ($p<0.0001$). *Summary/Conclusion.* The Bcl-6 negative relapses of DLBCL was associated with very aggressive lymphoma and significant increase of the biochemical parameters such as LDH, B2M, CRP, uric acid, ferritin and significant decrease of serum Fe, TIBC, hemoglobin resulting in anemia of chronic disease. Expression of the single gene Bcl-6 strongly predicts poor outcome of relapsed DLBCL. In human genome it is very rare that single gene can predict outcome of diseases such as Bcl6, but also can predicts relapse as showed in this report. Switching positive into negative Bcl6 expression was strong indicator of DLBCL relapse.

0948

OUTCOME OF PATIENTS WITH DLBCL AFTER ADDITION OF RITUXIMAB TO CHOP: A POPULATION-BASED TIME PERIOD ANALYSIS IN MANITOBA

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Background. Addition of Rituximab to CHOP chemotherapy (R-CHOP) as primary therapy for diffuse large B-cell lymphoma (DLBCL) is associated with improved survival. This benefit has been demonstrated in clinical trials where patients met strict selection criteria. Hence it is important to evaluate if similar outcomes continue in population based studies. *Aim.* To assess if R-CHOP administered to unselected patients with DLBCL living in a diverse geographic area leads to similar improvements as those reported in clinical trials. *Methods.* A population based retrospective study was undertaken of all patients diagnosed with DLBCL in the Province of Manitoba, identified by the Manitoba Cancer registry (MCR), and treated with CHOP or R-CHOP over a period of 36 months pre and post funding of Rituximab for this indication. Funding of Rituximab was approved for patients age 60-80 on 28 October 2002 and for all age groups on 22 June 2004. The provincial oncology drug program (PODP) database was used to identify patients who received the above treatment. Charts of all patients were reviewed to confirm the treatment received. *Inclusion criteria:* DLBCL patients who received at least one cycle of CHOP or R-CHOP as primary therapy and were Manitoba residents. *Exclusion criteria:* HIV +ve, primary CNS lymphoma, post-transplant lymphoproliferative disorders, transformed lymphomas, primary therapy with less intensive drugs. *Analysis.* The cohort was analyzed according to two characteristics: (1) A Time- Period Analysis, using the drug funding date as a cut-off point, in which all patients were included according to the time period, irrespective if they got Rituximab or not (an intention to treat analysis) (2) A Drug-Period Analysis, where in the Pre-Funding period, patients who received Rituximab were excluded and in the Post-Funding period, patients who did not get Rituximab were excluded. Overall Survival (OS) was calculated as time from the date of diagnosis to the date of death or the last day of follow-up (May 1, 2010). Multivariate analysis was performed using Cox proportional hazards model to assess the independent effect of treatment in the post rituximab period on OS, after controlling for age and sex. Data was analyzed using SAS version 9.1. *Results.* Patient characteristics: Time-Period Analysis- Total number 237 (male 118); Pre-rituximab 112 (median age 62 years, median follow up 55 months); Post-rituximab 125 (median age 65 years, median follow up 45 months). Drug-Period Analysis- Total number 196 (male 97); Pre-rituximab 94 (median age 62 years, median follow up 56 months); Post rituximab 102 (median age 64 years, median follow up 45 months). After controlling for age and gender, there was significant improvement in OS in the Post-rituximab era for both the Time-Period Analysis (hazard ratio, 0.53; 95% CI 0.31 to 0.92; $p = 0.02$) and the Drug-Period Analysis (hazard ratio, 0.52; 95% CI 0.28 to 0.98; $p = 0.04$). *Conclusion.* The study shows a significant improvement in OS of patients with DLBCL treated in multiple Community Cancer Centers and two Academic Centers in the Province of Manitoba, after the addition of Rituximab to CHOP as primary therapy.

0949

IMPACT OF AGE GROUP ON FEBRILE NEUTROPENIA (FN) RISK ASSESSMENT AND MANAGEMENT IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) TREATED WITH R-CHOP REGIMENS

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Background. Primary prophylaxis with granulocyte-colony stimulating factor (G-CSF) is recommended by the ASCO and EORTC guidelines to support chemotherapy delivery in patients with a high (≥20%) risk of FN. R-CHOP regimens used to treat aggressive non-Hodgkin lymphoma (NHL) are associated with high FN risk, and G-CSF prophylaxis is particularly important in dose-dense R-CHOP-14 to support full chemotherapy dose intensity. Older age is a known additional risk factor for FN. IMPACT NHL was an observational study designed to evaluate current clinical practice with respect to FN risk assessment in NHL patients treated with R-CHOP. *Aims.* To evaluate the impact of age group on FN risk assessment, G-CSF use, FN incidence, and chemotherapy delivery in patients with DLBCL receiving R-CHOP regimens. *Methods.* The study was conducted in Europe and Australia. All patients gave informed consent where required. Physicians assessed each patient's FN risk based on the planned chemotherapy regimen and individual patient risk factors. The primary outcome measure was the proportion of patients who had investigator-assessed individual patient risk of FN ≥20% per guidelines who received primary prophylaxis G-CSF; other outcome measures included primary prophylaxis by GCSF type used, incidence of FN, and measures of chemotherapy delivery. For this analysis, outcomes were calculated for patients aged <65 vs

Table 1. Outcomes by chemotherapy regimen and age.

	R-CHOP-14		R-CHOP-21	
	< 65 years (n=241)	≥ 65 years (n=168)	< 65 years (n=343)	≥ 65 years (n=361)
Mean (SD) age, years	49 (12)	72 (5)	52 (11)	73 (5)
Physician-assessed rate of FN ≥ 20%, n (%)	189 (78%)	134 (80%)	179 (52%)	255 (71%)
Received GSCF prophylaxis, n/N (%)	162/189 (86%)	108/134 (81%)	69/179 (39%)	136/255 (53%)
G-CSF prophylaxis and experienced FN, n/N (%)	32/162 (20%)	27/108 (25%)	11/69 (16%)	28/136 (21%)
Primary prophylaxis				
Pegfilgrastim, n (%)	129 (54%)	97 (58%)	60 (17%)	114 (32%)
Any daily GCSF, n (%)	79 (33%)	40 (24%)	30 (9%)	48 (13%)
FN rate				
cycle 1, n (%)	12 (5%)	12 (7%)	26 (8%)	37 (10%)
any cycle, n (%)	42 (17%)	39 (23%)	48 (14%)	85 (24%)
Chemotherapy RDI ≥ 90%, n (%)	176 (73%)	88 (52%)	275 (80%)	244 (68%)
Dose delay > 3 days, n/N (%)	107/240 (45%)	102/165 (62%)	128/342 (37%)	172/351 (49%)
Dose reduction ≥10%, n (%)	15 (6%)	40 (24%)	49 (14%)	93 (26%)

≥65 years receiving R-CHOP-14 or R-CHOP-21 regimens. *Results.* Out of a total of 1829 patients who initiated chemotherapy between Jan 2005 and Aug 2008, 1113 (61%) had DLBCL and received R-CHOP, and were included in this analysis. Demographics and outcome measures are shown in the table. Despite guideline recommendations, not all patients with investigator-assessed FN risk ≥20% received G-CSF primary prophylaxis. Of those who received R-CHOP-14, a similar proportion of younger and older patients were assessed with ≥20% risk of FN, although 14% of the younger patients and 19% of the older patients did not receive G-CSF primary prophylaxis despite meeting the criterion. Of those who received R-CHOP-21, a greater proportion of older than younger patients were assessed with ≥20% risk of FN, and G-CSF primary prophylaxis was more likely to be administered to older patients. Younger patients receiving R-CHOP-21 were the least likely group to be assessed as high risk. For both 14- and 21-day R-CHOP regimens, older patients had higher rates of FN; and, as a result of more dose delays and reductions, fewer patients received the planned RDI relative to younger patients. Unplanned hospitalizations occurred frequently and were more common in patients aged ≥65 years, occurring in 26% of younger and 40% of older R-CHOP-14 patients and 22% of younger and 36% of older R-CHOP-21 patients; neutropenia or FN was the most common reason in all age and treatment groups. *Summary/Conclusions.* Inconsistency was observed between overall FN risk assessment and use of G-CSF primary prophylaxis leading to lack of adherence to EORTC guidelines in both younger and older NHL patients. Elderly patients experienced more frequent neutropenia-related complications and reduced RDI in both 2-weekly and 3-weekly R-CHOP, suggesting that G-CSF primary prophylaxis may be particularly important in this patient population.

0950

RITUXIMAB MAINTENANCE THERAPY IN CD20+ B-CELL NON-HODGKIN-LYMPHOMA - FINAL RESULTS OF 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. *Aims.* To test for this hypothesis we performed a prospective randomized trial of rituximab maintenance therapy 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/m2) administered every 3 months for 2 years. Both pts after first line therapy and pts after relapse treatment were included in the study. Primary endpoint of the study was event free survival (EFS), secondary endpoints were relapse rate (RR), relapse free survival (RFS) and overall survival (OS). EFS and OS were analysed using an asymptotic logrank test, RFS using a competing risk model and RR using Fisher's exact test. *Results.* After recruitment of 171 pts the planned final analysis was performed on an intention to treat basis. Complete data sets of 163 pts were evaluable. At study entry, 120 pts (74%) were in CR, 2 pts (2%) in unconfirmed CR and 41 pts (25%) in PR. Histological subtypes included diffuse large cell lymphoma (67 pts), follicular lymphoma (35 pts), mantle cell lymphoma (18 pts), primary mediastinal lymphoma (16 pts), marginal zone lymphoma (7 pts), Burkitt's lymphoma (5 pts), and other lymphomas (15 pts). After a median follow up of 28 months, EFS (HR 0.50, 95% CI 0.23-1.09, p=0.037,) and RFS (HR 2.52, 95% CI 1.11-5.70 p=0.03) were superior for the maintenance group. In regards to diagnostic subgroups, EFS was in particular prolonged in pts with mantle cell lymphoma (p=0.055, one sided logrank test) and to a lesser extent in pts with follicular lymphoma (p=0.16) and diffuse large cell B cell lymphoma (p=0.18). Relapse occurred more often in the observation group than in the treatment group, however this effect was not significant (relapse rate observation group/treatment group = 2.31, 95% CI 0.86-6.75, p=0.08). There was no difference in OS between the two groups (p=0.74). Maintenance therapy was generally well tolerated: in 5 pts a WHO Grade 3 toxicity event occurred, which were arrhythmia, neuropathy, leucopenia (n=1 each) and infections (n=2). In one pt two WHO Grade 3 toxicities were observed (pain and infection). *5. Conclusion.* Rituximab maintenance therapy is feasible, safe and well tolerated and improves EFS in patients with CD20+ B-cell lymphoma.

0951

ORAL CYCLOPHOSPHAMIDE AND RITUXIMAB IN THE TREATMENT OF MARGINAL ZONE LYMPHOMA

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Introduction. MZL accounts between 5% and 17% of all non-Hodgkin lymphomas. MZL are most often very indolent malignancies that usually present with limited stage disease, which may be controlled with local treatment, with 10 years survival approximately 75-80%. Standard treatment option for stage I-II disease has not been yet established because the paucity of prospective trials, the heterogeneity of treatment and short follow-up and the indolent nature of disease justify a conservative approach. Patients with systemic stage III-IV disease should be considered for a more aggressive treatment. However a standard chemotherapic approach for MZL is missing. *Aim.* We are reporting a retrospective study on the use of oral cyclophosphamide (CTX) and rituximab in the treatment of advanced stage MZL patients. Cyclophosphamide is a nitrogen mustard alkylating agent identified as a lymphocyte cytotoxic agent. It determinates DNA crosslinks between (interstrand crosslinkages) and within (intrastrand crosslinkages) DNA strands at guanine N-7 positions in irreversible manner, leading to cell death. Adding rituximab allows targeting CD20 MZL positive cell, obtaining a synergistic effect on lymphoma cells. Patients and *Results.* 38 patients with MZL were included in the study and received 100 mg/die orally CTX for 15 days a month and 375 mg/sqm rituximab on day 8 of CTX therapy, monthly, for a 6 months treatment program. 31 patients are retrospectively valuable. The global overall response rate was 90.3%, with 51.6% complete response (CR) and 38.7% partial response (PR). The remaining 9.7% patients obtained a stable disease or a disease progression at the end of treatment. The principal side effects were recorded with rituximab use, with 4 patients that presented infusional reactions despite paracetamole and antihistamine premedication. One patients experienced herpes zoster reactivation. Patients achieving a response (CR or PR) underwent to follow-up, with a median of 10 mounts (mean 15). PFS presented a median of 23 months, and no statically significant differences documented between patients achieving a CR or a PR, due to short follow-up. All patients underwent at diagnosis to bone marrow aspiration and further analysis for IgVH rearrangement by PCR, to monitor minimal residual disease after therapy. Of the 31 pretreatment evaluable patients, 20 were positive and 11 negative at PCR. After treatment 10 patients were positive, 13 negative and 8 not evaluable. All PCR negative patients remained negative at the end of therapy. With regard to PFS, in the PCR negative subgroup we recorded 16.7% disease relapse, while 57.1% in the positive subset, confirming a predictive role of molecular biology status at the end of treatment (see figure). *Conclusions.* in the absence of comparative trial, it is difficult to know if any particular regimen should be preferred for the treatment of MZL. Oral cyclophosphamide combined

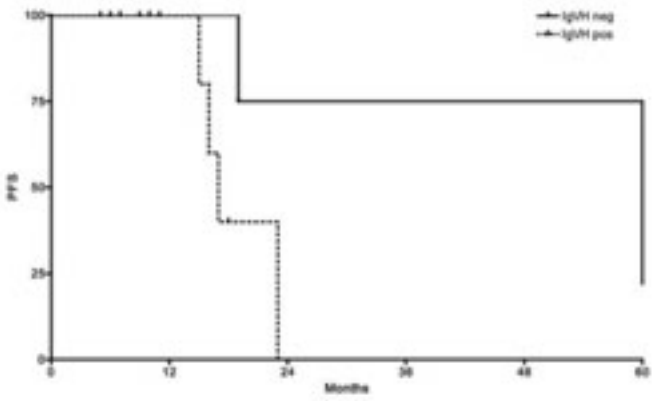

Figure 1. PFS according to minimal residual disease status.

with rituximab immunotherapy is effective, safe and reliable in the treatment of MZL. Minimal residual disease condition at the end of treatment may have a predictive role of disease relapse. Prospective randomized studies are necessary to validate our results and to define a standard approach in advanced stage MZL patients.

0952

CLINICAL IMPACT OF EBV-ENCODED LMP1 IN EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE

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Background. Extranodal NK/T-cell lymphoma, nasal type (ENKL) is a rare disease and more frequently develops in East Asia than in Western countries. Although more than 95% of the cases harbor Epstein-Barr virus (EBV) genome in the neoplastic cells, little is known regarding the roles of EBV-associated gene products in ENKL. *Aims.* To elucidate detailed expression profiles of EBV-associated gene products and their impact on clinical behavior in ENKL. *Methods.* ENKL cases referred to Juntendo University Hospital between August 1996 and June 2010 were retrospectively studied. All cases were diagnosed according to the latest World Health Organization classification. Expressions of EBV-associated gene products were evaluated by immunohistochemistry in formalin-fixed, paraffin-embedded tissue sections. This study was conducted with approval of the Institutional Review Board of Juntendo University, and informed consent was obtained in accordance with the Declaration of Helsinki. *Results.* Total 30 ENKL cases were analyzed. The cases consisted of 19 males and 11 females, and the median age at diagnosis was 62 years (range 27-85 years). All cases were positive for EBERs and lacked EBNA2 expression. ZEBRA was detected in four cases (13.3%). LMP1 and LMP2A were detected in 22 cases (73.3%) and 12 cases (40.0%) respectively. However, co-expression of LMP1 and LMP2A was observed in 10 cases (33.3%). LMP1-positive cases tended to have higher levels of phosphorylated Akt. The 2-year overall survival rates were 78.3% (95% CI, 59.3 to 97.3%) and 12.5% (95% CI, 0.0 to 35.4%) in LMP1-positive and -negative cases, respectively. Compared with LMP1-negative cases, LMP1-positive ones presented with limited disease (p=0.037, chi-square test) and showed better prognosis (p=0.001, log-rank test). *Summary/Conclusions.* Although the expression patterns of LMPs were heterogeneous, LMP1-positive cases showed more limited disease and had a survival advantage in ENKL.

0953

RELAPSE PATTERN AND PROGNOSTIC FACTORS OF PRIMARY CNS LYMPHOMA

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Background. Primary central nervous system lymphoma (PCNSL) rarely relapses in extracranial sites, but there has not been a specialized guideline for follow-up evaluation. High-dose methotrexate based chemotherapy and high-dose chemotherapy(HDC) followed by autologous stem cell transplantation (ASCT) were shown as alternative strategies for PCNSL recently, but data on prognostic factors with these treatment scheme are limited. *Aims.* The aim of this study was to evaluate the pattern of relapse and investigate prognostic factors for PCNSL with single institution experience. *Methods.* Between November 1995 and August 2010, 67 patients with newly diagnosed PCNSL at the Asan Medical Center, Seoul, Korea were included. *Results.* The median age was 54.5 years (range, 26-77 years) and 55 (78.6%) patients had intracranial lesions only. Nine patients had leptomeningeal involvement, 2 had ocular lesions, and one had spinal cord lesion. Twenty-nine patients (43.3%) were treated with chemotherapy only, 13 (19.4%) with chemotherapy followed by whole brain radiotherapy (WBRT), while 20 (29.8%) were given HDC followed by ASCT. Two patients received palliative WBRT only and 3 received best supportive care. While all patients achieved CR (complete response, 76.2%) or PR (partial response, 23.8%) with ASCT, overall response rate (ORR) to chemotherapy and chemotherapy followed by WBRT were 64.5% and 84.6%, respectively. No systemic relapse was noted among 27 patients experiencing relapse; intracranial lesion only in 23 patients, 3 with leptomeningeal involvement and one with ocular relapse. Median overall survival (OS) and failure free survival (FFS) of all patients were 35.8 and 13.1 months, respectively. Age< 60 years (44.9 ±12.41 versus 27.0 ± 6.93 months, p=0.040) and Eastern Cooperative Oncology Group performance status (PS) < 2 (44.9 ± 12.41 versus 13.2 ± 13.81 months, p=0.002) were the only variables related to prolonged OS. Other variables in the International Extranodal Lymphoma Study Group prognostic scoring system and Memorial Sloan-Kettering Cancer Center prognostic model were not predictors of survival in this group. Patients received ASCT had longer OS (58.6 ± 21.48 versus 33.3 ± 8.65 months) but without statistical significance (p=0.083), while they had significantly better FFS (35.5 ± 19.83 versus 9.9 ± 2.96 months, p=0.013). *Conclusions.* Considering no systemic involvement of relapsed PCNSL in current study, regular evaluation with computed tomography or positron emission tomography to investigate extracranial sites might not be necessary in PCNSL patients. Age and PS still retain prognostic significance irrespective of treatment scheme, and ASCT could lead to improve response rate and FFS.

0954

TOXICITY ADAPTED HIGH-DOSE THERAPY IN MANTLE CELL LYMPHOMA PATIENTS, USING SCHEMES EPOCH, HMA AND GIDIOX, AUTOLOGOUS STEM CELL TRANSPLANTATION AND RITUXIMAB

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Background. Mantle cell lymphoma (MCL) is aggressive B-cell neoplasm diagnosed predominantly among elderly men. (R)CHOP-like schemes are effective in remission induction, but progression free survival is short with median overall survival of 3-5 years. Upfront use of high-dose cytarabine (12 g/m^2), autoSCT and rituximab at all stages of therapy is the most effective treatment but possible only with younger patients. Decrease in AraC doses to 4 g/m^2 per cycle significantly reduce progression free survival. Prominent efficacy of gemcitabine-oxaliplatin combinations and irinitecan in relapsed and refractory MCL patients allowed including these drugs in first-line treatment in cases when the scheme R-HD-Met-AraC (Romanguera J. 2005) is impossible. *Aim.* toxicity and efficacy assessment of schemes R-EPOCH/R-GIDIOX and R-EPOCH/R-HMA in primary MCL patients eligible for autoSCT. *Methods.* Since May 2008 17 untreated MCL patients (average 55 years (29-63), 9 males and 7 females, MIPI: 35% high, 30 % intermediate, 35% low risk) were enrolled. After first R-EPOCH cycle (Wilson W. 2003) patients were stratified according to toxicity they had received either R-EPOCH/ R-HMA or R-EPOCH/R-GIDIOX (gemcitabine 800 mg/m^2 days 1 and 4, oxaliplatin 120 mg/m^2 day 2, irinotecan 100 mg/m^2 day 3, dexamethasone 10 mg/m^2 IV days 1-5, ifosfamide 1000 mg/m^2 days 1-5) for those who got higher hematological toxicity (grade 4 for more than 3 days). Depending on the terms of response, patients received 6-8 cycles of immunochemotherapy and autoSCT (BEAM) with *in vivo* purging by rituximab before harvest and reinfusion. Patients with residual tumor after autoSCT were consolidated with local radiotherapy. MRD was diagnosed and controlled by flow cytometry. Rituximab maintainance was performed every three months for 2 years. *Results.* 29 GIDIOX cycles were analyzed with evidence that GIDIOX is less intensive and toxic regimen than HMA, but in our selection hematologic toxicity in R-GIDIOX and R-HMA arms were similar due to less fit patients in R-GIDIOX arm, except thrombocytopenia, that was more prominent and lasting in R-HMA arm. Main non-hematological toxicity of GIDIOX was hepatic, with ele-

Figure 1. The scheme of treatment.

vated aminotransferases grades 1-2 and 3 in 48% and 3% of cycles respectively, without clinical signs. Complete responses were obtained for 16 out of 17 patients after a median of 6.5 cycles in both arms. The sources of stem cell were PB in 15 patients and BM in one case of harvest failure after GIDIOX. Median number of collected stem cells was $4.07x10^6$/kg. 8 patients achieved CR (MRD-) among 9 ones in HMA arm, 1 induction death after first HMA cycle (acute renal failure and septic shock), and one relapse in 8 months after autoSCT, with observation from 1 to 13 months. GIDIOX arm included 8 patients: OR 100%, 7 CR (including 1 patient who achieved CR after radiotherapy), one patient with partial remission continues to get treatment, observation from 1 to 23 months, without relapses. *Conclusion.* GIDIOX scheme is less toxic than HMA and equally effective in response induction and mobilizing, so it could be recommended for those in whom high-dose AraC and methotrexate can potentially cause severe adverse consequences.

0955

FEASIBILITY OF THE TNM-BASED STAGING SYSTEM OF OCULAR ADNEXAL EXTRANODAL MARGINAL ZONE LYMPHOMA OF MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT LYMPHOMA)

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Background. Malignant lymphomas of the ocular adnexa account for 1-2% of non-Hodgkin lymphomas and 8% of extranodal lymphomas. Although immunohistochemical and molecular analyses have resulted in ocular adnexal lymphoma (OAL) being diagnosed more frequently, previous studies do suggest that there has been a true and inexplicable rise in the incidence of these tumors in the last decades. Among OALs, the most common subtype by World Health Organization (WHO) classification is marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). Several studies have shown that the relative incidence of ocular adnexal MALT lymphoma (OAML) varies among different populations. The proportion of OAML among primary OALs is higher in Korea (86-98%) than in Western countries (50-78%). Although the prognosis of patients with OAML is generally favorable, some situations such as higher clinical stage, nonconjunctival primary site, nodal involvement, and bilaterality at presentation have been associated with a worse prognosis. However, based on the Ann Arbor staging system, two-thirds of OAMLs have been recorded as stage IE tumors, because the Ann Arbor staging system does not distinguish among OAMLs based on anatomic location, extent of primary tumor infiltration, multicentricity, or bilaterality. *Aims.* The Ann Arbor staging system is not particularly useful in determining the prognosis for these patients. The American Joint Committee on Cancer (AJCC) proposed the tumor, node, metastasis (TNM)-based clinical staging system for primary OAL to overcome the weak points of the Ann Arbor staging system, but the clinical impact of TNM-based staging on primary OAML has not been determined. This study is novel and demonstrates the feasibility of the TNM-based staging system for OAML. *Methods.* We performed this study to evaluate the feasibility of the TNM staging system for ocular adnexal extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (OAML). The data form 66 total eyes from 54 patients with biopsy-confirmed OAML according to World Health Organization classification were retrospectively analyzed. *Results.* Using the TNM staging system, we reclassified all patients into two categories: (1) T1N0M0 stage group (n=26), for patients with lymphoma involving only the conjunctiva; and (2) above T1N0M0 or bT1N0M0 stage group (n=28), for patients with lymphoma extending to the orbit, eyelid, or adjacent structures, and/or bilateral OAML. After a 24-month median follow-up period for all patients, the T1N0M0 group revealed higher progression- free survival (PFS) than the above T1N0M0 or the bT1N0M0 group (P=0.041). In a separate analysis of only 50 patients categorized as Ann Arbor stage IE, the T1N0M0 group demonstrated higher PFS (100%) than the above T1N0M0 or the bT1N0M0 group (84.7%; P=0.067). *Conclusions.* Our data show that the poor prognostic group classified as Ann Arbor stage IE can be further distinguished by using the TNM staging system. Thus, further studies to develop treatment strategies for reducing relapse after treatment for OAML should use the TNM staging system.

0956

TREATMENT OF BURKITT LYMPHOMA IN ADULTS USING AN ADAPTED PEDIATRIC ALL PROTOCOL: A SINGLE CENTER EXPERIENCE

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Background. Burkitt lymphoma is an aggressive B-cell malignancy that accounts for 1% to 2% of all adult lymphomas in Europe. Treatment consists of dose-intensive, multi-agent chemotherapy and is highly effective but associated with considerable toxicity and treatment-related mortality. Several therapeutic strategies are currently in use, but the optimum treatment has yet to be defined. *Aims.* We report the results of a series of adult patients treated for Burkitt lymphoma with a protocol adapted from the LMB regimens of the Société Française d'Oncologie Pédiatrique. *Methods.* Twenty-three patients, 18 male and 5 female, were diagnosed with Burkitt lymphoma or Burkitt leukemia between 1997 and 2009. Median age was 39 years (14 to 74 years). No patients were HIV-positive. Fifteen patients presented with stage III-IV disease, 8 of which had central nervous system involvement. According to the revised WHO 2008 criteria 5 cases would now be diagnosed as B-cell lymphoma unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. All patients were treated with an adapted LMB protocol consisting of an initial cytoreductive phase (COP), followed by 2 induction cycles (COPADM), 2 consolidation cycles (CYVE), and 4 maintenance cycles. Fourteen patients also received rituximab. Median follow-up was 31 months. *Results.* The adapted LMB protocol was well tolerated by most patients. Frequent toxicities included myelosuppression, neutropenic fever, and grade I-II mucositis and vasculitis. Three patients experienced grade III mucositis, 1 patient grade III neuropathy, and 2 patients nephrotoxicity (1x grade II, 1x grade III). Tumor lysis syndrome occurred in 3 patients. Eighteen out of 23 patients achieved a complete response (CR). Four patients had refractory disease. One patient achieved a partial response and received an autologous stem cell transplantation (SCT). He relapsed several years later and died during salvage therapy. Three patients underwent myeloablative allogeneic SCT: 2 in 1st CR because of extensive disease and the presence of a sibling donor, 1 following relapse after initial CR. Two of these patients died from treatment-related complications. The remaining 16 patients are alive and in CR as of today, including 4 out of 5 patients with B-cell lymphoma unclassifiable. The 2-year event-free survival and overall survival rates were 73.9% and 78.3% respectively. 2-year overall survival rates were significantly better for patients < 40 years (91.7% versus 50.0%), patients with early disease (100% versus 60.0%), and patients with low or low-intermediate IPI scores (100% versus 50.0%). *Conclusions.* The adapted LMB protocol is an effective regimen in adult patients with Burkitt lymphoma, Burkitt leukemia, and B cell lymphoma unclassifiable with a Burkitt-like phenotype. Survival rates are similar to the outcomes in case series using other treatment regimens such as CODOX-M/IVAC. However, compared to these studies we report a relatively low incidence of grade III-IV mucositis and grade III-IV neuropathy. The role of allogeneic SCT for Burkitt lymphoma should remain limited to salvage therapy.

0957

MSCT FOLLOW UP IN PATIENTS WITH MALIGNANT LYMPHOMA: DOES SEMI-AUTOMATED VOLUMETRY IMPROVE THERAPY RESPONSE CLASSIFICATION COMPARED TO MANUAL MEASUREMENTS?

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Background. Standardized staging and response assessment are crucial in lymphoma clinical trials in order to afford assessment of treatment strategies and facilitate comparison among studies. *Aims.* Impact of semi-automated volumetry compared to unidimensional measurements on therapy response classification in CT follow-up of malignant lymphoma. *Methods.* MSCT scans of 65 patients with malignant lymphoma prior to therapy (baseline) and after 2 cycles of chemotherapy

(follow-up) were included. A total of 313 target lymph nodes (56 cervical, 131 thoracic and 126 abdominal) were evaluated by two radiologists independently. Long axis diameter (LAD), short axis diameter (SAD) and volume were determined manually and using semi-automated segmentation software. Relative interobserver difference (RID) and time for manual and semi-automated segmentation were evaluated. Therapy response was calculated for each parameter based on "IWC" lymphoma-guidelines and "RECIST"-adapted guidelines. Mean of metric and volumetric measurements served as the reference standard. Statistical analysis encompassed intraclass correlation coefficients (ICC), t- and McNemar-test. *Results.* Over all regions mean lymph node size in baseline/follow-up was 23.8±10.3 mm/17.0±9.2 mm for LAD and 7.2±13.5 ml/3.4±9.9 ml for volume. RID was consistently low in baseline and follow up with high ICC>0.96 for semi-automated measurements. Mean evaluation time for semi-automated segmentation without need for correction was shorter (16.6±11.7 sec) than for manual measurements (29.0±14.5 sec). In 65% of all lymph nodes correction was necessary and evaluation time increased to 39.5±25.9 sec. Regarding therapy response, semi-automated volumetry obtained significantly more accurate classifications than semi-automated and manual LAD and SAD (e.g. volume 87.8% vs. semi-automated LAD 83.8%, manual SAD 78.9%, all p<0.05). *Summary/Conclusions.* Semi-automated lymph node volumetry is more accurate for therapy response classification in patients with malignant lymphoma as compared to established LAD.

0958

OUTCOME OF NON-HODGKIN LYMPHOMA (NHL) AND CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IN THE SPANISH BENDAMUSTINE REGISTRY

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Background. Bendamustine (B) is a purine analog/alkylator hybrid with antitumor activity. B is currently licensed by EMEA for use in NHL, CLL and multiple myeloma. *Aim.* Our aim was to analyze retrospectively the efficacy and toxicity of B for NHL and CLL in Spain. *Patients and Methods.* From June 2009 to September 2010, a questionnaire form was sent to Spanish centers in which B had been used as Compassionate Use Program. Patients with relapsed or refractory NHL or CLL after at least 1 prior treatment regimen were eligible. Any B regimen was included. *Results.* 109 patients (pts) were included from 22 institutions. Histology: 42 pts CLL; 18 pts aggressive NHL; 49 pts indolent NHL. Median time from diagnosis to B treatment was 4.9 years (range 1-24). Median number of previous treatment regimens was 3 (range 1-11). 44 pts (40%) were refractory to prior treatment. The most frequent used regimen was rituximab plus B (RB) independently of the histology. 63% of the pts had adverse events grade 3 or 4 (mainly hematology toxicity). Overall response rate (ORR) was 66% (30% complete response (CR)). Response rate was higher in mantle cell lymphoma (86%). ORR observed in refractory pts was 45%, including fludarabine resistant pts. The median progression-free survival (PFS) was 12.7 months (95% CI%, 7.14 to 18.23). Among follicular NHL and CLL pts, median PFS time was 12.4 vs 8.9 months, respectively. The factors significantly affecting PFS were number of treatments prior to B, resistance to prior chemotherapy and type of response achieved to B therapy. *Conclusions.* 1. Bendamustine containing therapy achieved a high response rate in this heavily pretreated CLL and NHL pts with an acceptable toxicity profile. Responses were seen in indolent and aggressive histology, and also in patients with chemoresistant disease to previous regimen. 2. Histology and number of prior treatment were the most important factors affecting PFS.

0959

FDG-PET/CT STAGING IN FOLLICULAR LYMPHOMA AND IMPACT ON FLIPI SCORE

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Background. [F18]fluorodeoxyglucose positron emission tomography (FDG-PET) is currently recommended by the International Harmonisation project for staging of Hodgkin Lymphoma and Diffuse Large B-cell Lymphoma. However the role of FDG-PET in the staging of Follicular Lymphoma (FL) has not yet been clearly defined. Despite this FDG-PET and PET/CT are increasingly being used by multiple centres in the staging of FL. *Aims.* To investigate the impact of FDG-PET/CT on the staging of FL and to evaluate the potential changes in the FLIPI score. *Methods.* Between 2008 and 2010, 40 patients with grade 1-3 FL (either newly diagnosed or at relapse) underwent whole body FDG-PET/CT, a contrast enhanced CT (ceCT), a bone marrow aspirate and trephine (BMT). We compared the number of lymph node regions and the site of extranodal involvement assessed by FDG-PET with those as assessed by ceCT and BMT. *Results.* FDG-PET identified 160 involved lymph node regions compared to 137 by ceCT. In 7% of patients with a negative BMT, FDG-PET showed bone marrow involvement by demonstrating focally increased glucose metabolism. However, FDG-PET failed to identify bone marrow involvement in 17% of patients with a positive BMT. FDG-PET upstaged 12% of patients compared to ceCT while 15% were downstaged. The management of 2 patients was affected by a change in stage due to the identification of extranodal involvement on FDG-PET not detected on ceCT. The FLIPI score was altered in 20 % of patients by using FDG-PET/CT: the prognostic group according to the FLIPI changed from low to intermediate risk in 5% of the patients, and from intermediate to poor risk in 10%. *Conclusion.* FDG-PET/CT resulted in a change of stage in 27% of FL patients but this resulted in a change in the management in 2 patients only. As a result of identifying more involved lymph node regions, the FLIPI score was altered. The issue of whether FDG-PET or ceCT only is performed for staging needs to be taken into account when using the FLIPI to define and compare study populations.

Table 1. Percentage of patients in each risk group.

Risk group FLIPI	ceCT	FDG-PET/CT
Low (0 - 1)	42.5 %	30.0 %
Intermediate (2)	15.0 %	17.5 %
High (3 - 5)	42.5 %	52.5 %

0960

POPULATION BASED ANALYSIS OF CHEMO-IMMUNOTHERAPY IN ADULT BURKITT LYMPHOMA AND LEUKAEMIA (BLL)

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Background. Adult sporadic BLL is a rear and highly aggressive malignancy. It is known to strongly express surface CD20 antigen. Recent data indicates that the outcome of adult BLL patients can be improved by the addition of anti-CD20 antibody rituximab to multi-agent chemotherapy. The population based outcome data of BLL is rarely presented and may

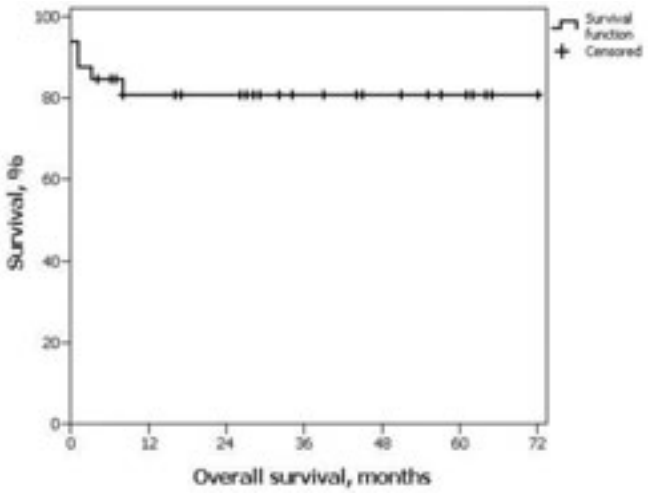

Figure 1. Overall survival of all patients.

help to avoid reporting bias. *Aim.* The outcome of patients 18 years of age and older diagnosed with BLL. *Methods.* Adult BLL cases diagnosed in Lithuania (average annual population 3.34 million) in 2004-2010 were retrospectively identified through Hematology Monitoring System of Lithuania and their medical records were reviewed. BLL was diagnosed according to the World Health Organization criteria. *Results.* 32 adults were diagnosed with BLL (26 lymphoma and 6 leukemia). The median age was 32 years (range 18-81). 24 (75%) patients were male, 3 (9%) were HIV positive. 20 (77%) of lymphoma patients had St. Jude stage III-IV disease. The main location of lymphoma was gastrointestinal tract 11 (42%). 2 (6%) patients had neuroleukaemia. Two (6%) patients were not treated: one refused therapy and one died before treatment and 30 (94%) patients received short, intensive high-dose methotrexate, fractionated alkylator based therapy. Rituximab 375 mg/m2 (off-label use) was administered 1 day before each chemotherapy cycle in 27 (90%) of the treated patients. Autologous stem cell transplantation was used to consolidate 2 (7%) partial remissions. The median follow-up was 28.5 months (range: 0 - 71). Among the treated patients, 24 (80%) and 2 (7%) achieved complete and partial remission, respectively and 3 (10%) patients had primary progressive disease. 6 (19%) patients died during the first 8 months of the diagnosis: 2 were not treated, 3 deaths were due to primary disease progression and one was due to traumatic subdural hematoma. 2 year overall and progression free survival were 81% (95% CI: 62% - 91%) in all patients (Figure 1). In patients who received treatment, 2 year overall and progression free survival were 86% (95% CI: 67% - 96%). *Conclusion.* Our population based analysis confirms favorable outcome of adult BLL receiving brief intensive chemotherapy combined with anti-CD20 immunotherapy. 10% of the patients have primary refractory disease.

0961

RESULTS OF A LYMPHOBLASTIC LEUKAEMIA-LIKE CHEMOTHERAPY PROGRAMME WITH RISK-ADAPTED MEDIASTINAL IRRADIATION AND STEM CELL TRANSPLANTATION FOR ADULT PATIENTS WITH LYMPHOBLASTIC LYMPHOMA

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Background. In lymphoblastic lymphoma (LL) the role of mediastinal radiotherapy (mRT) and stem cell transplantation (SCT) remains controversial. In a risk-oriented design, we adopted a flexible treatment in which (1) patients with persistent mediastinal abnormality, evaluated by postinduction CT scan, received mRT; and (2) those with persistence of MRD, evaluated by molecular analysis of BM, underwent stem cell transplantation (SCT). *Aims.* To evaluate the clinical outcome, prognostic factors and the pattern of recurrence adopting this innovative strategy. *Methods.* Between 2000 and 2008, 6 B-LL and 24 T-LL untreated pts, median age 27 years (range, 16-57), M/F 17/13, 21 with mediastinal and 12 with BM involvement (<20%) were enrolled in the NILG 09/00 protocol (Bassan, *et al.*, Blood, 2009). The treatment included an induction/early consolidation with Ida/V/P/Asp/Cy (blocks 1-3, 5, 6, 8), HD-MTX/Ara-C (4, 7), CNS phase and mRT (Gy 24-32) for pts with pathological postinduction CT scan. Postconsolidation therapy was MRD/risk oriented. MRD negativity (Mneg) was defined by low positive (<10-4) and negative determinations obtained before blocks 6 and 8, respectively. Mneg pts received maintenance, while Mpos underwent family-related/unrelated SCT (or 2-4 autologous stem-cell supported hypercycles [H/C: L-PAM/VP/6MP/; HD-MTX/Ara-C] followed by maintenance). Pts with undefined MRD were scheduled to receive maintenance if standard risk (SR -only the pts with CD10+ B-LL), and SCT if high risk (HR-all other pts). *Results.* Twenty-eight pts (93%) (T-LL, n=24; B-LL, n=6) achieved CR and 2 had refractory T-LL and died. Of 21 pts with mediastinal mass, 13 (62%) achieved a CR after chemotherapy alone, whereas 6 (28.5%) required additional mRT. Eleven pts were evaluated for MRD: 6 were Mneg and 5 Mpos. On the basis of MRD findings and clinical risk characteristics, 14 pts underwent SCT, 13 received maintenance chemotherapy, and one had local RT. Five pts relapsed. Among the 14 nonirradiated pts with T-LL, the mediastinal recurrence rate was only 7%. After a median follow-up of 3.9 years (range, 0.8-9.2+ years) 22 pts (73%) who responded were alive: 21 in 1st and one in 2nd CR. Postremission failure was due to recurrence (n=5, 18%) in the BM (B-LL, n=1; T-LL, n=3; 1 irradiated and 2 non-irradiated patients) or mediastinum (n=1, non-irradiated T-LL patient), and other malignancy (n=2, 7%; 1 sAML and 1 sDLBCL). The projected 5-year OS, DFS, and relapse rate were 72% (83% for B-LL and 69% for T-LL), 77% (83% for B-LL and 71% for T-LL), and 18%, respectively. The probability of OS and DFS was not significantly affected by any of examined prognostic factors, however the achievement of Mneg status was associated with a better outcome in comparison with Mpos cases (5-yrs DFS 80% vs. 60%, P=ns). *Conclusions.* This programme induced high remission and survival rates, with an acceptable toxicity profile. Avoidance of mRT and SCT in patients with an early response in the mediastinum and without molecular evidence of disseminated LL limits treatment-related toxicity and warrants high cure rates in a significant proportion of patients exposed to standard chemotherapy only.

0962

THERAPY WITH 90Y IBRITUMOMAB TIUXETAN IN RELAPSED/REFRACTORY NON-HODGKIN LYMPHOMA. ANALYSIS OF RECENT OUTCOMES

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Introduction. Add-on treatment of follicular non-Hodgkin's lymphoma (NHL) with 90Y Ibritumomab tiuxetan (Zevalin®) has become an efficient alternative. The aim of this study is to analyze our updated information of patients treated with 90YIbritumomab/tiuxetan in a prospective study according clinical practice setting and to analyze treatment outcome. *Subjets and Methods.* 87 relapsed/refractory lymphoma patients were included in a clinical protocol conducted by a multidisciplinary team and treated in the same centre. According the inclusion criteria: found relapsed/refractory CD20+ NHL patients with neutrophils ≥ 1,5 x 109/L, platelets ≥ 100 x 109/L, bone marrow lymphocytes CD20+ ≤ 25%. All patients received 0,3 or 0,4 mCi /kg IV (88%) of 90YIbritumomab/tiuxetan and response evaluation was performed 12 weeks after. Period of study: September 2005/September 2010. Endpoints: objective response rate (ORR), time to relapse (TTR) overall survival (OS) and safety. Other clinical prognostic factors were observed to assess their possible influence upon treatment value. *Results.* Until September 2010, 87 patients had received treatment with 90YIbritumomab/tiuxetan, and were considered to analysis; M/F 52.6%/47.4%; mean age 61.88 years (30-86); ECOG 0-1 96.2%; 68 follicular NHL (63.8%), 8 mantle cell NHL (13.8%), 9 BDLG NHL (15.5%) and 2 Hodgkin Lymphoma (6.9%). According FLIPI score distribution: (0-1) 70.2%, FLIPI (>1) 29.8%, ECOG 0-1 96.2%, Previous therapy schedules 1-2 (44.8%), >2 (55.2%). The median follow-up time: 30.23 months,

medianTTP: 34.4 months (95% CI: 29.7; 39.0). Mean OS 46.0 months (95% CI: 38.0; 53.9), median: NA for FNHL. Completed response (CR) was different according lymphoma subtype: 58 (CR) follicular NHL, 7 (CR) mantle cell NHL and 6 (CR) NHL BDLG. ORR: 76.8%. Complete response 73.4%, partial response 3.4% and relapsed 23.1%, 11.3% patients have died or withdraw. Median TTR was 42 months for follicular NHL vs 15 months for other NHL subtypes. Safety: thrombocytopenia being the most frequent (27.6%) haematological toxicity, median time to G3-4: fourth week, and neutropenia (22.4%), the median time to recover normal values was 4.2 and 2.6 weeks respectively. In 10.3% of patients red blood cell transfusion was required, and platelet transfusions in 27.6%. The most frequent non haematological toxicity was asthenia. One patient developed a severe mucositis. Two patients have concomitant associated tumours (colon and prostate) and two patients developed secondary malignancies (skin and lung tumours). *Comments*. In our experience 90Y Ibritumomab tiuxetan (Zevalin®) is a safety and effective therapy in relapsed NHL, especially in patients with follicular non-Hodgkin's lymphoma that can obtain higher complete response than other types of lymphoma and prolonged median survival time.

0963

EARLY STAGE FOLLICULAR LYMPHOMA: ROLE OF MOLECULAR MONITORING IN PATIENTS TREATED WITH LOCAL RADIOTHERAPY ± RITUXIMAB

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Background. Conventional treatment of stage I-II follicular lymphoma (FL) is local radiotherapy (RT), which allows eradication of the disease in about 50% of patients. *Aim.* To evaluate the role of anti-CD20 MoAb and of minimal residual disease (MRD) in this setting of patients. *Methods.* 41 consecutive patients with a confirmed diagnosis of stage I/II FL were investigated by PCR in order to identify the presence of Bcl-2 rearranged cells in the bone marrow (BM) and/or peripheral blood (PB). All patients were treated with involved field RT (36 Gy). Subsequently, MRD was evaluated every 6 months in patients positive at baseline. *Results.* PCR analysis revealed Bcl-2 rearranged cells in 24/41 patients (58.5%) at presentation. After irradiation of the sole site of the disease, Bcl-2 rearranged cells disappeared in 15 of the 24 (62.5%) patients positive at baseline; in 8 (19.5%) MRD was positive, while 1 patient refused the test. After a median follow up of 50 months, 5 patients (12.2%) had a clinical relapse. MRD evaluation demonstrated that: - 17/41 Bcl-2 negative patients at the basal evaluation were not subsequently retested; only 1/17 patients had a clinical relapse (the new biopsy documented a mantle cell lymphoma). - Of the 15 patients positive at baseline and who became negative after RT, 3 have had a molecular relapse during the follow-up, leading in one case to an overt clinical relapse. - Of the 8 patients persistently Bcl-2 positive after radiotherapy, 3 had a clinical relapse. Rituximab (375 mg/m2 x 4) was administered to 5 patients with a persistently positive Bcl-2 after RT: 3 of them became Bcl-2 negative. *Conclusions.* Viable Bcl-2+ cells can be demonstrated in the BM and/or PB of the majority of stage I-II FL patients (despite a negative BM biopsy). Irradiation of the sole nodal/extranodal disease sites allows disappearance of Bcl-2+ cells in the majority of previously positive patients (62.5%). Pre-treatment Bcl-2 BM and/or PB evaluation has a prognostic role: no clinical relapses were observed in Bcl-2 negative cases at baseline except for one patient, relapsed as mantle cell. MRD evaluation has a prognostic role: among 32 Bcl-2 negative patients after treatment, 2 relapses (6.2%) were observed (1 relapsed as mantle cell), while among 8 Bcl-2 positive patients after treatment 3 relapses (37.5%) were observed. Prognosis of early stage FL treated with local RT ± rituximab is excellent: only 5 patients have so far relapsed at a median follow up of 50 months.

0964

RITUXIMAB RETREATMENT IN PATIENTS WITH RELAPSED OR REFRACTORY B CELL NON-HODGKIN'S LYMPHOMA: PREDICTIVE FACTORS AND SAFETY

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Background. Use of rituximab, a monoclonal antibody targeting CD20, is a milestone for B-cell non-Hodgkin's lymphoma (NHL) treatment. In patients with relapsed B-cell NHL, retreatment of rituximab, with or without salvage chemotherapy, has been shown to exert promising safety and efficacy. However, a substantial proportion of patients would eventually fail by this approach. *Aims.* In this retrospective study, we aimed to explore factors predictive for response of rituximab retreatment and determine whether response could be translated into progression-free survival (PFS). A second objective is to re-exam the

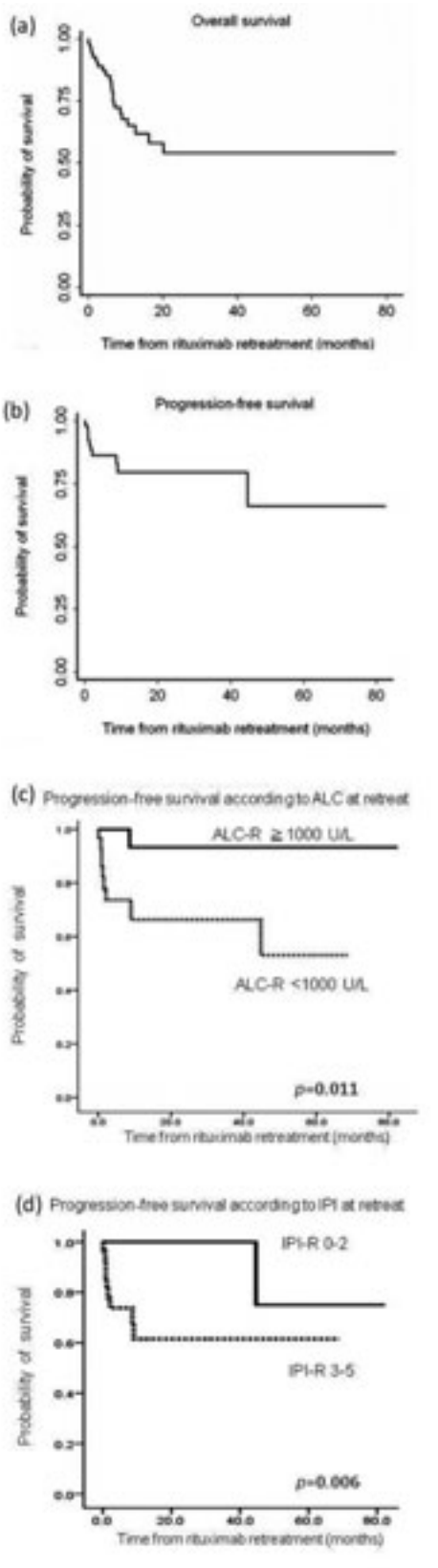

Figure 1. Response and factors of rituximab retreatment.

safety and efficacy of rituximab retreatment in a hyperendemic area of chronic hepatitis B virus infection. *Methods.* This is a single institute study that retrospectively analysis patients with relapsed or refractory B cell NHL, who had received retreatment of rituximab either alone or in combination with salvage chemotherapy. All patients had received first-line rituximab-containing treatment. Patient's characteristics at initial diagnosis and at relapse were collected. Response to treatment was evaluated by chart review and was defined as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). For those who reached complete response and partial response were defined as responder. *Results.* A total of 54 patients who received rituximab retreatment were identified. The overall response rate to first-line rituximab-containing treatment was 88.9% (CR 27.8%, PR 61.1%). Most patients (92.5%) received rituximab retreatment in combination with salvage chemotherapy. The overall response rate of rituximab retreatment was slightly lower than the first-line treatment (61.1%) but the CR rate was similar (27.8% vs 29.6%). Median PFS and median overall survival (OS) from rituximab retreatment were not reached, with a 5-year PFS rate of 81.5% and a 5-year OS rate of 63.0%. Factors associated with better response to rituximab retreatment were high absolute lymphocyte count at retreatment [(ALC-R), defined as absolute lymphocyte count ≥1000/UL, p=0.006) and low IPI at retreatment [(IPI-R), defined as IPI ≤2, p <0.001). Both of which were significant independent predictive factors in multivariate analysis. Incidence rate of febrile neutropenia was 38.8% but was not associated with response rate, PFS or OS. Moreover, a high incidence of herpes zoster reactivation (14.8%) and a low incidence of HBV reactivation (3.7%) were observed during rituximab retreatment. *Conclusion.* For patient with relapsed/refractory B cell NHL, retreatment with rituximab-containing regimen is a promising and generally tolerable salvaging approach. Patients with low ALC-R and high IPI-R might predict worse response to rituximab retreatment. Febrile neutropenia and herpes zoster reactivation are the most common adverse events during or after rituximab retreatment. Acute exacerbation of hepatitis B virus infection or other opportunistic infections were uncommon. Further prospective controlled trials are needed to warrant our findings.

0965

COST-EFFECTIVENESS OF FIRST-LINE RITUXIMAB MAINTENANCE TREATMENT FOR FOLLICULAR LYMPHOMA

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Background. Rituximab (R) and chemotherapy induction treatment has been recommended for patients with follicular lymphoma (FL). Just recently, guidelines have started to recognize the first-line R-maintenance treatment (1LRMT). FL patients receiving no 1LRMT are likely to relapse earlier compared to the patients receiving 1LRMT. *Aims.* To project the lifetime health outcomes for the 1LRMT and observation, and to compare the lifetime cost-effectiveness of the 1LRMT and observation in patients presenting with FL in Finland. *Methods.* A probabilistic Markov state-transition model based on the PRIMA (Primary RItuximab and MAintenance) phase III trials' 38 months first-line results and EORTC20981 phase III 60 months' subsequent-line trial results and literature was developed in order to perform the analysis from the Finnish public health care payer perspective. The model was used to simulate patients' transitions between first-line progression-free (PF1), PF2, progression and death states using a second order Monte Carlo simulation (2000 simulations recorded), one month cycle, and half cycle correction. Parametric extrapolation for the PRIMA PF1 results was done. The best fitting survival model was Gompertz and the maximum of 4 year treatment benefit was assumed for R in PF1 state due to the immature PRIMA data. After PF1, eligible patients were assigned for second R-maintenance based on the PRIMA results and the recent ESMO guideline for FL. For PF2, the recently published Finnish modelling results based on the EORTC20981 5-year data (maximum 5 year treatment benefit assumed) were used. Age-dependent transition to death was set equal to the larger of EORTC20981 trial or Finnish background mortality. Case-mix adjusted Finnish treatment, safety, monitoring, management and test costs were included in 2010 real value, and the most affordable public drug costs (2/2011; drug wastage included) were used. EQ-5D-based utilities were used. Discounting with 3% per annum was used for costs and health outcomes. The impacts of various assumptions (e.g. discounting with 0%, and PF1 function forms as Weibull, Exponential, Log Logistic, Log Normal and Gamma) were assessed. *Results.* The difference in survival estimates was significant for 1LRMT in comparison to observation; 1LRMT was projected to result to expected 8.56 (95%CI 7.85-9.38) quality-adjusted life-years (QALY) and 11.25 (95%CI 10.40-12.25) life-years meanwhile observation resulted to 7.18 (95%CI 6.38-8.16) QALYs and 9.61 (8.57-10.98) life-years. The incremental cost-effectiveness ratio (ICER) for 1LRMT vs. observation was €10025 per QALY gained and €8431 per life-year gained. According to the cost-effectiveness acceptability frontier, 41.6%, 96.1% and 99.9% of patients with 1LRMT were cost effective (i.e. had lower ICER than the willingness to pay threshold) at the WTP levels of €10000, €15000 and €20000 per QALY gained, respectively (Figure). The expected value of perfect information was highest with the ICER value of €10025 per QALY gained (€1830 per patient; Figure). The relative results were robust according to the sensitivity analyses. *Conclusions.* 1LRMT was projected to result to significant gains in health outcomes. In addition and irrespective of the conservative assumptions, 1LRMT was a potentially cost-effective treatment option for the FL.

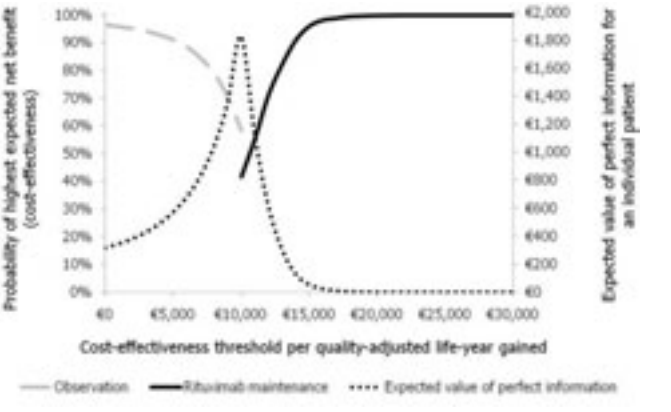

Figure 1. Probabilistic sensitivity analysis results.

0966

TEMPORAL AND GEOGRAPHIC VARIATIONS OF WALDENSTRÖM'S MACROGLOBULINEMIA INCIDENCE: A LARGE POPULATION-BASED STUDY

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Background. Waldenström's macroglobulinemia (WM) is a non-Hodgkin lymphoma (NHL) subtype. Little is known about the incidence and trends for this disease in the United States.*Methods.* Twenty-year data from the Surveillance, Epidemiology, and End Results (SEER) program were used for this study. SEER*Stat was used for data analysis. *Results.* Of the 95 797 cases of NHL diagnosed between 1988 and 2007 in nine SEER registries, 1835 (1.9%) were new cases of WM. Median age at diagnosis of WM was 73 years. The overall annual age-adjusted incidence was 0.38 per 100 000 persons per year, which increased with age, ranging from 0.03 in patients <50 years to 2.85 in patients ≥80 years. The incidence of WM was higher in men (0.54) than in women (0.27) (P < .001) and was higher in whites (0.41) than in African Americans (0.18) or other races (0.21) (P < .05). The annual percent change (APC) for the whole population was 1.01% (P > .05). The APC was 1.21% for whites (P < .05) and 0.80% (P > .05) for non-White. Significant APC increases were seen in the 70-79 age group (1.24%; P < .05) and in three geographic registries (P < .001). *Conclusions.* Although the overall incidence of WM remained steady over time, significant increases in incidence were seen over the past 20 years in whites, in those aged 70 to 79 years, and in three geographic registry areas.

Red cell clinical and transfusion

0967

KNOWLEDGE DEFICITS IN BLOOD COMPONENT TRANSFUSION AMONG JUNIOR DOCTORS IN THE UNITED KINGDOM

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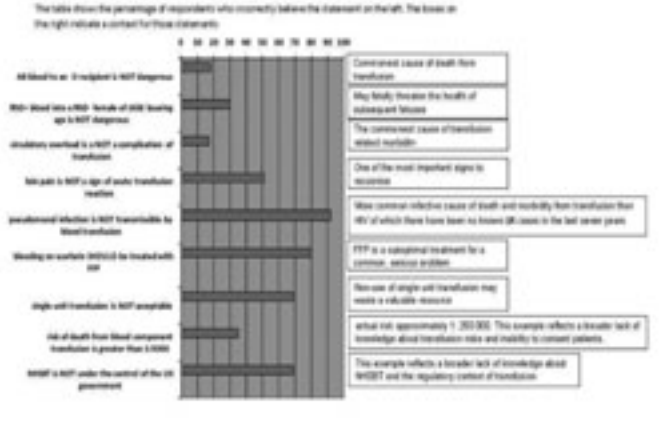
Background. 2.9 million units of blood products are administered annually to approximately 400 000 patients in the UK, usually prescribed by junior doctors. Most adverse events reported are, at least partly, attributable to human error. However, blood transfusion occupies a very small part of the undergraduate curriculum and most postgraduate exams. No published data exists on the level of knowledge junior doctors have of blood product transfusion, its indications and risks. *Aims.* To determine knowledge among non-consultant grade doctors of the indications for, risks, compatibility, complications and broader context of blood component transfusion. *Methods.* A questionnaire distributed to non-consultant, non-haematology trainee, but otherwise unselected, hospital doctors at 6 hospitals, three with medical schools and three without, The questionnaire contained 14 multiple response questions covering 1. Respondent qualifications, grade and specialty 2. Indications for transfusion of platelets, FFP and Packed red cells 3. Nature and magnitude of risks associated with use of these products 4. The products themselves and issues of compatibility 5. Recognising complications of transfusion 6. National Health Service Blood and Transplant (NHSBT) and the regulatory context of blood transfusion. The responses were compared to pre-determined correct answers drawn from the annual UK Serious Hazards of Transfusion reports, official government statistics, UK transfusion guidelines, current legislation and information that could reasonably be expected to be taught to medical staff or be available in standard medical textbooks (especially ABO compatibilities). *Results.* 205 valid questionnaires were returned (approximately 40% response rate). No respondent correctly identified all the indications for transfusion of blood components. 6.5% would inappropriately use red cell transfusion to promote wound healing. 80% would use FFP to treat major haemorrhage due to warfarin, an inferior approach. 35% overestimated the risk of death from blood component transfusion by at least 50 times, 7% thought contracting HIV from blood component transfusion was as likely as dying in a car accident. 30% identified imaginary complications of transfusion, such as acute psychosis or transfusion associated acute liver disease (TaLiD), as real while 17% did not identify volume overload, the commonest cause of transfusion related morbidity, as real. 18% did not recognise the most frequently fatal ABO incompatibility of AB blood to an O recipient as dangerous while 30% did not identify RhD+ blood to an RhD- female recipient of child bearing age, which would risk haemolytic disease of the fetus or newborn in subsequent pregnancies, was dangerous. 69% believe the UK NHSBT is controlled directly from Europe. Figure 1 shows responses to some of the questions asked. *Conclusion.* Appropriate use, avoidance of waste, informed consent from patients and recognition of complications and risks are important aspects of blood transfusion yet it appears that the main prescribers of blood products in the UK have incomplete and sometimes poor understanding of these issues. These deficiencies need to be addressed urgently in undergraduate and postgraduate medical curricula and there should be greater oversight of junior doctor involvement in blood component use.

Table 1. Selected knowledge deficits amongst junior doctors.



0968

KINETICS OF CLOTTING FACTORS INACTIVATION DURING PASTEURIZATION AS APPLIED TO FLEBOGAMMA® AND FLEBOGAMMA® DIF MANUFACTURING PROCESSES

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Background. An increased rate of thromboembolic adverse events associated with the use of an IVIG from a specific manufacturer has been ascribed to increased residual FXIa, and possibly other impurities, in that product. Pasteurization is a process with demonstrated capacity to inactivate clotting enzymes during IVIG production (José *et al.* WebmedCentral Immunotherapy 2010;1(12):WMC001425. Available at: www.webmedcentral.com/wmcpdf/Article_WMC001425.pdf). *Aims.* In this work we describe the kinetics of Pasteurization-induced inactivation of procoagulant activity, in the conditions applied during the production Flebogamma® and Flebogamma® DIF, two pasteurized IVIGs from Grifols. *Methods.* Plasma fraction (Fr) II+III derived samples, containing artificially activated clotting factors, were spiked 1/5, 1/10, 1/20 (v/v) in samples taken from the IVIG manufacturing materials before acid pH treatment (4.5h, pH 4.0, 37°C) or before Pasteurization treatment (10h, 60°C) were applied to the mixtures. The following activation markers were studied: non-activated partial thromboplastin time (NaPTT) with platelet poor plasma (PPP, either neat or after dilution in the assay buffer) or with FXI deficient (FXIdef) plasma, PKA, "kallikrein-like" activities, thrombin generation (TGT) standard test or with FXIdef plasma, and FXI antigen (FXI:Ag, ELISA). The Pasteurization effect was also assessed at different time points (0, 0.5, 2, 3, 5 and 10 h) in 1/5 (v/v) spiked samples and in non-spiked controls as well as in equivalent samples spiked with 1 or 0.1 nM FXIa. *Results.* Samples of industrial materials before acid pH treatment or before Pasteurization spiked with artificially activated FrII+III derived samples showed positive results when assayed for coagulation markers in all tests performed (i.e., in the spike 1/5 samples values ranged 24-77 s for NaPTT-PPP neat, 43-155 s for NaPTT-PPP diluted, 53 - 114 IU/ml for PKA, 0.015-0.038 ∅ AU/min for "kallikrein-like", 89 s for standard TGT, 331-502 s for FXIdef plasma TGT). With the exception of PKA, which was significantly reduced to <2 IU/ml, levels of markers in all the spiked mixtures remained practically unchanged after acid pH treatment even at the lowest spike proportion (1/20), thus pointing out a very limited effect of this treatment on coagulation factor inactivation. In contrast, Pasteurization rendered the spiked mixtures negative for all assays even at the highest spike proportion (1/5). Moreover, after only 2 h of Pasteurization, activation markers were already negative for NaPTT -both tests-, TGT -both tests-, PKA and "kallikrein like"tests, while after 3 h Pasteurization thrombin generation showed a reduction of 80-90%, being undetectable after 5 hours. *Conclusion.* Even after only 2h of treatment, Pasteurization effectively inactivated relevant concentrations of procoagulant activities in artificially spiked FrII+III samples, higher than those found in the real Flebogamma® and Flebogamma® DIF production conditions. Acid pH treatment showed a very limited capacity to separate or inactivate these compounds.

0969

DOES FIBRINOGEN CONCENTRATE REDUCE BLOOD PRODUCTS USE IN MAJOR OBSTETRIC HAEMORRHAGE?

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Background. In July 2009, the Irish Blood Transfusion Service (IBTS) replaced cryoprecipitate with fibrinogen concentrate with the aim of reducing the potential risk of pathogen transmission. Fibrinogen concentrate appears to have similar efficacy in treatment of major haemorrhage but there is limited data about its use in Major Obstetric Haemorrhage (MOH). *Aims.* The aim of this study was to assess the impact of this change on estimated blood loss (EBL) and blood product use in MOH. *Materials and Method.* Prospective detailed audit of MOH began at our institution in January 2009. Cases are defined by EBL of 2.5 litres, transfusion of 5 or more units of Red Cell Concentrate (RCC) or treatment of a coagulopathy. The EBL and the use of blood products were compared between those that sustained an MOH with associated hypofibrinogenemia before and after cryoprecipitate was replaced with fibrinogen concentrate. *Results.* 59 cases of MOH were identified in 2 years (3.3/1000 deliveries). 29 required treatment for hypofibrinogen-

emia; Cryoprecipitate (14) and Fibrinogen concentrate (15). The two groups were similar in age, parity, ethnicity and gestation at delivery. The main cause of bleeding was uterine atony followed by retained placental tissue. Medical and surgical management were similar among the two groups. Haemostasis was achieved in all cases. Minimum serum fibrinogen level recorded was 0.19g/l. The estimated blood loss was found to be higher in the cryoprecipitate group (mean+/-SD=5+/-4 versus 3.5+/-2.6). The use of red cells and octaplas were similarly higher in the cryoprecipitate group, however there was no difference in the use of platelets among the two groups. *Conclusion.* The replacement of cryoprecipitate with Fibrinogen concentrate in MOH was associated with a reduction in EBL and a reduction in use of RCC and Octaplas. These cases, however, were diverse and complex and fibrinogen replacement was only one factor in the overall management but replacement with a small volume bolus that can be administered rapidly without thawing may facilitate more rapid correction of coagulopathy and earlier haemostasis.

0970

EXPERIENCE IN THE MANAGEMENT OF LOOKBACKS IN A TERTIARY HOSPITAL TRANSFUSION SERVICE

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Background. Donor selection and the use of serological and nucleic acid testing in blood donations have greatly reduced the infectious window for several transfusion-transmitted pathogens; however, this window has not been totally eliminated. Lookback programs involve identification and notification of components collected from donors that are later found to be reactive in specific serological tests. This strategy is supposed to prevent progression of disease in a transfusion recipient who has unknowingly been infected, especially if the treatment provides a clear benefit to the patient (e.g. syphilis). Although mandatory under European law, the process is complex and laborious, and its real utility and cost-effectiveness remain doubtful. *Aims.* To analyze the impact of lookbacks on the transfusion service of a tertiary hospital. To report the effectiveness of the protocol and screening results. *Methods.* We reviewed lookbacks managed in 2009 and 2010 in the transfusion service of Hospital Gregorio Marañon (Madrid, Spain) which performs 36.645 transfusions annually. Lookbacks are reported from the regional transfusion centre to the transfusion service, which reviews all available records to identify the recipient. Once a recipient is identified, the hospital information system is searched to determine the recipient's last known status (living or dead). If the recipient is recorded as living we have 2 options: 1.Alert the patient's physician and request that the patient be notified and tested; 2. Notify the recipient directly. If the first strategy is not effective, we apply the second: Notify the recipient directly and if it is not possible, we send a letter to the recipient or to the patient's physician. The regional transfusion centre offers testing to all recipients. *Results.* The regional transfusion centre reported a total of 51 notifications distributed as shown in Table 1. A flow chart depicting the overall results of the lookbacks is presented in Figure1. We were able to ascertain test results for only 15 of 25 living recipients (60%) of which 13 were obtained by notifying the recipient directly (first or second strategy), and 2 by alerting the patient's physician. None of the recipients tested had a positive serology result. *Conclusion.* Our success ratio of 60% is reasonable and consistent with findings in the literature. Nevertheless, the low serological positivity (0%) and the effort involved calls into question the cost-effectiveness of this process. -In our experience, contacting the recipient directly was much more successful than alerting the patient's physician, although this could generate concern for the patient. These findings lead us to question whether it might be necessary to change our protocol in the future.

Table 1.

YEAR/PRODUCT	2009	2010	PLATELETS	RED CELLS	WHOLE BLOOD	PLASMA
TOTAL	18	33	41	8	1	1
ELISA Syphilis +	9	29	35	2	0	1
Anti-HIV PCR +	8	2	6	4	0	0
Anti-HBc PCR +	1	1	0	1	1	0
HLTV I-II	0	1	0	1	0	0

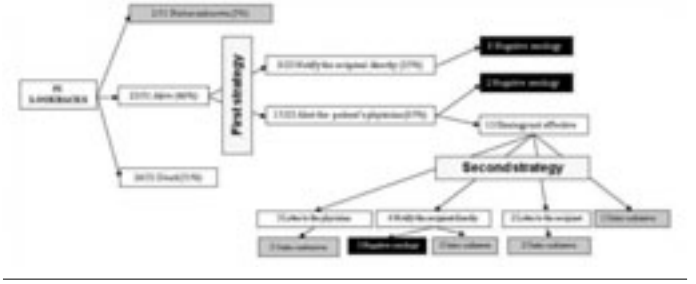

Figure 1.

0971

HAEMOVIGILANCE IN THE GLOBALISATION CONTEXT: THE ANDALUSIA EXPERIENCE. FOCUS ON TRALI

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Background. Haemovigilance is a tool to improve the quality of the blood transfusion chain focusing on safety. Some common efforts have been made but the approach to haemovigilance is different in each country. *Aims.* Our purpose is to evaluate the development of our regional haemovigilance system, at an early stage, in the context of the national haemovigilance network, and to compare it with a well consolidated system as SHOT (Serious Hazards of Transfusion) in the UK (United Kingdom), with emphasis on TRALI. *Methods.* We performed a descriptive analysis of transfusion-related incidents reviewed between 2006-2009, in the Andalusian, Spanish, and English haemovigilance reports, considered as such adverse reactions (major and minor), transfusion errors and near miss events, regardless the degree of imputability. We focused on TRALI, because of its special importance as the current most common cause of transfusion-associated major morbidity and death. The data has been extracted from official sources, such as the Ministry of Health of Spain (http://www.msps.es/profesionales/saludPublica/medicina Transfusional / publicaciones/publicaciones.htm), the Regional Ministry of Health of Andalusia, and the SHOT report (http://www.shotuk.org/home/). *Results.* The main data from the haemovigilance systems through the study period are as follow: in Spain, the rate of reports has progressively risen from 1 per 1.562 transfusions to 1 per 909; in the UK, the increase was from 1 per 2.325 transfusions to 1 per 1.176. In Andalusia, from 1 per 1.000 transfusions to 1 per 602. In Granada, from 1 per 3.448 to 1 per 1.492. Reporting on TRALI has been greater in Spain (1 per 71.4289, on average) than in the UK (1 per 166.666), during the study period. There were no reported cases of TRALI in Andalusia until 2009 (1 per 90.909 transfusions). Granada reported no TRALI cases for the whole period. The results are shown in the table below. *Conclusions.*The transfusion activity

Table 1.

			GRANADA	ANDALUSIA	SPAIN	UNITED KINGDOM
2006	TRI	CR	13	351	1.501	1.279
		NBCI	45.132	337.781	2.331.303	3.002.797
		R/1.000	0.29	1	0.64	0.43
	TRALI	CR	0	0	32	10
		R/1.000	-	-	0.014	0.003
2007	TRI	CR	15	397	1.622	1.341
		NBCI	44.793	285.881	2.390.436	2.914.228
		R/1.000	0.33	1.4	0.68	0.46
	TRALI	CR	0	0	32	24
		R/1.000	-	-	0.013	0.008
2008	TRI	CR	23	464	1.781	2.177
		NBCI	33.692	279.409	2.000.131	2.845.459
		R/1.000	0.68	1.66	0.88	0.77
	TRALI	CR	0	0	30	17
		R/1.000	-	-	0.015	0.006
2009	TRI	CR	24	465	2.252	2.475
		NBCI	36.060	280.307	2.015.408	2.903.760
		R/1.000	0.67	1.66	1.1	0.85
	TRALI	CR	0	3	36	21
		R/1.000	-	0.011	0.018	0.0072

TRI: Transfusion-Related Incidents
CR: Cases reviewed
NBCI: Number of blood components issued
R/1.000:Reports/1.000 units

and the rate of reports during the 2006-2009 period have pointed to an increase in all haemovigilance systems analyzed, showing a growing awareness of the need to improve transfusion safety. Globally considered, the rate of reports in Spain and the UK are similar, highlighting a positive growth of the Spanish haemovigilance system. The number of reports on TRALI have grown in all systems, beeing higher in Spain which can be attributed to the different transfusion policies of both countries. Haemovigilance systems provide a mechanism for monitoring the transfusion activity that allow comparisons between countries and help to develop policies to make blood as safe as possible.

0972

ADVANCED RED BLOOD CELL INDICES AND IRON STATUS AS COMPLEMENTARY FACTORS IN THE MANAGEMENT OF REGULAR BLOOD DONORS

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Background/Aims. Frequent blood donations may lead to depletion of body iron stores resulting in development of anaemia. Currently, screening for iron deficiency (ID) is done by determination of haemoglobin concentration (Hb) prior to each donation. Hb measurement has limitations due to the late response in case of an iron deficient state. Several methods for early detection of iron deficiency are available but lack practicability. We therefore evaluated the association of iron status with advanced red blood cell (RBC) indices in regular whole blood donors. *Methods.* In a prospective study, the iron status including serum ferritin and soluble transferrin receptor (sTfR) was tested in 1308 healthy blood donors after written informed consent. The ferritin index is representing the most relevant marker for ID and was calculated as the ratio of sTfR to the logarithm of ferritin. Full RBC count including percentage of hypochromic mature erythrocytes (%HYPOm) and reticulocyte haemoglobin content (CHr) was determined on the automated haematology system Advia 2120i (Siemens Healthcare Diagnostics). Gender matched study populations were assessed for signs of ID and impaired haemoglobinisation. The areas under the receiver operating characteristic (ROC) curves were calculated using CHr and %HYPOm to assess their practicability in ID diagnosis. *Results.* Cut-off values to detect an ID were generated by evaluating the 97.5th percentile of the ferritin index obtained in controls (male (m): 0.960, female (f): 1.621 donors). Considering these cut-offs, the highest percentage of ID (54.5%) was obtained in male donors with the highest allowed donation frequency (6 times per year). Interestingly female donors displayed the highest rate of ID already after three donations per year (24.7%), and ID rate was not higher after further donations. Among these iron deficient donors reduced levels of CHr and increased rates of %HYPOm were observed. Using CHr as a marker for ID, areas under the ROC curves were 0.790 (m) and 0.907 (f). When %HYPOm was used, areas under the ROC curves were 0.808 (m) and 0.922 (f). *Conclusion.* A strong correlation between iron status and RBC indices was not found. However, measurement of RBC indices allows for an early estimation of impairment in red blood cell haemoglobinisation, especially while donors may show normal haemoglobin values in absence of clinical signs and symptoms for ID. Additional testing of CHr and %HYPOm is feasible for routine screening of regular whole blood donors to better prevent the development of ID based anaemia at a very early stage.

0973

NON-ABO RED BLOOD CELL ALLOANTIBODIES AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. The appearance of alloimmune hemolytic anemia (AHA) due to anti-A and anti-B antibodies in patients undergoing a major or minor ABO-incompatible allogeneic HSCT is one of the most common and dangerous immunohematological complication. Less frequently, other red blood cell (RBC) antigen systems have been implicated in the development of AHA. Although the hemolytic complications following ABO-incompatible allogeneic HSCT have been investigated by several authors, the development of alloantibodies against RBC antigens other than ABO has been less well investigated. *Aims.* We were

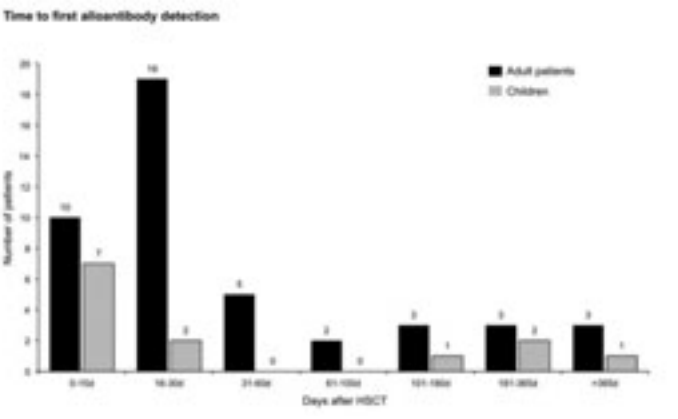

Figure 1. Time to first alloantibody detection.

therefore interested to examine in a single center study the presence of non-ABO RBC alloantibodies in patients treated with allogeneic HSCT between 1996 and 2009. *Methods.* This retrospective cohort study is based on standardized, prospectively collected clinical data from the stem cell transplant unit of the University Hospital Basel, Switzerland, and serological tests from the database of the Blood Bank Basel, Switzerland. Between January 1996 and September 2009, 514 adult patients and 73 children received 604 respectively 85 HSCT. *Results.* In the first two years after allogeneic HSCT the cumulative incidence for the development of non-ABO RBC alloantibodies was 7.27% (95% CI 7.25-7.29%) in adult patients respectively 14.15% (95% CI 13.87-14.43) in children. The most common detected alloantibodies were Anti-E and Anti-Lua. In all the patients in whom antibody specificity was identified, the antibody was directed against RBC antigens absent in donor or recipient's RBCs. However in 43 (74% of the patients with alloantibodies) respectively 11 (19%) patients the alloantibody was directed against an antigen present in the transfused RBC units respectively platelet (PLT) units. In the remaining 7% of the patients the causes of alloimmunization remain unclear. The mean time between transplant and antibody detection was 18 days (range 1-2239 days), and the most alloantibodies were detected in the first 30 days after allogeneic HSCT (s. Figure 1). In the univariate analysis, GvHD prophylaxis, conditioning regimen, and number of transfused RBC units were associated with a higher risk of development of alloantibodies. After stepwise introduction in the multivariate model, the number of RBC units remained the only variable that significantly influenced the formation of non-ABO RBC alloantibodies. In fourteen patients (24% of the patients with alloantibodies) there was evidence of hemolysis after antibody detection; two of these patients developed a documented severe immune hemolytic anemia in the early post transplant period and died. *Conclusions.* To the best of our knowledge this is the largest cohort study describing the development of non-ABO RBC antibodies after allogeneic HSCT. In our analyze, the cumulative incidence of alloantibodies formation was with 7% in adult patients and 14% in children higher in comparison with those reported by other authors. The only risk factor for developing non-ABO RBC antibodies was the amount of transfused RBC units. Virtually all the antibodies resulted following blood products (RBC and PLT) positive for the respective antigen; no antibodies emerged in an antigen-mismatched situation between donor and recipient. However, the most common alloantibodies detected, Anti-E and Anti-Lua, are potentially naturally occurring antibodies, not necessarily due to transfusion.

0974

AUDIT OF MEAN ANNUAL PRE-TRANSFUSION HB LEVEL OF PATIENTS WITH B THALASSEMIA MAJOR AT THALASSEMIA CENTRE

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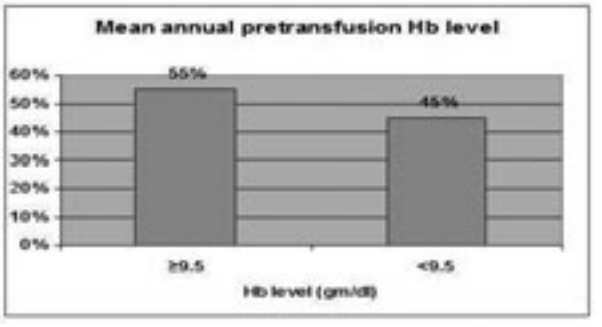
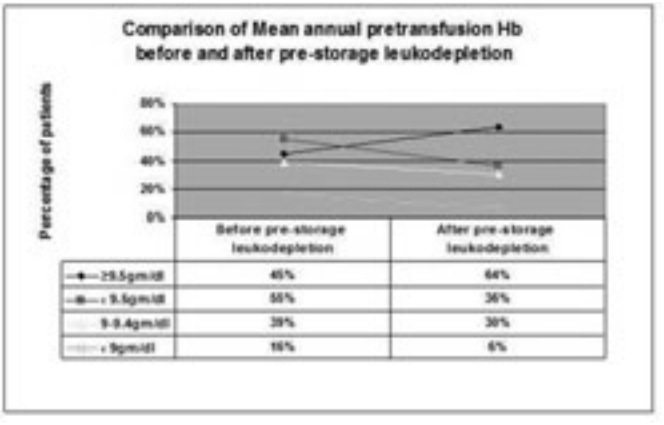
Background. Thalassemia center transfusion guidelines assess the efficiency of the blood transfusion program implemented at the centre through maintaining the mean annual pre-transfusion Hb ≥9.5gm/dl. This is essential in the management of B Thalassemia major patients to improve wellbeing and overall outcome. In April 2009, pre-storage leukocyte depleted packed RBCs were introduced while bedside blood



Graphs 1.



Graphs 2.

filters were used previously. *Aims.* To evaluate the mean annual pre-transfusion Hb level of patients with B Thalassemia major transfused at the center and the impact of pre-storage leukocyte depleted blood on the mean annual pre-transfusion Hb. *Methods.* This was a retrospective audit where pre-transfusion Hb was obtained form Oct-2008 till end of Sep 2009 by reviewing patients' files. Random sample of 100 B Thalassemia major patients on regular blood transfusion every 3-4 weeks at thalassemia centre were selected. Patients with increased risk of further Hb drop other than the original disease were excluded. *Results.* 55% of patients had mean annual pre-transfusion Hb levels of ≥9.5gm/dl (graph 1). Analyzing the remaining 45%, 80% (36patients) of them had mean annual pre-transfusion Hb level between 9-9.4 gm/dl and 20% (9 patients) < 9gm/dl. Further analyses to correlate patients' age with the mean annual pre-transfusion Hb level revealed that 72% of adults had their mean annual pre transfusion Hb levels ≥9.5gm/dl compared to 30% of pediatrics counterpart. Studying the overall effect of pre-storage leukodepleted blood introduction revealed that the mean Hb level of all patients was significantly higher after introducing pre-storage leukodepletion (P<0.0001), (graph 2). *Conclusions.* The mean annual pre-transfusion Hb level of patients with B-Tthalassemia major at Thalassemia Centre still is not meeting the standard as only 55% had Hb level > 9.5 gm/dl, although the majority of patients maintained their Hb level > 9gm/dl. Surprisingly this failure to achieve the target was mainly observed in the pediatrics population. This could be attributed to children being under transfused to avoid blood wastage. Pediatric blood units that would allow the transfusion of the full calculated amount with minimal waste were not available so this audit has exposed the importance of provision of these bags to meet the guidelines. Positive impact of pre-storage leukodepletion on the mean annual pre-transfusion Hb was an interesting outcome that needs to be studied further.

0975

A NOVEL RHD VARIANT WITH RHD G339V

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Background. Due to the strong immunogenicity of D antigen, about 80% of RhD-negative patients are at risk for anti-D alloimmunization when exposed to RhD-positive erythrocytes. Therefore, the accurate determination of the RhD type is of clinical significance. *Aims.* We experienced a novel RhD variant by RhD typing using the serologic and molecular methods. *Methods.* The patient was a 41-year old male on the waiting list for liver transplantation. The ABO and RhD blood typing with anti-D reagent (Millipore Ltd, UK) were performed. By using gel cards (Bioclone, Ortho-Clinical diagnostics, US), the Rh subgrouping was done. Direct anti-globulin test and antibody screening test (DiaMed AG, Switzerland) for unexpected blood group antibodies were also performed. Serologic test for partial D was accomplished with ID-Partial D Typing cards and Extended Partial D Typing cards (DiaMed AG). The RhD zygosity type by sequence-specific priming polymerase chain reaction (SSP-PCR, BAG Healthcare GmbH, Germany) and molecular determination of partial D type test (BAG Healthcare GmbH) were accomplished. In addition, direct DNA sequencing (Bristol institute, UK) of all 10 exons of RHD gene was done. *Results.* The blood was typed as A and considered D-negative by RhD typing. The erythrocytes revealed positive reaction to indirect antiglobulin test for the presence of weak D. In the Rh subgrouping, the phenotype was D/C/E/c/e (-/+/-/+/+). It showed negative reaction to direct Coomb's test and to antibody screening test for unexpected blood group antibodies. By using ID-Partial D Typing cards and Extended Partial D Typing cards, DFR type was suspected but didn't completely agree with that type. RHD positive/RHD negative (Dd) was determined by SSP-PCR. In molecular determination of partial D test, it revealed standard RHD. In direct DNA sequencing of RHD gene, a novel RHD allele was identified. It had a single nucleotide substitution of G to T at 1016 in exon 7, resulting in glycine-to-valine amino acid substitution at amino acid residue 339 (p.G339V). *Conclusions.* Thus, c.1016G>T(p.G339V) mutation found in this patient is identified as a novel partial D variant.

0976

HEPARIN INDUCED THROMBOCYTOPENIA: MEASURES TO IMPROVE PRE-TEST PROBABILITY SCORING, APPROPRIATE REQUESTING AND EFFICIENCY OF LABORATORY TESTING

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Background. Heparin Induced thrombocytopenia (HIT), an immune mediated thrombocytopenia paradoxically associated with thrombosis, is caused by development of IgG antibodies against platelet factor4 (PF4)-heparin complex. Combination of well defined pre-test probability scores and interpretation of laboratory HIT assays determines the overall probability of HIT. The BCSH HIT guideline recommends use of the 4T's score which relies on adequate clinical information. Our centre provides testing service for entire West of Scotland. We introduced a new HIT assay request form in order to obtain accurate clinical information, improve accuracy of 4Ts scores, assess appropriateness of requests, and provide guidance for requesting clinicians. *Aim.* To compare the quality of clinical information provided and appropriateness of requests for HIT assays since introduction of new request form. *Method and Results.* We split data from 1st October 2010- 21st Feb 2011 in 2 phases. As of 16th January 2011 we asked requesters to use the new forms which explicitly required information on indication, type and route of administration of heparin, platelet count kinetics and documented scoring for each of the 4T categories discouraging testing in low-probability cases. 84 samples (51 phase 1 and 33 phase 2) were analysed during that time period using the PF4 enhanced ELISA kit which identifies IgG, IgA and IgM antibodies. Overall 28 samples (33%) tested positive using an optical density (OD) cut-off value of 0.40. The median OD was 0.62(0.42-3.5). Indication for heparin was given in 65% of patients during phase 1, increasing to 76% in phase 2. 51% of requests had HIT scores provided in phase 1, increasing to 73% in phase 2. Likewise requests for low pre-test probability scores (0-3) reduced from 6 to 2. Interestingly requests with scores >3 increased from 37% (19/51) to 66% (22/33) in phase 2, with positive results unexpectedly dropping from 48% to 28% respectively. We were able to review clinical information provided and rescore ourselves 19 of 33 phase 2 requests: 63% (12/19) scores led to a change with 9 downgraded (4/9 from intermediate to low probability and all had correspondingly negative test results). 3 were upgraded (1/3 changed from intermediate to high probability). *Conclusion.* Our audit highlights that introduction of the new forms improved the quality of clinical information provided. However, the scoring was inaccurate in a significant number of phase 2 requests

with a tendency towards higher pre test probability, but drop in percentage of positive results which raises the possibility of scores being skewed towards higher probability. We aim to overcome this by screening all requests and communicating with requesting team. Given the high percentage of negative results we aim to introduce a sensitive rapid gel agglutination card technique as a screening method subsequently confirming positive results with an IgG specific ELISA including confirmation with heparin inhibition. Given the current workload we hypothesize that further improved communication we will reduce the number of inappropriate requests and introduction of rapid screening will significantly reduce staff time and cost. We aim to re-audit once the above are implemented.

0977

COMPARISON OF *IN VITRO* EFFECTS OBTAINED BY COLLAGEN STIMULATION OF FRESH WHOLE BLOOD AND RECONSTITUTED WHOLE BLOOD

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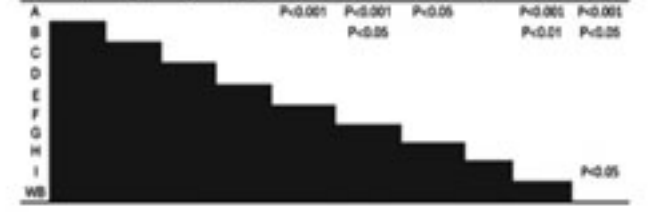
Background. The use of whole blood (WB) has nearly completely been replaced by blood component therapy in transfusion medicine. However, in situations where blood components are not available or affordable, i.e. military condition or major catastrophes, enlarged documentation of the quality of fresh WB is warranted. Also, there is an increasing debate regarding the storage lesion of blood component, e.g. reduced quality of both stored red blood cell (RBC) concentrates and platelets. Even regarding fresh frozen plasma, there is an issue because the thawing time may be critical in massive bleedings - and the concept of pre-thawed plasma has been introduced, although debated. *Aims.* Based on these considerations we performed a study to compare; (i) *in vitro* responses to collagen stimulation in fresh WB, with (ii) responses obtained by stimulation of reconstituted WB of different compositions. Furthermore, we investigated potential varying in responses between age of RBC and the platelets in the units. *Methods.* Nine groups of reconstituted WB with different compositions of platelets, RBCs, and plasma were compared (Table). Platelets stored for 1, 3, and 5 days were combined with RBC stored for 0-4 days, 12-16 days, and 26-35 days. Platelet concentrations for all samples were determined by the impedance method. Platelet aggregation was expressed as percentage of single platelet disappearance (SPD). Thrombelastography (TEG) was performed on fresh WB samples and reconstituted WB samples, and the effects of storage time on fresh WB were tested to preclude this as a source of error. *In vitro* thrombin generation was estimated by thrombin-antithrombin (TAT), quantified by standard enzyme-linked immunosorbent assay (ELISA). *Results.* A significant decrease (P<0.001) in SPD between unstimulated samples and samples stimulated with collagen was seen in all groups, with exception of E, F and I. SPD was found to be lowest in WB and the groups containing the oldest RBC- and platelet concentrates. Regarding the stimulated samples, significant differences were found between WB vs. group A and B, in addition to group A vs. E, F, and I, and group B vs. F and I. Results from TAT complexes showed that thrombin generation decreased according to storage time in stimulated reconstituted WB samples, corresponding to the results obtained from the SPD. TAT complexes were significantly lower in fresh WB as compared to group A and B after collagen stimulation. None of the groups show significant deviations from standard TEG parameters. *Conclusion.* The results show that storage time of both RBCs and platelets may influence the effects of blood component therapy. Results indicate that the use of WB over reconstituted WB will result in decreased SPD. Further studies to clarify the clinical relevance of these effects are warranted.

Table 1. The composition of the groups - with SPD.

	Platelets	Red Blood Cells	Plasma	% Single Platelet Disappearance: Addition of 5 µl collagen	Addition of 10 µl collagen	Addition of 20 µl collagen
Group A	1 day	0 - 4 days	Fresh	71.3	75.7	81.3
Group B	1 day	12 - 16 days	Fresh	54.2	68.8	75.6
Group C	1 day	26 - 35 days	Fresh	39.4	56.9	77.9
Group D	3 days	0 - 4 days	Fresh	48.0	52.5	61.5
Group E	3 days	12 - 16 days	Fresh	32.4	37.9	59.7
Group F	3 days	26 - 35 days	Fresh	29.4	35.1	56.4
Group G	5 days	0 - 4 days	Fresh	45.3	48.4	70.1
Group H	5 days	12 - 16 days	Fresh	52.8	63.0	69.6
Group I	5 days	26 - 35 days	Fresh	24.1	31.5	40.9
WB	-	-	-	29.9	29.8	35.1



0978

FREQUENT USE OF BLOOD TRANSFUSIONS IN CURRENT TREATMENT PRACTICE FOR CHEMOTHERAPY-INDUCED ANEMIA COUNTERACTS TREATMENT RECOMMENDATIONS AIMING FOR LESS TRANSFUSIONS

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Background. Patients with cancer frequently experience chemotherapy-induced anaemia (CIA) and iron deficiency (ID). Clinical evidence suggests intravenous (I.V.) iron supplementation of erythropoiesis-stimulating agents (ESAs) as effective treatment for CIA. However, an analysis of patient records across Europe revealed that markers of ID are underused in CIA patients (e.g. transferrin saturation tested in only 12%) and only a minority receives I.V. iron whereas the majority of patients receive ESAs and blood transfusions as part of their treatment. *Aim.* This analysis aimed to quantify the role of blood transfusions and explore reasons for their regular use in current treatment practice. *Methods.* Eligible onco-hematologists were recruited at random and completed records on their last five patients treated for CIA in two waves: Wave one (France, Germany, Spain, Switzerland, UK; Jun-Oct 2009), Wave two (Austria, Italy, Netherlands, Sweden; Aug-Nov 2010). Overall results are presented as median between and [range] across countries and detailed results of wave two countries as % of all cases and [range] across countries. *Results.* 375 physicians recorded 1730 cases of CIA, 58% [38-77%] in patients with solid tumors of whom 52% [30-60%] had metastatic disease. At diagnosis of anemia, 14% [8-25%] of patients presented with severe anemia (Hb <8 g/dL) and 20% [8-41%] of ferritin-tested patients with absolute ID (ferritin <30 µg/L). 52% [11-93%] received a transfusion at some stage and 73% [15-100%] received an ESA. Iron was given to 22% [11-61%] and thereof only 19% [4-77%] received I.V. iron. Of all patients receiving transfusions, 60% [11-100%] received a combination with ESA and 27% [7-51%] with iron. Detailed questions regarding the use of blood transfusions by physicians participating in Wave two (131 physicians, 651 cases) show that blood transfusions were given as regular treatment in 76% [48-85%] of cases and except for Italy (44%) only rarely as emergency treatment (11-13%; 15% of all cases). In 45% [26-53%], blood transfusions were given at least once every three months and in 3% [0-7%] even weekly or more often. Over a 12-month period prior to the survey, transfused patients received 5 units [2-6 units] blood concentrates. Among the options in the questionnaire, 'easily available' (47% [15-68%]) and 'uncomplicated use' (34% [0-52%]) were most commonly selected as reasons for administration of blood transfusions. Further reasons were that anemia was not controlled by ESAs alone (29% [14-69%]) or by the given iron treatment (24% [0-39%]). In 82% [77-84%] of these iron-treated patients, oral iron was administered at high total doses of 16.6g [10.6-32.8g] given over 12 weeks [9-20 weeks]. *Conclusions.* More than half (52%) of the patients treated for CIA received blood transfusions while ESAs were given to 73% and iron (mostly oral) to only 22% of patients. Frequently, transfusions were given on a regular basis and not only as a rescue therapy reflecting the suboptimal results obtained with ESAs alone or in combination with oral iron. As I.V. iron supplementation of ESAs improves hematologic response, awareness of this option should be increased in order to minimize the use of red blood cell transfusions.

0979

ECULIZUMAB EFFICACY AND SAFETY IN PATIENTS WITH ATYPICAL HEMOLYTIC UREMIC SYNDROME RESISTANT TO PLASMA EXCHANGE/INFUSION

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Background. Atypical hemolytic uremic syndrome (aHUS) is a rare, life-threatening disease, characterized by systemic thrombotic microangiopathy (TMA), which is caused by chronic uncontrolled terminal complement activation. Systemic TMA presents as endothelial damage, hemolytic anemia and platelet consumption and leads to progressive renal disease, multi-organ damage and, ultimately death. Importantly, many patients with aHUS receive chronic plasma exchange/infusion, but despite this, still continue to have persistent TMA and poor clinical outcomes, and up to 60% of patients develop end-stage renal disease or die within 1 year of diagnosis. *Aims.* In a phase II trial, we evaluated the efficacy and safety of eculizumab, a terminal complement inhibitor, in plasma exchange/infusion-resistant aHUS patients. *Methods.* This is a 26-week, controlled, open-label, single-arm trial. Patients were enrolled who were ≥12 years and had plasma exchange/infusion-resistant aHUS (persistent TMA despite ≥4 plasma exchange/infusion sessions 1 week before screening). The eculizumab intravenous dosing schedule was 900mg/week for 4 weeks, 1200mg at week 5, then 1200mg q2 weeks. All patients received a meningococcal vaccine. The primary endpoint was the change in platelet count (a measure of TMA) over 26 weeks. Secondary endpoints included TMA event-free status (≥12 weeks of stable platelet count, no plasma exchange/infusion and no new dialysis), TMA intervention rate (number of plasma and new dialysis events/patient/day), renal function, pharmacokinetics/pharmacodynamics (PK/PD), health-related quality of life ([HRQoL] measured by ED-5D) and safety. *Results.* A total of 17 patients were enrolled; 15 received eculizumab for the entire 26-week period. Median age was 28 years (range 17-68 years), 29% were males, 76% had an identified complement regulatory factor mutation, 41% had a kidney transplant, and 29% were on dialysis immediately prior to eculizumab. Following eculizumab treatment, platelet count increased from baseline to week 26 by a point estimate 73x109/L (95%CI: 40-105x109/L) (primary endpoint; p=0.0001). The increase in platelet count was seen as early as Day 7 and maintained throughout the study. Platelet count was normalized in 13/15 patients (87%) who had abnormal platelets at baseline. A total of 15 patients (88%; 95% CI 64%-100%) became TMA event-free and median TMA intervention rate decreased from 0.88 to 0 events/patient/day (p<0.0001). Ten patients (59%) had a sustained improvement in chronic kidney disease by ≥1 stage. Importantly, 4 of 5 patients became dialysis-free. For all endpoints, eculizumab was efficacious in patients regardless of the presence of identified complement regulatory factor mutations. PK/PD blood sampling confirmed that eculizumab provided complete and sustained inhibition of terminal complement activation and validated the selection of the dosage. The improvement in HRQoL using EQ-5D scores was highly statistically significant; mean change from baseline to week 26 was 0.33±0.09 (p<0.0001). The most frequently reported adverse events were headache, anemia and diarrhea (all mild-moderate in severity). *Conclusions.* In summary, the primary and secondary endpoints were achieved with high clinical and statistical significance. Eculizumab prevented TMA, restored renal function, removed the need for plasma exchange/infusion and improved HRQoL. Eculizumab was also well tolerated.

0980

A PHASE II STUDY OF ECULIZUMAB IN PATIENTS WITH ATYPICAL HEMOLYTIC UREMIC SYNDROME RECEIVING CHRONIC PLASMA EXCHANGE/INFUSION: INTERIM ANALYSIS

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Background. Atypical hemolytic uremic syndrome (aHUS) is a rare, life-threatening disease that can occur in adults and children of any age. In aHUS, chronic uncontrolled terminal complement activation causes systemic thrombotic microangiopathy (TMA). Systemic TMA, through haemolytic anemia and platelet consumption, leads to progressive kidney destruction as well as cerebral and cardiac damage. Many aHUS patients receive chronic plasma exchange/infusion, but despite this, patients still have uncontrolled terminal complement activation, which means the TMA remains so within 1 year of diagnosis most aHUS patients (up to 60%) present with end-stage renal disease or die. *Aims.* In a phase II trial, we evaluated the efficacy and safety of eculizumab, a terminal complement inhibitor, in aHUS patients receiving chronic plasma exchange/infusion. *Methods.* In this 26-week, controlled, open-label, single-arm trial, patients enrolled were ≥12 years and receiving plasma exchange/infusion for aHUS. After an 8-week observation period, during which the platelet count and plasma intervention frequency were stable, patients discontinued plasma exchange/infusion and started eculizumab dosed at 900mg/week for 4 weeks, 1200mg at week 5, then 1200mg q2 weeks). Patients also received a meningococcal vaccine. The primary endpoint was TMA event-free status defined as ≥12 consecutive wks of stable platelet count, no plasma exchange/infusion and no new dialysis. Secondary endpoints included TMA intervention rate (no. of plasma and new dialysis events/patient/day), renal function, pharmacokinetics and pharmacodynamics (PK/PD) and safety. *Results.* In this interim analysis, 20 patients received eculizumab; 15 had ≥12 weeks of follow-up. Median age was 28 years (range: 13-63 years), 70% had an identified complement protein mutation(s), 40% had a prior kidney transplant, 55% had eGFR levels <30 ml/min/1.73 m2, 10% were on dialysis, and 45% were receiving 2-3 plasma exchange/infusion sessions/week at baseline. The primary endpoint was highly statistically significant with 87% of patients (13/15; 95% CI 60%-98%) becoming TMA event-free following eculizumab treatment. Median TMA intervention rate decreased from 0.16 to 0 events/patient/day (p<0.0001, sign-rank test). eGFR stabilized or increased with eculizumab vs. chronic plasma exchange/infusion, which was given during the observation period (median 31 vs. 27 ml/min/1.73 m2). Eculizumab was similarly effective in patients with/without an identified complement regulatory factor mutation. PK/PD blood sampling confirmed that eculizumab provided sustained terminal complement blockade and validated the selection of the dosage. Eculizumab was well tolerated. The most frequently reported adverse events were diarrhea, headache, hypertension and nausea (mild-moderate in severity). *Conclusions.* In this interim analysis, treatment with eculizumab sustained suppression of TMA, led to permanent discontinuation of chronic plasma exchange/infusion, stabilized or improved renal function, and was well tolerated.

0981

ECLIPSE: A FRENCH STUDY CONCERNING THE DIAGNOSIS OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

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Aim. PNH is a rare life-threatening disease with prevalence between 7.3 and 15.9 cases/million. Diagnosis is difficult and often delayed because of clinical polymorphisms. ECLIPSE is a French study aimed to evaluate the delay between the onset of PNH symptoms and diagnosis, to identify the clinical signs leading to diagnosis and to determine which medical specialists are seen first by PNH patients. *Patients and Methods.* 4920 physicians were asked to participate: 992 hematologists, 1638 internists, 1155 gastroenterologists, 697 nephrologists, 438 neurovascular physicians. Physicians were divided into 3 groups: (A) having diagnosed PNH at least once, (B) having suspected a PNH without having confirmed the diagnosis, (C) neither having suspected nor diagnosed PNH. *Results.* 528 physicians accepted to participate in the study (overall response rate: 10.7%). 507 answers were analyzed. Among the 507 physicians, 108 (21 %) belonged to group A, 213 (42 %) to group B, and 186 (37 %, CI95% [32.49 % - 41.05 %]) to group C. In group A, clinical signs and symptoms leading to diagnosis were: pancytopenia (44%), anemia (37%), haemolysis (23%), peripheral venous thrombosis (18%), hepatic vein thrombosis (14%) and hemoglobinuria (14%). Clinical situations raising the suspicion for diagnosis within group A physicians were: unexplained thrombosis (86%), hemoglobinuria (84%), aplastic anemia (83%), Coombs negative anemia (80%), cytopenias (71%). Physicians were also asked to describe the circumstances of their latest PNH diagnosis. The patient was referred to the physician by the Emergencies (23%), a haematologist (22%) or internist (21%). Most frequent functional symptoms were: fatigue (39%), anemia (24%), abdominal pain (20%) and thrombosis (14%). 7% of patients were asymptomatic. PNH diagnosis was confirmed in a mean time of 9.32±11.46 months after the onset of symptoms, and a maximum delay between first symptoms and diagnosis being 60 months. Biological signs raising the suspicion for a PNH were: anemia (80%), increased LDH (60%), increased biluribin (44%), thrombocytopenia (41%) and/or neutropenia (28%). Confirmation of PNH diagnosis was made by flow cytometry in 87% of the cases. Among the 213 physicians belonging to group B, 50% had suspected at least 5 times PNH without confirmation. Clinical and biological signs prompting group B physician to suspect PNH were: Coombs negative anemia (48%), pancytopenia (42%) and/or aplastic anemia (38.5%), myelodysplastic syndrome (18%), hemoglobinuria (15.5%), increased LDH associated with venous or arterial thrombosis (15%), abdominal pain (14%), dark urine (12%) or jaundice (11%). 186 physicians belonged to group C. 6.5% of physicians had never heard about PNH. *Conclusions.* PNH was mainly diagnosed by hematologists. Frequent symptoms leading to diagnosis were unexplained thrombosis, hemoglobinuria, Coombs negative anemia, aplastic anemia, cytopenias and myelodysplastic syndrome. Flow cytometry, the gold standard for PNH testing, was only used in 87% of cases. Diagnosis was usually delayed with a maximum of 5 years between onset of symptoms and diagnosis. Fatigue and abdominal pain were commonly reported and should therefore be more routinely assessed.

0982

HIGH PREVALENCE OF IRON DEFICIENCY ACROSS DIFFERENT TUMORS CORRELATES WITH ANEMIA, INCREASES DURING CANCER TREATMENT AND IS ASSOCIATED WITH POOR PERFORMANCE STATUS

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Background. Iron deficiency (ID) with or without anemia affects physical function and quality of life. In patients with cancer or chronic inflammatory diseases, impaired iron utilization can limit effective erythropoiesis. Presently, only limited data on iron status and its relation to hemoglobin levels, clinical status and applied cancer treatment are available. *Aim.* This analysis aimed to assess the prevalence of ID and its relation to hemoglobin levels, performance status, cancer type, and disease and treatment status in a large cohort of unselected cancer patients with different cancer types. *Patients and Methods.* 1528 cancer patients that presented consecutively from October 2009 to January 2010 at our center were evaluated for ECOG performance status, cancer type, stage at initial cancer diagnosis, status of disease at evaluation (CR/no evidence, persistent, or progressive disease) and for time of last treatment. Further, iron parameters and hemoglobin levels were assessed. The following definitions were applied: anemia (hemoglobin, Hb ≤12 g/dL), ID (transferrin saturation, TSAT <20%), absolute ID (AID, serum ferritin <30 ng/mL or TSAT <10% if ferritin was not available) and functional ID (FID, ferritin ≥30 ng/mL or TSAT >10%). *Results.* 1053 patients presented with solid tumors and thereof 48.2% had metastatic disease. Anemia and ID were most prevalent in pancreatic cancer (≥50%; Table). Anemia was more prevalent in patients with stage IV (42.2%) compared to those with stage I/II or stage III disease (23.8%). The prevalence of FID increased with stage (29.1%, 35.3% and 45.6% in stage I/II, stage III and stage IV respectively) whereas AID was comparable across stages (6.7%, 10.0%, 7.7%, respectively). The prevalence of anemia and FID correlated with worse ECOG performance status (ECOG 0-1: 29.3% anemic, 36.1% FID; ECOG 2-4: 61.1% anemic, 52.8% FID). A concomitantly higher prevalence of anemia and FID was also observed in patients receiving anticancer treatment within 12 weeks prior to evaluation compared to patients without therapy (anemia 48.6 vs. 32.4%; FID 42.3 vs. 38.4%). Among patients who have received treatment more than 13 weeks prior to evaluation, anemia, FID and AID were less prevalent (17.1 %, 33.9%, 3.8%, respectively). Persistent and progressive disease at time of evaluation was associated with high rates of anemia and ID (47.1% and 56.8%, respectively). Notably, although 77.8% of patients in complete remission achieved normal Hb levels, 36.4% remained iron deficient. *Conclusion.* This analysis shows a high prevalence of iron deficiency, in particular functional iron deficiency, across different tumor types. The prevalence of ID correlates with the prevalence of anemia and progression of the disease. ID is also associated with worse performance status. In patients receiving anticancer treatment, the higher prevalence of anemia is paralleled by a higher prevalence of ID.

Table 1. Prevalence of ID across different tumor types.

	% ID	% AID	% FID	% Anemic
Pancreatic cancer	63,1	10,5	52,6	50,0
Colorectal cancer	51,9	11,5	40,4	35,0
Lung cancer	51,3	9,2	42,1	43,4
GI/Esophageal cancer	49,0	7,8	41,2	49,0
Genitourinary cancer	43,9	1,5	42,4	31,8
Breast cancer	39,6	6,0	33,6	27,5
Gynecological cancer	34,5	6,9	27,6	41,4
Testicular cancer	19,4	0,0	19,4	3,2
Other	41,8	4,1	34,7	23,5

ID iron deficient, AID absolute ID, FID functional ID, GI Gastrointestinal

0983

FERRIC CARBOXYMALTOSE FOR THE CORRECTION OF CANCER AND CHEMOTHERAPY-ASSOCIATED ANEMIA IN CLINICAL PRACTICE

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Background. Anemia and iron deficiency are frequent complications in cancer patients. Intravenous iron as a supplement to erythropoiesis-stimulating agents (ESAs) has been shown to improve hemoglobin (Hb) levels, reduce the number of blood transfusions and decrease the need for ESAs in anemic cancer patients. *Aim.* This observational study evaluated the effectiveness and tolerability of ferric carboxymaltose (FCM) in routine treatment of anemia in cancer patients. *Methods.* Adult cancer patients with anemia were enrolled from Dec 2008 - Jul 2010 at 68

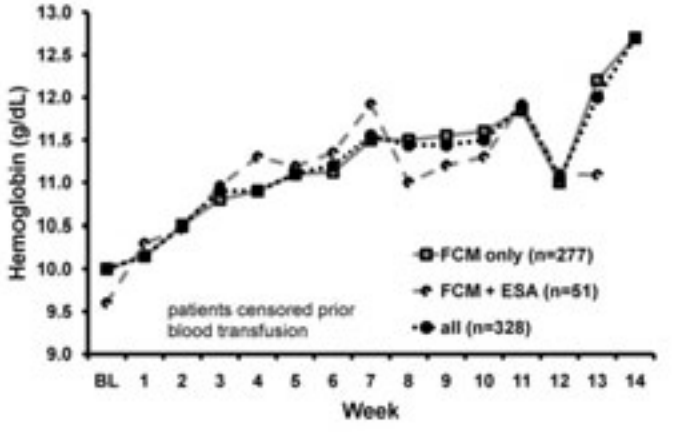

Figure 1. Median Hb over time.

German hematology/oncology practices and observed until Week 12 (+2) post inclusion or the termination visit. FCM was administered without restriction on dosing, concomitant use of ESAs or transfusions. Of 639 enrolled patients, 364 with available Hb measurements at baseline and at least one follow-up visit were analyzed for Hb increase (primary endpoint), 420 with available Hb measurements at baseline for secondary effectiveness parameters and 619 who received at least one FCM dose for safety. Effectiveness analyses included stratification by baseline Hb-, ferritin levels and subgroups who received FCM only or FCM and ESAs. Patients who received blood transfusions were censored prior to the transfusion. Data are shown as median values (25%, 75% quartiles) unless otherwise stated. *Results.* Most patients in the effectiveness population (female 54.8%, 67 years [58, 73]) presented with solid tumors (91.2% total; 25.2% breast, 19.8% colorectal, 8.8% stomach), of which 61.0% were metastasized. 74.3% received concomitant cytotoxic chemotherapy and 24.3% had received at least one anemia treatment during four weeks prior to study inclusion (13.1% transfusions, 8.3% ESAs, 4.0% i.v. or oral iron, 0.7% others). Median baseline Hb in the effectiveness population was 10.0g/dL (9.1, 10.6), 37.5% of tested patients had a ferritin ≤100ng/mL and 75.6% a transferrin saturation <20%. Median total iron dose per patient was 1000mg (600, 1500). Median increase in Hb levels was 1.4g/dL (0.2, 2.3) in the overall population, 1.4g/dL (0.2, 2.3) in patients who received FCM only, 1.6g/dL (0.7, 2.4) in patients who received FCM plus ESAs, and 1.4g/dL (0.3, 2.3) in patients censored for transfusions during the study. Hb levels improved steadily after the first FCM administration. From Week 5 onwards, mean Hb levels remained stable in the range of 11-13g/dL and were comparable between patients treated with FCM alone or concomitant ESAs as well as in patients with mild (baseline Hb 10-11g/dL) and moderate-to-severe (baseline Hb <10g/dL) anemia. Patients with baseline ferritin levels <100ng/mL achieved Hb levels >11g/dL earlier (Week 3-4) than those with baseline ferritin ≥100ng/mL (Week 7). FCM was well tolerated. Possibly or probably drug-related adverse events (AEs), mainly nausea and diarrhea, were reported for 2.3% (n=14) of patients. Three serious AEs (SAEs) comprised one fatal case after a possibly related respiratory insufficiency and two unlikely related events of tachycardia and dyspnea. *Conclusions.* FCM effectively improved and stabilized Hb levels of anemic cancer patients at 11-13g/dL in routine clinical practice, even without concomitant ESAs. Furthermore, the results of this observational study suggest that FCM can provide benefit to cancer patients independent of baseline Hb levels.

0984

THE EFFECT OF HEMOGLOBIN LEVEL AT DARBEPOETIN ALFA INITIATION ON TRANSFUSION REDUCTION AND POTENTIAL COST-SAVING IMPACT IN THE TREATMENT OF CHEMOTHERAPY-INDUCED ANEMIA

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Background. Patients with cancer who receive chemotherapy may develop chemotherapy-induced anemia (CIA). Efficacy of erythropoiesis-stimulating agents (ESAs) in reducing the incidence of transfusions and increasing hemoglobin (Hb) levels has been demonstrated (Aapro, The Oncologist. 2008;13 (suppl 3):33-36). According to current guidelines in the US and Europe, ESA treatment should be initiated as the Hb level approaches either 10 g/dL (NCCN, USA) or between 9-11 g/dL (EORTC, Europe) in patients with anemia-related symptoms. *Aims.* The objective of this study was to understand the impact of Hb level at the start of darbepoetin alfa (DA) treatment on transfusion reduction and to identify the cost-saving impact of this reduction on CIA treatment costs. *Methods.* Two separate systematic literature reviews were performed. We conducted a systematic review of the clinical literature in PubMed, as well as ASCO, ASH, ESMO, ESMO/ECCO, and EHA conference abstract databases between 2006 and 2010. Search terms included "DA"and "CIA". DA is an ESA with dosing regimens up to every three weeks. A systematic review of economic studies on cost-of-transfusion was performed using a PubMed search from 2000-2010. Reference lists of retrieved studies from this review were scanned to identify additional articles. Mesh terms included "anemia/economics". *Results.* Eight studies were retrieved from the clinical literature review; six full-text articles and two conference abstracts. Six were based on clinical trials whereas two were based on observational studies. Despite the differences in baseline patient characteristics, length of the studies and analytical techniques, the need for transfusions decreased across all studies when DA initiation occurred at higher Hb levels. Twenty-one studies met the inclusion criteria for the economic literature review. These studies indicated that the cost of one unit of red blood cell (RBC) transfusion ranged from €240-414 in Europe, USD$107-529 in USA, £90-402 in UK, CAN$280-456 in Canada and AUS$143 in Australia. Few studies reported actual number of units transfused. When the number of transfusions was reported, more than one unit was usually transfused in the majority of patients. To estimate potential savings in CIA treatment associated with Hb level at the time of DA initiation, the difference in transfusion rates was multiplied by the midpoint of transfusion cost range in Europe (€327). An illustrative example using two of the eight identified clinical studies (latest studies) is presented in Table 1. Potential cost savings ranged from €589-3,548 for every 10 patients treated. We performed the same analysis using the remainder of identified studies and found the same trend of cost-savings (data not shown). *Conclusions.* The findings of the clinical systematic review suggest that transfusion incidence decreases with higher Hb levels at DA initiation. Cost of transfusions was found to vary from country to country and depended on cost items included (e.g. direct costs, indirect costs). Our findings suggest that the resulting cost-savings depends on the number of RBC units transfused and cost items included. Initiation of DA according to guidelines is important in terms of reducing the number of transfusions as well as the potential cost-saving impact on CIA treatment.

Table 1.

Impact of different Hb levels at DA initiation on transfusion rate and cost of CIA treatment

	Canon 2010			Eisterer 2011		
	Baseline Hb at DA initiation					
	<9 g/dL	9 to <10 g/dL	≥ 10 g/dL	<9 g/dL	9 to 10 g/dL	>10 g/dL
Transfusion rates (TR) (%)	62%	35%	19%	50%	19%	10%
Difference in % points of TR		27%	16%		31%	9%
Cost-saving for every 10 patients(€)(2 units)*		1,766	1,046		2,027	589
Cost-saving for every 10 patients(€) (3.5 units)**		3,090	1,831		3,548	1,030

Canon 2010 reports Kaplan- Meier % TR for W1-W13 (Week)

Eisterer 2011 reports % TR for W1-W12

Number of units transfused reported as 2 units* in Cremieux 2000 and 3,5 units** in Eisterer 2011

Canon, et al. (2010) ASCO
Eisterer, et al.(2011) Curr Med Res Opin.27(2), p.285.
Cremieux, et al.(2000) J Clin Oncol,18(14), p.2708.

Stem cell transplantation - Clinical 2

0985

DONOR ALLELE (GT)16 IN THE PROMOTER/ENHANCER REGION POLYMORPHISM OF FOXP3 GENE IS ASSOCIATED WITH A HIGHER INCIDENCE OF RELAPSE AFTER MYELOABLATIVE HLA-IDENTICAL SCT

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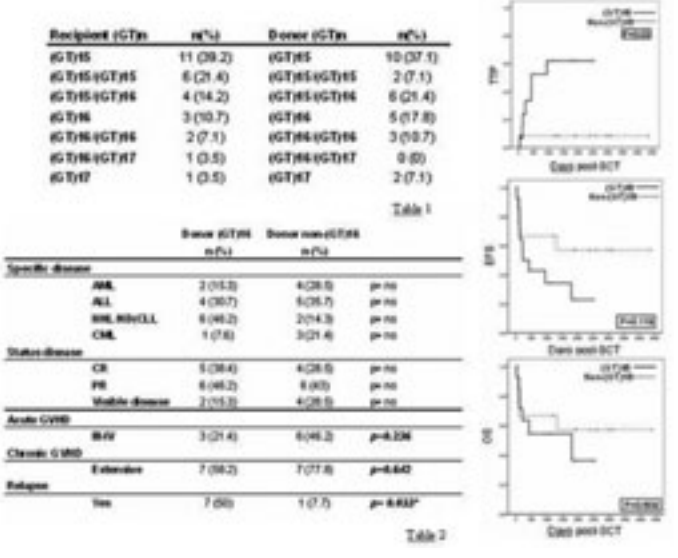
Introduction. The FOXP3 gene located on chromosome Xp11.23, encodes a protein that is a member of the forkhead/winged-helix family of transcriptional regulators. It is mainly expressed in CD4+CD25+ regulatory T cells, and is involved in the regulation of T-cell activation after Allogenic Stem Cell Transplantation. A microsatellite (GT)n polymorphism of the promoter/enhancer region of FOXP3 gene, located on the intron zero is associated with the degree of inmunological reactivity mediated by this protein. Some studies suggest a lower reactivity when (GT)16 alele is present. However, the influence of the polymorphism in donor and recipient cells on the immunological fenomena developed after Allo-SCT is unknown. *Objetive.* The main objective of this study was to analyze the role of FOXP3/Scurfin gene intron zero (GT)n polymorphism in the outcome of myeloablative HLA-identical Allo-SCT. *Patients and Methods.* Twenty-seven patients submitted to myeloablative HLA-identical Allo-SCT at our institution were included in the analysis with a median follow up of 424 days (23-4369 days) were included in the analysis. Genomic DNA was purified from peripheral blood using a QIAamp DNA extraction kit (Qiagen). Genotyping of intron zero (GT)n polymorphism in the FOXP3 gene was performed by fluorescent PCR revealed by capilar electroforesis as described by Bassuny *et al.* (Immunogenetics 55:149, 2003). *Results.* Distribution of (GT)n alleles in donors and recipients are summarized in Table 1. Since the FOXP3/Scurfin gene is located on the X chromosome, females can be homozygous or heterozygous while males are hemizygous. The presence of allele (GT)16 in the donor is associated with a higher relapse rate (50% vs 7.7 %; p=0.033), and a lower Time to Progression (TTP; 426 days vs not reached, p=0.03). Patients transplanted from donors with allele (GT)16 showed less gr.III-IV acute and extensive chronic graft versus host disease incidence (p=NS; Table 2). The higher incidence of relapse led to a worse event free survival (EFS) and overal survival (OS), although statistical significance was not achieved. *Conclusions.* The microsatellite (GT)n polymorphism in the promoter/enhancer region of the FOXP3 gene seems to influence outcome of myeloablative HLA-identical Allo-SCT. Indeed, the presence of allele (GT)16 in the donor is associated with a higher incidence of relapse and a lower TTP. In the case these observations are confirmed in a larger patient cohort, they may be useful for an individualized management of transplanted patients.

Tables.



0986

OPTIMAL CUTOFF VALUE OF THE HEMATOPOIETIC PROGENITOR CELL (HPC) COUNT FOR EFFICIENT AUTOLOGOUS STEM CELL HARVEST

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Background. Even if enumeration of CD34+ cells in peripheral blood is a reliable index for timing efficient autologous stem cell collection (ASCC), flow cytometric techniques to measure CD34+ cells are complex and costly. We previously have shown that hematopoietic progenitor cell (HPC) count enumerated by SE-9000 automated hematology analyzer is a useful surrogate for the timing of ASCC. *Aims.* We aimed to fine-tune cutoff value of HPC in predicting successful ASCC. *Methods.* Between May 2002 and January 2011, 378 patients (median age, 50 years; Male:Female = 230:148) with hematologic malignancy including 152 patients with multiple myeloma, 196 with Non-Hodgkin's lymphoma, 23 with Hodgkin's lymphoma and 7 with POEMS syndrome, underwent ASCC in the Asan medical center. A receiver operating characteristic (ROC) curve was used to define a threshold value of collected CD34+ cell count on day 1 and HPC for optimal ASCC (total CD34+ cell ≥ 5.0 x 10^6/kg). The involvement of the various factors that may affect ASCC was analyzed by a multiple logistic regression analysis. *Results.* In a series of consecutive 378 patients, 1116 leukapheresis were performed. The median number of harvested CD34+ cells was 11.27 x 10^6/kg (range, 0.01-172.7) with the median number of collection of three (range, 1-8). The ROC curve revealed that the most reliable parameter for optimal CD34+ cell collection was a number of CD34+ cells collected on day 1 ≥2.0 x 10^6/kg (AUC 0.803, 95% confidence interval [CI] 0.759 - 0.848, p <0.001) and the best cutoff value of HPC was 20 x 10^6/L (HPC 20) for the collected CD34+ cell count on day 1 ≥2.0 x 10^6/kg (sensitivity of 70.0% and specificity of 77.7%). On the basis of HPC 20 (HPC ≥20 vs. HPC <20), optimal collection rates were 94.8% and 75%, respectively. Failure to achieve optimal CD34+ cell collection was significantly associated with prior exposure to alkylating agents (p <0.001, odds ratio [OR] 2.86 [95% CI, 1.64 - 4.92) and the number of prior chemotherapeutic regimens (<2 vs. ≥2, p <0.004, OR 2.30 (95% CI, 1.30 - 4.06) in the multiple logistic regression analysis. *Summary/Conclusions.* We defined cutoff value of HPC to be 20 x 10^6/L for optimal ASCC. Prior exposure to alkylating agents or heavy treatment was a significant cause for inefficient ASCC.

0987

EFFICACY AND TOXICITY OF THE FLAMSA/RIC REGIMEN IN 40 PATIENTS WITH HIGH-RISK AML: A SINGLE CENTRE EXPERIENCE

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Background. Sequential use of chemotherapy and reduced-intensity conditioning (RIC) for allogeneic stem cell transplantation (SCT) in high-risk leukemia patients (pts) represents a promising approach (Schmid *et al.*, JCO 23, 2005: 5675-87). Here we present our experience with this therapy at cohort of 40 pts with acute myeloid leukemia (AML). *Methods.* High-risk was defined by progressive or refractory AML (n = 23), AML on the second or third remission (n = 8), or AML on the first remission with unfavorable cytogenetics (n = 9). Fludarabine (30 mg/m2), cytarabine (2 g/m2), and amsacrine (100 mg/m2) for 4 days (FLAMSA) were used for cytoreduction. After 3 days of rest, RIC consisting of 4 Gy TBI, anti-thymocyte globulin (ATG-Fresenius) 10-20 mg/kg/day for 3 days, and cyclophosphamide 40-60 mg/kg/day for 2 days followed. Prophylactic donor lymphocyte transfusions (pDLT) were given from day +120 in pts who were free of immunosupressive medication for at least 30 days without developing GVHD. We analyzed 40 pts with AML undergoing FLAMSA/RIC in our centre from March 2006 to March 2010. Disease status before SCT was: CR1, n=9; CR2, n=6; CR3, n=1; PR1, n=1; refractory/progressive disease, n=23. Median age of pts was 49 years (range 25-62). Types of donors and used grafts were as follows: HLA identical sibling, n=10; unrelated donor, n=30; PBSCs, n=37; BM, n=3. *Results.* The median time of neutrophil engraftment (above 0.5x10E9/L) was 16 days, 34 pts engrafted, 6 pts died in aplazia on days 1, 3, 7, 8, 10 and 19 after SCT. Incidence of acute GVHD was

evaluated in 33 pts: 58% (19/33) of pts had GVHD (grade I+II in 16 pts, grade III in 3 pts). Incidence of chronic GVHD was evaluated in 28 pts, 64% (18/28) of pts had GVHD (limited in 14 pts, extensive in 4 pts). Eight pts fulfilled the criteria for pDLT and received pDLT (median 2 doses). So far, 2 pts of 8 have got AML relapse and they died, 6 pts of 8 are alive in remission of AML. The cumulative incidence of non-relapse mortality (NRM) at 1 year and 2 years was 28% (11/40) and 30% (12/40). Causes of death were refractory GVHD (n=3), septic shock (n=4), multiorgan failure (n=3), brain hemorrhage (n=1) and posttransplant lymphoproliferative disease (1). The other most frequent toxicities were grade III/IV infections according to common toxicity criteria in 22 of 34 pts and gastrointestinal toxicity (grade III in 10 of 34 pts). Treatment response was evaluated in 34 pts: remission was achieved in 28 pts (82%), 6 pts (18%) had progression. With the median follow-up from SCT of 33.5 months (range 10-60), the 1- and 2-year progression-free survival (PFS) was 44.0% and 38.0%, and the overall survival (OS) was 46.0% and 41.0%, respectively. Twenty-four pts died (12 deaths from NRM, 12 deaths from relapse or progression), 16 pts are alive and disease free. *Conclusion.* FLAMSA/RIC regimen seems to be feasible and effective alternative for pts with high-risk AML with acceptable toxicity and high response rate (82%), 2-year OS after SCT is 41%.

0988

ALLOGENEIC STEM CELL TRANSPLANTATION FOR ADULTS WITH MYELODYSPLASTIC SYNDROMES: RELEVANCE OF PRE-TRANSPLANT DISEASE STATUS

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Background. allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only potential curative therapy for myelodysplastic syndromes (MDS). Approximately 40% of patients may be cured with HSCT, although advanced age, medical comorbidities and the lack of suitable donor limit this strategy to a selected minority of patients. Aim of the study was to investigate the outcome of adult patients with MDS who received an allogeneic HSCT in two Italian hematological centers. *Methods.* We retrospectively analyzed 77 adult MDS patients (median age 53, 30-70 years) receiving an allogeneic HSCT between January 1995 and September 2010 at two main Piedmont Hematological Institutions. Patients were classified according to standard FAB criteria: 8 (10%) had refractory anemia, 3 (4%) refractory anemia with ringed sideroblasts, 36 (47%) refractory anemia with excess of blasts, 8 (10%) refractory anemia with excess of blasts in transformation, 14 (19%) chronic myelomonocytic leukemia and 8 (10%) had MDS not otherwise specified. At the time of diagnosis, 3 patients (4%) had IPSS low-risk disease, 19 (25%) had intermedate-1, 25 (33%) intermediate-2 and 9 (12%) high risk disease; 49 out of 77 patients (64%) received active treatment before HSCT including hypomethylating agents and AML-like induction chemotherapy. At the time of HSCT, 22 patients (29%) were in complete remission, 12 (16%) were untreated, while 43 (56%) had relapsed/refractory disease. Peripheral blood stem cells was the graft source in 68 cases (88%), bone marrow in 8 (10%) and umbilical cord blood in 1 case (2%); 50 patients (65%) received grafts from an HLA-identical sibling, 24 (31%) from a matched unrelated donor and 3 (4%) from a partially matched related donor. Forty-three patients (56%) received myeloablative preparative regimens and 34 (44%) received reduced intensity conditioning. *Results.* primary neutrophil engraftment was achieved in 71 patients (92%) at a median of 16 days after transplantation (range 11-30 days) and platelet engraftment was achieved in 57 patients (74%) at a median of 14 days (range 7-51 days). The cumulative incidence of acute graft-versus-host disease (aGVHD) by day +100 and chronic GVHD by 1 year were 30% (19%-41%, 95% confidence interval [CI]) and 57% (45%-68%, 95% CI) respectively. The cumulative incidence of transplant-related mortality (TRM) at 100 days and 1 year were 13% (5%-21%, 95% CI) and 20% (11%-29%, 95% CI) respectively. The 2-year progression free survival (PFS) and overall survival (OS) were 41% (30%-52%, 95% CI) and 48% (36%-59%, 95% CI) respectively. On multivariate analysis, advanced disease stage at transplantation was the major independent variable associated with an inferior 2-year PFS (HR 4.48, 2.13-9.45 95% CI, p<0.001) and OS (HR 4.11, 1.94-8.70 95% CI). The use of a donor other than an HLA-identical sibling (HR 2.81, 1.04-7.63, 95% CI, p 0.04) was the independent variable associated with TRM. *Summary/Conclusions.* our data suggest that disease status at the time of transplant is the major predictor for improved PFS and OS, and treatments required to reach this goal may have value in leading to improved outcome. Additional studies are justified for clarify the role of HSCT in MDS.

0989

DIAGNOSTIC STRATEGIES AND RISK FACTORS FOR CYTOMEGALOVIRUS INFECTION IN PATIENTS WITH LYMPHOMA UNDERGOING AUTOLOGOUS TRANSPLANTATION: A RETROSPECTIVE ANALYSIS FROM THE ROME TRANSPLANT NETWORK

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Background. Routine monitoring for Cytomegalovirus (CMV) infection are considered unnecessary in patients undergoing autologous hematopoietic stem cell transplantation (ASCT) because of the low likelihood of progression from infection to disease, with the exception of high-risk subgroups, including those receiving CD34-selected grafts and prior treatment with Fludarabine, Cladribine or Alemtuzumab. However, current data on CMV infection and disease following ASCT for malignant lymphoma are very limited. The starting point for this study was the quality accreditation process of the Rome Transplant Network (RTN) according to the JACIE standards, considering that two different CMV infection diagnostic strategies were set within the participating Institutions: a clinically driven diagnostic approach based on plasma quantitative Polymerase Chain Reaction (PCR) assay in patients with clinical signs suggesting a CMV infection and a CMV surveillance based on a routine monitoring of all patients by plasma quantitative PCR assay. *Aims.* The aim of the study was to compare two different CMV infection diagnostic strategies (i.e. clinically driven vs surveillance) in terms of CMV symptomatic infection and/or end-organ disease incidence, diagnostic timing, transplant-related mortality (TRM), CMV-related mortality (CMVRM), PCR testing cost and to provide insights on the risk factors for CMV symptomatic infection or end-organ disease and TRM. CMV symptomatic infection and end-organ disease were defined according to published recommendations. *Methods.* We perform a retrospective analysis on 144 adult patients (median age 47 years, range 17-71) with diagnosis of malignant lymphoma consecutively undergoing non-selected peripheral blood ASCT in 3 Hematology Institutions participating in the RTN. *Results.* Considering the two CMV infection diagnostic strategies, PCR testing cost was significantly higher in the surveillance arm (€199 vs 824, p=0.000), whereas no statistically significant difference was observed concerning CMV symptomatic infection or end-organ disease incidence (11.5 vs 10.5%, p=1), diagnostic timing (day 33 vs 26, p=0.294), TRM (7 vs 3.5%, p=0.422) and CMVRM (4.9 vs 0%, p=0.116). In multivariate analysis, the HBcIgG seropositivity [HBcIgG- HR 0.13 (95% CI: 0.03-0.6), p=0.004] and conditioning regimens containing 90Y-Ibritumomab Tiuxetan (Z-BEAM or Z-FEAM) [HR 28.5 (95% CI: 2.9-278.6), p=0.004] or non-Carmustine-based [HR 34.8 (95% CI: 5.2-231.9), p=0.000] proved to be independent significant variables for the risk of developing CMV symptomatic infection or end-organ disease. The number of Rituximab administrations as risk factor remains controversial, since the multivariate analysis (p=0.124) did not confirm the predictive value observed in univariate analysis (p=0.002). Overall, 1 out of 5 (20%) patients with CMV end-organ disease and 3 out of 11 (27%) with symptomatic infection died, demonstrating the clinical impact of CMV indirect effects such as secondary infections. In multivariate analysis, the occurrence of CMV symptomatic infection or end-organ disease was the only factor influencing the risk of TRM (6-fold risen, p=0.017). *Summary/Conclusions.* Routine monitoring for CMV infection following ASCT should be carried out not only in lymphoma patients grafted with CD34-selected cells or previously treated with Fludarabine, but also in lymphoma patients HBcAb+. From our study, 90Y-Ibritumomab Tiuxetan in conditioning and non-BCNU-based regimens should be considered as further risk factors for CMV symptomatic infection or organ disease.

0990

INTRABONE CORD BLOOD TRANSPLANT: PRELIMINARY RESULTS FROM A PROSPECTIVE PHASE II STUDY

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Background. Intrabone transplantation has been described as an efficient way to infuse cord blood. *Aims.* The study was a phase II prospective monocenter study approved by local ethical Committee and registered at http://clinicaltrials.gov/; primary endpoint was engraftment rate and 17 patients are planned. *Methods.* the All patients signed a written informed consent. End of recruitment is planned within may 2011.Conditioning regimen was myeloablative (Bu- Cy or unfractioned TBI-Cy); prophylaxis of GVHD was CsA, micophenolate 30 mg/kg/die and ATG-F 30 mg/kg, total dose. G-CSF was routinely given from Day +7 until recovery.CB processing was as described by Frassoni *et al.* Briefly, cord blood units were thawed, washed with the Rubinstein solution to remove DMSO and reduced the final volume up to 30 ml.Infusion was performed in operating room using a monitored anaesthesia care sedation with propofol and remifentanil.Median age was 36 years (29-54), median weight of recipient was 60 kg (51-93); diagnosis were AML (8) ALL (4) MM (1) CML (1). Phase at transplant was mainly advanced (for AML 6 with resistant/relapsed disease, 2 II CR; for ALL 1 II CR and 3 resistant disease; MM: 1 progressive disease and CB for CML). 3 patients had a previous allotransplant and 3 a autotransplant. All CB units were 4/6 except for one (5/6). *Results.* Median total cell infused was 1.91 x 10e7/kg and median CD34 pos cells 0.52 x 10e5/kg.Median time to 0.5 x 10e9/L ANC was 21 days and median time to 20x 10e9/L and 50 x10e9/L platelets were 46 and 60.5 days, respectively. At day +100 the evaluable patients had 121x 10e9/L plt (range 104-192). Three patients died before engraftment for CNS bleeding (+6) or for myocarditis (+16) and sepsis (+6)One patient didn't graft and a second unit, again via intrabone, was given, after 2 months and with a RIC regimen, with successful engraftment.All the evaluable patient achieve a complete response (CR), except for the MM pt, who obtain a nCR); two patients relapse at 4 and 7 months from transplant. GVHD occurrence was very low: no severe acute GVHD and only one case of extensive GVHD were recorded. *Summary/Conclusions.* Preliminary results of this study with advanced disease suggest that intrabone injection of CB resulted in short term good engraftment, expecially for platelets, low GVHD and good outcome. Longer follow up is needed to estimate the actual antileukemic effect.

0991

UNRELATED STEM CELL TRANSPLANTATION IN ADULTS WITH HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA: HLA-MATCHING DEGREE AND GRAFT SOURCE-BASED ANALYSES OF LONG-TERM OUTCOMES

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Background. Adults with high-risk acute lymphoblastic leukemia (HR-ALL) have a poor outcome with standard chemotherapy and usually undergo unrelated donor stem cell transplantation (URD-SCT) if a matched sibling donor is not available. However, long-term results of URD-SCT, especially based on HLA-matching degree and graft source, are scarce in adult HR-ALL patients. *Aims.* We report long-term outcomes in 106 consecutive adult HR-ALL patients who received URD-SCT using bone marrow (BM; n=67) or peripheral blood progenitor cells (PBPC; n=39) at our center between 2000 and 2008. *Methods.* Median age was 24 years (range, 15-54 years). All patients had one or more high-risk features, including adverse cytogenetics [t(9;22), t(4;11), t(8;14), complex, Ho-Tr], older age (≥35 years), high leukocyte counts, or delayed first complete remission (CR1). Eighty patients (75.5%) were transplanted in CR1; 9 (8.5%) in CR2; and 17 (16.0%) in advanced status. Using allele-level typing (for HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci), graft sources were classified as (1) 8/8-matched BM (n=30), (2) 8/8-matched PBPC (n=17), (3) 7/8-matched BM (n=35), and (4) 7/8-matched PBPC (n=24). All patients received TBI (≥12 Gy)-based myeloablative conditioning or reduced-intensity conditioning (fludarabine plus melphalan) regimens. Graft-versus-host disease (GVHD) prophylaxis was uniformly attempted by administering tacrolimus plus methotrexate. Antithymocyte globulin (2.5 mg/kg) was administered to 59 patients who received 7/8 matched grafts. All patients and donors provided written informed consent, and the treatment protocol was approved by the institutional review board of The Catholic University of Korea. *Results.* Compared with 8/8-matched transplants (BM and PBPC) and 7/8-matched BM transplants, 7/8-matched PBPC transplants were older (P=0.050) and received a reduced-intensity conditioning regimen (P=0.015). Disease status at the time of transplantation was more advanced for 7/8-matched BM transplants (P=0.009). After a median follow-up of 53 months (range, 24-124 months) for surviving transplants, the 5-year cumulative incidence of relapse and non-relapse mortality were 31% and 25%, respectively, and the 5-year disease-free survival (DFS) rate was 52%. The risk of relapse was higher for 7/8-matched transplants (42%; 48% for PBPC and 37% for BM) than for 8/8-matched transplants (19%; 18% for PBPC and 20% for BM; P=0.019), while the risk of non-relapse mortality was similar between groups. As a result, DFS at 5 years was lower using 7/8-matched grafts (44%; 42% for PBPC and 46% for BM) than 8/8-matched grafts (62%; 59% for PBPC and 63% for BM; P=0.041). In each group of patients showing the same HLA-matching degree, overall transplantation outcomes were similar between PBPC transplants and BM transplants. Regardless of HLA-matching degree and graft sources, disease status at transplantation (CR1 versus beyond CR1) was an independent predictive factor affecting relapse (HR 3.21, 95% CI 1.46-7.07; P=0.004) and DFS (HR 2.75, 95% CI 1.41-5.36; P=0.003) in multivariate analysis. The presence of chronic GVHD was also associated with lower relapse (HR 5.03, 95% CI 2.01-12.61; P=0.001) and better DFS (HR 2.37, 95% CI 1.23-4.57; P=0.010). *Summary/Conclusions.* Our long-term data suggest that outcomes are similar for transplantation using PBPC or BM sources in the setting of 8/8-matched or 7/8-matched URD-SCT for adult HR-ALL.

0992

PHASE II STUDY OF YTTRIUM-90-IBRITUMOMAB TIUXETAN IN REDUCED INTENSITY CONDITIONING (RIC) ALLOGENEIC TRANSPLANT IN RELAPSED OR REFRACTORY AGGRESSIVE B-CELL LYMPHOMA: A GEL/TAMO PHASE II CLINICAL TRIAL

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Background and Objectives. Radiolabelled immunotherapy is a promising treatment in malignant lymphomas. We designed a Phase II clinical Trial with Yttrium-90 (Y-90)-ibritumomab tiuxetan as a part of a RIC regimen associated with melfalan and fludarabine (Clinical Trials Identifier: NCT00644371). The main objective was evaluate one year Progression free Survival (PFS), and secondary objectives: toxicity, engraftment, acute and chronic GVHD and one year non relapse mortality (NRM) and overall survival (OS). *Patients and Methods.* 20 patients from 10 Spanish centers with aggressive non-Hodgkin's lymphoma (NHL), including diffuse large B-cell, follicular grade 3, Burkitt and mantle-cell lymphomas, were eligible for the trial. Inclusion criteria were: to reach less than a partial response (PR) after two lines of chemotherapy, relapse after an autologous SCT (ASCT), positive PET before or after ASCT, or failure in mobilization of stem-cells for ASCT. Conditioning regimen consisted of rituximab 250 mg (day -21), Y-90 ibritumomab IV (2,08 mg, day -14) plus Fludarabine 30 mg/m^2 IV (days -3,-2) plus Melphalan 70mg/m^2 IV (days-3,-2) or only -2 plus tiotepa 10 mg/kg (day -8). All donors were family matched. We report here preliminary analysis of results. *Results.* Median age was 49 years (range 32-63). At the moment of transplant, 7 patients (39%) were in complete remission (CR), 7 (39%) in PR and 4 had refractory disease (RD). Five patients had a prior ASTC. Y-90 ibritumomab infusions were well tolerated, without immediate reported adverse reactions. All patients engrafted, and median of days to reach more than 500x10^9 granulocytes and more than 20x10^9 platelets were 15 (12-24) and 12 (2-19) respectively. Incidence of grade 3-4 acute GVHD was 31.2%. With a median follow-up of alive patients of 17 months (range 3 to 27 months), 11 patients (55%) are alive, 9 of them (82%) in CR. Global postransplant re-

lapse was 25% and at one year, PFS was 44%. At day +100, NRM was 15% and overall NRM was 25% (2 patients due to acute GVHD and 3 infectious complications). Median OS has not been reached. 2-year estimated OS was 54%. *Conclusions.* Our results show that yttrium-90-ibritumomab tiuxetan as a component of reduced intensity conditioning for allogeneic transplantation is feasible in patients with high-risk relapsed or refractory aggressive B-cell lymphoma. Longer follow up is needed in order to design future trials.

0993

CLINICAL IMPACT OF GLUTATHIONE S-TRANSFERASE M1 POLYMORPHISM ON OUTCOME AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Despite significant progress in allogeneic hematopoietic stem cell transplantation (allo-HSCT), this procedure is still associated with substantial morbidity and mortality. Various pretransplantation and transplantation-related clinical risk factors have been implicated, but so far there is no method to estimate the occurrence and severity of transplant-related complications. The most common complication after allo-HSCT which affects survival remains graft versus host disease (GvHD). Single nucleotide polymorphisms in genes coding cytokines and chemokines have been shown to influence GvHD and outcome after allo-HSCT. Besides pharmacogenomics is a new field investigated in the HSCT setting. This study aimed to determine association between polymorphism of glutathione S-transferase M1 (GSTM1) genotypes with the outcome of allo-HSCT performed between May 2001 and October 2010 in our center. *Materials and Methods.* The allelic variants of GSTM1 gene were determined in 83 patient/donor pairs by real-time polymerase chain reaction. Patients characteristics were: median age 36 (range 18-65), underlaying diseases-hematologic malignancies in 77 and aplastic anemia - in 6 cases. Donors were HLA-identical sibling-in 61 and unrelated-in 22 transplants, the median age-37 (range 14-65). The studied end points were GvHD, haemorrhagic cystitis, toxicity and venooclusive disease (VOD) of the liver and survival. The Spearman's rank correlation test was used in statistical analysis. *Results.* The allelic distribution of examined polymorhisms was similar to that reported in Caucasoid population. Acute-GvHD was recognized in 35/83 (42,2%) and chronic-in 43/83 (51,8%) of patients. Overall survival in analysed group was 60,2%. Our study revealed significant correlation between the occurrence and severity of aGvHD and the GSTM1-positive genotype compared with those with the GSTM1-null genotype (P=0.00786). Besides we found a significant correlation between the GSTM1-null genotype and increased overall survival obtained by the Kaplan-Meier method (P=0.016). We couldn't find any correlation between: 1) examined polymorphism and the rest of analysed end points, 2) donor genotype and HSCT complications (P=ns). *Conclusion.* Our data revealed that GSTM1 gene polymorphism is correlated with aGvHD, and overall survival after allo-HSCT. These findings confirmed some previously published data, but results remains variable between investigators, so should be studied prospectively and in the larger group of patients.

0994

THE PROGNOSTIC SIGNIFICANCE OF PET SCAN PRE- AND POST- HIGH DOSE THERAPY AND HEMATOPOIETIC STEM CELL TRANSPLANTATION (HDT/ASCT) IN RELAPSED/REFRACTORY HODGKIN LYMPHOMA (HL) PATIENTS

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Background. The prognostic significance of PET-scan in advanced stage HL patients treated with ABVD as first line treatment is well established. However, the prognostic impact of PET scan in the relapsed setting is not clearly defined, while chemosensitivity before HDT/ASCT - as assessed with conventional imaging - is one of the most important prognostic factors for outcome. *Aims.* To study the prognostic significance of PET scan before and after HDT/ASCT in patients with relapsed/primary refractory HL. *Methods.* Clinical staging with computed tomography (CT) and clinical examination, as well as PET-scan were performed after salvage chemotherapy just prior to ASCT and at 3 months post transplant and 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. Chemosensitive patients were considered those who had achieved complete or partial remission, whereas chemoresistant those with stable or progressive disease by conventional clinical staging pre-ASCT. Failure-free Survival (FFS) was calculated from ASCT to relapse, progression, death from any cause or last follow-up. *Results.* Sixty-one patients were retrospectively studied. Eighty % were chemosensitive by CT criteria. PET scan was available in 52 patients before HDT/ASCT, 59 after HDT/ASCT and in 50 at both time points. Pre-ASCT PET scan was positive or MRUp in 23/42 chemosensitive patients, vs 10/10 chemorefractory. The analysis of the prognostic significance of pre-transplant PET scan revealed: three relapses were observed among 19 PET negative patients, vs 17 among 33 MRUp or positive ones, leading to a 2-year FFS of 77% vs 45%, respectively (p=0.02). Post ASCT PET scan had a strong prognostic impact on outcome: 2-year FFS was 87% for PET negative or MRUp patients vs 6% for positive ones, (p< 0.0001). The analysis of the 50 patients who had a PET scan available both pre- and post-transplant disclosed: a. One relapse was recorded for those who were PET scan either positive or MRUp pre-ASCT and became negative or MRUp after ASCT (1/16 patients), b. 15 patients relapsed among 16, who were PET scan positive or MRUp pre-transplant and remained or became positive post-transplant c. 2 relapses were recorded among 16 patients who were negative both pre- and post-ASCT. These differences were statistically significant (p<0.0001). *Conclusions*. PET scan positivity prior to ASCT does not preclude a favorable outcome in patients with primary refractory/relapsed HL, since approximately 50% remain disease free after ASCT. Patients who remain or become PET scan positive after ASCT have a dismal outcome in contrast to those who become PET negative.

0995

EXTRA- AND INTRA-CELLULAR IRON DISTRIBUTION IN PRE-TRANSPLANT BONE MARROW TREPHINE BIOPSIES AND OUTCOME AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Limited data are available on the patterns of pre-transplant iron deposition in the bone marrow (BM) [interstitial and/or in macrophages] and their impact on outcome [survival, non-relapse-mortality (NRM), and graft-versus-host-disease (GvHD)] after allogeneic hematopoietic cell transplantation (HCT). Also, the correlation of BM iron stores (Fe-S) with inherited (HFE-genotype), treatment-related factors [pre-transplant units of packed red blood cells (URB) transfused] and serum ferritin (SF) is not clearly defined. Therefore BM-biopsies of 90 patients (AML, n=68, MDS, n=22), taken for diagnostic purpose at a median of 24 days pre-HCT, were analysed for iron deposition (Perls` reaction) and macrophage identification (CD68 immunostaining). Grading (G0-G5) for Fe-S, and (G0-G3) for interstitial iron (Fe-I) was used to express iron deposition. The percentage of iron storing macrophages in relation to all CD68+ macrophages (Fe-Mac) and of CD68+ macrophages in relation to all haematopoietic cells (Mac) was expressed as percent. Peripheral blood parameters were measured 7 days pre-HCT with a median C-reactive protein of < 5 mg/l [48 males/42 females, median age 57 years]. Donors were matched-related (MRD) in 17 (19%) and matched-unrelated (MUD) in 73 (81%). Preparative regimen was conventional conditioning with 12 Gray TBI/cyclophosphamide 120 mg/kg in 32 (35.6%) and reduced intensity conditioning with fludarabin 30mg/m2/day for 3 days and 2 Gray TBI in 58 (64.4%). Mutated HFE-genotype was found in 27 patients pre-HCT (heterozygosity (het) for H63D,n=15, het C282Y,n=7, het S65C,n=2, and homozygosity for H63D,n=3). Median SF was 2009 (range 36-16900 ng/ml) with 25% of patients having a SF >3000ng/ml. Median URB was 26 (0-115) units. Correlations between

Fe-S and both Fe-I and Fe-Mac (r=0.8, p<0.0001) and between Fe-I and Fe-Mac (r=0.8, p<0.0001) were strongly significant but augmented Fe-S was not associated with increasing Mac (p=0.4). Also, higher SF values were associated with higher grades for Fe-S (p=0.002), Fe-I (p=0.01), and Fe-Mac (p=0.004) but the correlation was poor (r=0.3). There was a significant correlation of SF level (r=0.6, p<0.0001) but not of Fe-S and URB transfusion. Additionally, Fe-S was not influenced by HFE-genotype. After a median follow-up of 24 months, acute and chronic graft-versus-host-disease (GvHD) occurred in 70% and 53% of patients respectively. Survival was 58% and NRM was 22%. Fe-S, Fe-I, and Fe-Mac did not correlate with GvHD, survival or NRM. However, elevated SF levels were highly predictive for inferior survival (p=0.002) and higher NRM (p=0.007) as in patients with SF >3000ng/ml, survival and NRM were 37% and 49% versus 78% and 9% respectively in patients with lower SF (p=0.001). In addition, SF levels >3000ng/ml were associated with acute GvHD (p=0.002) but not with chronic GvHD. In the face of transfusional iron overload, iron is deposited in the bone marrow equally both interstitially and in macrophages. Iron overload measured by an elevated SF is usually accompanied by augmented marrow iron stores but the correlation remains poor. The pre-transplant marrow iron status, unlike serum ferritin, could not predict outcome after HCT. Keeping the well-known limitations in mind, serum ferritin remains a cheap and non-invasive tool to measure iron overload.

0996

IRON OVERLOAD IN HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT). SINGLE-CENTER EXPERIENCE

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Background. Serum ferritin is sensitive but not specific for iron overload and is a poor predictor of body iron burden. However, accumulating evidence has established the negative impact of elevated pretransplantation serum ferritin on transplant related toxicity and mortality (MRT), related in particular to graft-versus-host disease (GVHD), infection, nonrelapse mortality and survival, but this relationship is not clear. *Aims.* The aim of the present study was to study impact of elevated pretrasnsplantation serum ferritin level on transplant toxicity and mortality in our single-center serie. *Methods.* We retrospectively evaluated the prognostic effect of serum ferritin level on the post-transplantation outcome (overall survival, transplant related mortality, infection related mortality, and acute graft versus host disease) of 187 patients who underwent transplantation between 2006 and 2010 in our center. Serum ferritin was measured within the 3 months before transplantation. Patients were divided in 3 groups depending on the serum ferritin level (<500 ngr/mL; 500-1000 ngr/mL; >1000 ngr/mL). We also analyzed our serie with 2 of the most commons cut-off points described in literature (600 and 1000 ngr/mL). Kaplan-Meier and log rank test were used to analyze survival, and χ^2 or Mann-Whitney test to analyze association with infection and aGVHD. *Results.* The HSCT grafts were autologous in 114 patients, and allogeneic in 73 (43% non-myeloablative).

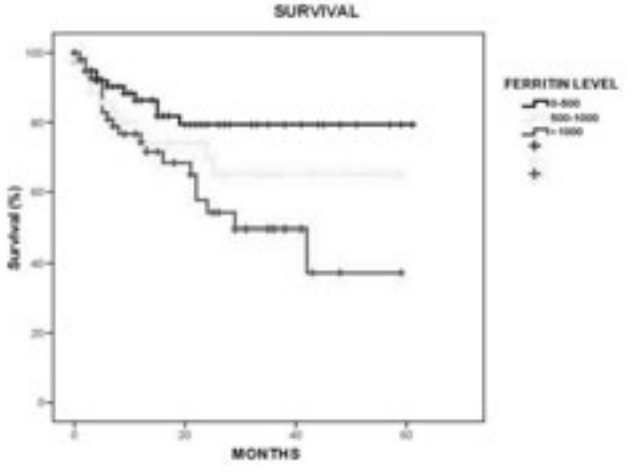

Figure 1.

Follow up was 17,24 months (0.-61). Survival in the entire group was 75,9% (142 patients). MRT was 5,3% and MRT-100 3,2%, mainly due to infection and acute GVHD. Mean (SD) pre-HSCT serum ferritin concentration was 951 (1125) ng/mL in the entire group, 704 (929) ng/mL in autologous recipients, and 1337(1291) ng/mL in allogeneic recipients. Ferritin level was <500 ngr/mL in 82 patients (43,9%), 500-1000 in 46 patients (24,6%), and >1000 in 59 patients (31,6%). High ferritin concentrations (500-1000 ngr/mL and >1000 ngr/mL) were significantly associated with overall survival (79,3%,65,1% and 37,2% respectively; log rank test p=0,044) (figure 1). Ferritin level >600 ng/mL was significantly associated with higher mortality secondary to infection (89% vs 72,4%;p=0,033). We didn't found association between serum ferritin level and MRT, MRT-100 or aGVHD (grade 0-2 vs 3-4). *Summary/Conclusions.* in our serie pre-transplantation serum ferritin level divides patients into 3 groups of risk with significantly different survival. Ferritin level was also associated with higher risk of mortality secondary to infection.This study suggest a predictive role of pre-transplantation ferritin levels in selecting a subset of patients at increased risk for HSC, and could be useful in making treatment decisions for individual patients.

0997

THE IMMUNE PHENOTYPIC PROFILE OF HEMATOPOIETIC STEM CELLS IN DIFFERENT MOBILIZATION REGIMENS AND GENE EXPRESSION PROFILES OF G-CSF MOBILIZED PERIPHERAL HEMATOPOIETIC STEM CELL PRODUCTS

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Background. There is no detailed information about immune phenotypic profile of hematopoietic stem cells (HSC) in different mobilization regimens and gene expression profiles of G-CSF mobilized HSC products. The effects of these properties on the outcome of transplantation largely unknown. *Aims and Methods.* In this prospective study, surface immune phenotypic features (PE-CD11a, FITC-CD18, PE-CD31, APC-CD38, FITC-CD44, APC-CD62e, FITC-CD62L, FITC-CD90, PE-CD117, PE-CD135, PE-CD184) of HSCs which had been mobilized with three different regimens (group I: growth factor alone, group II: cyclophosphamide + growth factor, group III: ESHAP + growth factor) from a total of 44 patients (median age: 46 F/M: 18/28) have been investigated. CD34+ cell sorting was done by flow cytometry (BD FACSAria cell sorter). The relationship between HSC immune phenotype and the duration of the neutrophil and platelet engraftment has also been studied. Additionally, in 9 stem cell products (without cell sorting) which had been mobilized by using G-CSF alone, whole genom expression profiling was carried out. Moreover the effects of these gen profiles on graft versus host disease (GVHD) was evaluated. *Results.* The median duration of neutrophil engraftment after transplantation was 12 (9 - 21) days and platelet engraftment was 12 (7 100) days. The immune phenotypic features of group I, II and III mobilized HSCs were not significantly different. The surface antigens most commonly expressed by CD34 positive stem cells were CD31, CD44, CD90, CD117 and CD135. The CD31 (platelet endothelial cell adhesion molecule-1) positivity ratio of the HSCs were inversely correlated with the duration of the neutrophil (r= -0.32, p= 0.03) and platelet (r= -0.36, p = 0.02) engraftment. No relationship was found between engraftment duration and the expression status of CD184 (CXCR4). There was a relationship between the acute GVHD and the increased expression of genes that are associated with immunity, cell communication and metabolic processes. Another relationship was also found between the development of chronic GVHD and the expression levels of genes which are related to growth and metabolic processes. *Conclusions.* As a result we found that the surface immune phenotypic profiles of CD34+ peripheral HSCs harvested by different mobilization regimens were not different. To our knowledge, it has been demonstrated for the first time that CD31 expression of HSC could positively affect both neutrophil and platelet engraftment. Additionally, CD184 (CXCR4) expression ratio and even CD184 negativity of HSCs have found no effect on neutrophil or platelet engraftment. When these results are evaluated, additional surface antigens (such as CD31) might be more effective in the homing process. More studies investigating the effects of stem cell products whole genom expression on graft versus host disease are needed.

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0998

IS THERE STILL A ROLE FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA?

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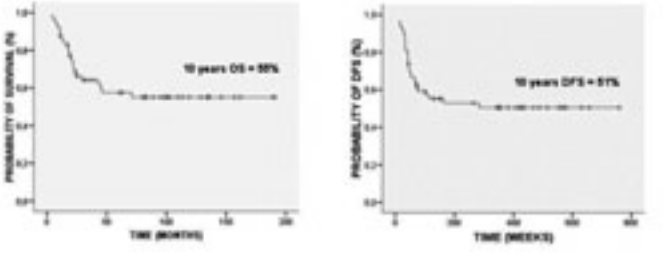
Background. the role of autologous stem cell transplantation (ASCT) in the treatment of patients with acute myeloblastic leukemia (AML) remains unsettled. *Aims.* we report our results of ASCT in patients with AML during the last 15 years. *Methods.* between December 19, 1994 and May 10, 2010, a total of 63 patients with AML without HLA-matched donor in the department of Hematology and Transfusion Medicine, University Hospital, Bratislava, received an ASCT. The median patient age was 41 years (range 20-61 years). 35 (56%) males and 28 (44%) females. At the time of ASCT, 50 (79%) patients were in first complete remission (CR), 11(18%) patients were in second CR and 2(3%) patients were in relapse. The median time interval from CR to ASCT was 107 days (range 48 - 281). Five (8%) patients received bone marrow (BM), 53 (84%) patients received peripheral blood stem cells (PBSC) and 5 patients (8%) received BM plus PBSC. Patients were stratified into three risk groups; poor, intermediate and good-risk groups on the basis of cytogenetic and molecular analyses at diagnosis. *Results.* with a median follow-up of 92 months (7.6 years), the 10 year overall survival (OS) and disease free survival (DFS) of all patients is 55% and 51%, respectively (Figure 1.). Transplant-related mortality is 6%. The relapse rate is 38% and 9 years probability of relapse is 44%. Low white blood cell count (WBC) at diagnosis, favorable and intermediate cytogenetic-risk was independently associated with clinical outcome by univariate analysis. *Conclusion.* we conclude that ASCT is still an effective post-remission treatment in AML patients without HLA-matched donor; with the possibility of long-term survival or even cure in remarkable proportion of patients with AML, particularly in patients with favorable and intermediate cytogenetic risk.



The 10 years Kaplan-Meier estimated probabilities of overall survival (OS) and disease free survival (DFS) after ASCT for the whole group of patients

Figure 1.

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DEVELOPMENT OF RISK SCORE TO PREDICT SIGNIFICANCE OF POSITIVE FUNGAL ISOLATES IN RECIPIENTS OF ALLOGENEIC AND AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background. Invasive fungal infections are a major cause of mortality following stem cell transplantation (SCT). The significance of a positive microbiological isolate with yeasts and molds in this population remains uncertain. *Aims.* to address the clinical significance of isolation of yeasts and mold species from any site in SCT recipients. *Methods.* we retrospectively reviewed all microbiological data, including cultures and direct microscopic examination for yeasts and mold species in 760 autologous SCT (auto-SCT) and 455 allogeneic SCT (allo-SCT) recipients at our institution between 1997 and 2006. Specific fungal species, predisposing factors and survival rates at 100 days after a positive fungal isolate were analysed. *Results.* a total of 494 positive fungal isolates were documented in 187 patients. Fungal isolates were reported in 100 (22%) allo-SCT and 87 (11.4%) auto-SCT recipients. Of these, 407 were yeasts and 87 filamentous fungi. Non-albicans Candida accounted for 57% of all yeasts and Aspergillus species for 74% of filamentous fungi. The 100-day overall survival after a positive fungal isolate was 56.2% for auto-SCT and 59.4% for allo-SCT recipients (p=0.72). In allo-SCT recipients with a positive fungal isolate, the following risk factors were associated with significantly lower 100-day survival on univariate analysis: Neutropenia (37% vs. 51%, p=0.024), immunsuppressive treatment (29% vs. 67%, p=0.033), fungemia (28.6% vs. 57.1%, p=0.035), creatinine >120 µmol/L (35.7% vs. 63%, p=0.013), isolation of non-albicans Candida and aspergillus species (aspergillus species 57.4% vs. non-albicans Candida 46.8% vs. C. Albicans and other yeasts 75%, p=0.021), *in vitro* resistance to >1 antifungal drug (none=63% vs. one=50% vs. two=33.3%, p=0.009), lymphopenia <1x109/L (41% vs. 56%, p=0.003), concurrent fever for >96 hours (32% vs. 66%, p=0.005), respiratory symptoms (20% vs. 78%, p=0.001) and presence of a central line (43% vs. 53%, p=0.004). In auto-SCT recipients with a positive fungal isolate, the only factors associated with lower 100-day survival were creatinine >120 µmol/L (p= 0.0004) and fungemia (P= 0.003). Previous history of positive fungal culture, multiple isolates, abnormal chest X-ray or CT scan were not found to be associated with lower survival. In multivariate analysis only three factors, namely lymphopenia, creatinine >120 µmol/L and respiratory symptoms at the time of isolation remained significant for day-100 survival. Based on these results, we developed a fungal isolate risk score for allo-SCT recipients where each variable was assigned two scores; the presence of respiratory symptoms (yes=2 or no=0), creatinine >120 µmol/L (yes=1 or no=0) and lymphopenia (yes=1 or no=0). Patients with a score of 0-1 (n=18) had a significantly improved 100-day survival (100%) compared to patients who had a risk score of 2-3 (53.7%; n=67). None of the patients with a score of 4 (n=8) survived at 100 days. We propose that based on this risk score, patients with a positive fungal isolate and a score of 0-1 do not require treatment whereas patients with a score of 2-4 are at increased risk of mortality and should be considered for anti-fungal therapy. A prospective study to validate the fungal isolate score is underway.

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OUTCOMES OF REDUCED-INTENSITY STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA WITH T(8;21)- IN COMPARISON WITH CONVENTIONAL STEM CELL TRANSPLANTATION

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Background. Patients with t(8;21) generally have a favorable prognosis with a higher rate of first complete remission (CR1) and higher rate of cure with high-dose cytarabine (HDARA-C), precluding these patients as candidates for stem cell transplantation (SCT). However, some studies raised questions regarding improved survival with autologous SCT (ASCT) in these patients. Moreover, there has been some concern that the rate of relapse after HDARA-C is higher than previously reported and although approximately 50% of CBF-AML patients achieve long-term survival, disease relapse is still a major cause of treatment failure. Of CBF-AML, t(8;21) patients are reported to be related to worse outcome than those with inv(16). Therefore, patients with t(8;21) might benefit from more intensive treatment such as SCT. To date, few studies have analyzed the outcome of reduced intensity conditioning (RIC)-SCT for this population specifically. *Aims.* From this point of view, we performed a study to evaluate the efficacy and safety of RIC-SCT for AML with t(8;21) and compared the results with those of the historical cohort who treated with conventional SCT (ASCT or myeloablative-SCT) in our institution. *Methods.* We transplanted 19 adult patients with AML who had t(8;21) after RIC since Mar 2007. RIC regimen consisted of fludarabine, busulfan and low dose total body irradiation. Their clinical features were compared with historical patients who were transplanted with autografting or myeloablative conditioning (MC) in our institution from 2001. *Results.* Probability of overall survival (OS) and disease free survival (DFS) was 77.5 ± 9.9% and 78.2 ± 9.7%, respectively, with a median follow-up of 34 months (range: 13.5+ to 45.2+ months) for surviving patients. 2-year cumulative inci-

dence of relapse (CIR) and nonrelapse mortality (NRM) was 11.9 ± 7.9%, respectively. By univariate analysis, age seems to be a dominant factor affecting DFS and OS in RIC-SCT and ASCT group ($P = 0.025$ and 0.029, respectively, for RIC-SCT group, and $P = 0.036$ and 0.013, respectively, for ASCT group). In ASCT group, presence of -Y was associated with inferior DFS ($P = 0.049$), whereas this difference disappeared when the patients were transplanted with RIC or MC. For patients with autografging, CIR was significantly lower in patients without -Y than with -Y ($P = 0.012$), while there was no difference in NRM between the two subgroups. *Summary/Conclusions.* RIC-SCT is effective treatment modality for AML with t(8;21), judging from little differenes in CIR and survival in comparison with those of MC-SCT, but greater effort should be put to reduce NRM further. Because ASCT was associated with extremely low NRM and demonstrated comparable efficacy to those of allo-SCT, this modality might be a preferable treatment option for this disease except for the subgroup of patients with -Y. In our study, patients with -Y demonstrated inferior survival only in the setting of ASCT, and these differences disappeared in allo-SCT groups, justifying that allo-SCT should be considered in this subgroup of patients. Finally, MC-SCT is not recommended in patients with t(8;21) in CR1 because it has no advantages in terms of relapse and NRM.

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TWO DAYS OF ATG IN THE CONDITIONING REGIMEN IS ASSOCIATED WITH A REDUCED INCIDENCE OF BOTH ACUTE AND CHRONIC GVHD WITHOUT INCREASING RELAPSE IN RIC TRANSPLANT FOR HEMATOLOGICAL DISEASES

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Background. the best combination of Fludarabine-Busulphan-ATG (FBA) in reduced-intensity conditioning for allogeneic hematopoietic stem cell transplantation (RIC-AlloSCT) is unknown, busulphan dose being crucial for disease control but limited by a dose-dependent toxicity and ATG playing a pivotal role in the prevention of both acute and chronic GvHD but with a potential higher relapse rate associated with higher doses. *Aims.* here we compared two FBA regimens among adult patients transplanted at our Institution for a hematological malignancy. We aimed identifying the impact of ATG dose on GvHD and other transplant outcomes. *Methods.* ATG was administered at a total dose of 2.5 mg/kg (1-day ATG) or, more recently, 5 mg/kg (2-day ATG). The effect of ATG dose on transplant outcomes was analyzed, both in univariate and multivariate analysis. *Results.* 124 patients and 105 patients were included in the 1-day and 2-day groups, respectively. The two cohorts significantly differed for: follow-up duration, year of transplantation, patient's age, patient/donor gender matching, busulfan formulation and donor type. No significant difference in OS, NRM, relapse/progression between the two ATG groups was observed, both in univariate and multivariate analysis. On the other hand, incidence of acute and chronic GvHD differed among the two groups, with a significant reduction of acute and chronic GvHD in the 2-day ATG patients, both in univariate and multivariate analysis. Multivariate hazard ratio (95% CI) of grade 2-4 acute GvHD was 0.27 (0.12 - 0.59) and for grade 3-4 acute GvHD was 0.26 (0.10 - 0.69); p=0.001 and p=0.007 respectively. The same protective effect played by ATG 5 mg/kg was also found on chronic GvHD, both overall and extensive form. Multivariate hazard ratio (95% CI) of extensive chronic GvHD was 0.23 (0.12-0.46) in the 2-day ATG vs. 1-day ATG group, with a p<0.0001. It is worth noting that the increased ATG dose was not associated with a higher risk of relapse or progression. *Conclusions.* in this monocenter series it seems that a higher dose of ATG significantly reduces the incidence and severity of acute and chronic GvHD, without increasing disease relapse. Taken together, present results show that a FBA-based regimen including two days of ATG appears to confer better outcomes in terms of GvHD and disease control and might represent an optimal dosage. Prospectively assessment is of interest.

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STUDY OF THE EFFECT OF TEMPERATURE IN PRE-FREEZING OVERNIGHT STORAGE IN 268 AUTOLOGOUS LEUKAPHERESIS PRODUCTS

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Background. In autologous peripheral blood progenitor cells (PBPCs) overnight storage of leukapheresis (LAPH) products is necessary when immediate processing is not possible. Properties of PBPCs, could be altered by storage before cryopreservation. *Aims.* The aim of this study is to evaluate the effect of overnight (ON) storage, at 4°C (group A) and 20°C (group B), comparing the results with those of immediate freezing (IF) as well as to analyze the influence of the storage temperature. The viability, percentage of CD34+ cells, CFU-GM and bacterial contamination were analyzed. *Methods*. The LAPH performed, for PBPCs autologous transplantation, in our hospital in the last ten years were reviewed. Only those patients in which both, IF and ON storage, were done are studied in this report. PBPCs were cryopreserved with DMSO at final concentration of 10% plus autologous plasma or blood bank ABO isogroup plasma. CD34-positive cells were counted following the International Society for Haematotherapy and Graft Engineering protocol. CFU-GM assay was performed in agar-gel feeder-layer or enriched semisolid culture media, Methylcellulose Medium. The same culture method was always used to compare pre-freezing and post-thawing samples of the same cryo-preserved cell batch. Cell viability was determined by 0.2% Trypan blue dye exclusion test. Bacterial cultures were done immediately after mixing PBPCs with DMSO and in the post-thawing sample. The results pre-freezing and post-thawing were compared, as well as recovery in both storage temperatures. T-test was used to compare CD34+ cells, CFU-GM and viability, pre-freezing and post-thawing, and the recovery with both temperatures. Chi-square-test was used for bacterial contamination comparison. *Results.* A total of 123 patients, with 268 PBPCs LAPH (92 in group A and 176 in group B), were included. The median, aged was 49 years (3-73). Diagnoses were: 40 MM, 23 breast cancer, 22 NHL, 12 HD and 26 others diagnoses. Mobilization was done with: chemotherapy+G-CSF (17%), cyclophosphamide (1.5/m2)+G-CSF (57%) or G-CSF (26%). Half of the LAPH were IF and half were ON stored: 46 LAPH at 4°C, and 88 at 20°C.In the group A there were 92 LAPH (46 IF and 46 ON), and in the group B there were 176 (88 IF and 88 ON). In group A no significant difference was found between the IF CD34+ recovery (127.7±67.4) compared with those ON stored at 4°C (112.1±43.2). There were not significant differences in group B between IF (121.5±91.7) and 20°C (118.7±123.6).When the recovery of CFU-GM in group A, IF and ON storage, were compared no difference was found; the same occurred in group B. However, the pre-freezing viability was significantly decreased in the ON samples in both groups, in comparison with IF, (86.7±56.2% vs 92.3±5.7% in group A) and (76.3±15.7% vs 86.0±11.7% in group B). No statistical difference was found when viability, CFU-GM and CD34+ cells recovery between both groups were compared. The bacterial contamination incidence was not different in both groups. *Conclusions.* These data suggest that the ON pre-freezing temperature does not affect seriously the PBPCs quality. However, viability decreases when freezing is not performed immediately.

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EFFECT OF THROMBOCYTOPENIA IN HEMATOPOIETIC STEM CELL MOBILIZATION WITH PLERIXAFOR PLUS G-CSF

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Background. High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) is considered standard of care for a variety of hematologic malignancies including Multiple Myeloma (MM), Non-Hodgkin Lympoma (NHL) and Hodgkin Lymphoma (HL). A significant proportion of patients fail to mobilize and collect a minimum of $2x10^6$ CD34+ cells/kg and a number of factors have been reported to

impact hematopoietic stem cell (HSC) mobilization, including baseline platelet counts. Thrombocytopenia prior to mobilization with G-CSF±chemotherapy is a significant predictive factor for mobilization failure in patients with Hodgkin's and non-Hodgkin's lymphoma *(Hosing C, Am J Hematol. 2009). Aims.* The purpose of this retrospective analysis is to assess the efficacy of HSC mobilization with plerixafor plus G-CSF in patients with low platelet counts. *Methods.* Patients who had failed at least 1 previous HSC mobilization were remobilized with plerixafor plus G-CSF as part of the European compassionate use program (CUP). G-CSF (10µg/kg) was administered subcutaneously (SC) every morning for 4 days and plerixafor (0.24 mg/kg SC) was administered in the evening on Day 4. On Day 5, G-CSF was administered and apheresis was initiated. Plerixafor, G-CSF and apheresis were repeated daily until patients collected the minimum of $2x10^6$ CD34+ cells/kg. *Results.* Data on platelet counts prior to mobilization are available for 189 patients from our European CUP database. Of these, 86 patients presented platelet counts ≤150 $x10^9$/L, median 115, range 18-150; NHL=40, MM=32, HL=10, other=4; 55% male; median age 57 years, range 20-75; median number of prior therapy regimens 3, range 1-10, and 103 patients had normal platelet counts >$150x10^9$/L, median 225, range 151-442; NHL=45, MM=37, HL=21; 53% male; median age 54 years, range 24-72; median number of prior therapy regimens 2, range 1-8. Following a similar number of aphereses in both cohorts (median 2, range 1-4), the median CD34+ cell yield was significantly higher in the normal platelet group ($3.2x10^6$/kg, range 0.5-32.6) than in thrombocytopenic patients ($2.56x10^6$/kg, range 0.18-9.2; p<0.001). Also a significantly higher proportion of patients achieved the target cell dose of $2x10^6$ CD34+ cells/kg in the normal platelet group (86% versus 58%, p<0.001). Sixty-five per cent of patients with normal platelet counts and 45% in the thrombocytopenic group have undergone ASCT, with similar time to neutrophil and platelet engraftment in both groups. *Conclusions.* In keeping with previous reports in first line mobilization, our data suggest that thrombocytopenia prior to remobilization with plerixafor and G-CSF remains a significant predictive factor for mobilization failure compared with patients with normal platelet counts. Nevertheless, 58% of such thrombocytopenic patients, who have already failed prior mobilization attempts, can be successfully rescued with plerixafor and G-CSF to collect ≥$2x10^6$ CD34+ cells/kg. Overall, this strategy provides a remarkable success rate in the mobilization of these challenging patients and subsequently proceeding to transplantation, compared with published alternatives for thrombocytopenic patients.

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VARIABLES AFFECTING HEMATOPOIETIC STEM CELL MOBILIZATION FOR HIGH DOSE CHEMOTHERAPY AND STEM CELL RESCUE IN CHILDREN

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Background. A SDF-1alpha/CXCR4 binding inhibitor has been recently approved for adult patients diagnosed with multiple myeloma or lymphoma to enhance mobilization of hematopoietic stem cells (HSC) to peripheral blood (PB). Experience in children, however, is extremely limited. We have conducted a retrospective study in order to analyze variables related to poor mobilization in children to identify those patients who will likely benefit on the use of this new drug. *Aims.* To analyze variables related to poor mobilization of peripheral blood progenitor cells in children. *Methods.* We analyzed data prospectively recorded, since January 2000, from 183 consecutive patients mobilized with filgrastim at different doses prior to HSC collection. CD34+ cells on PB before the first apheresis were evaluated to consider poor mobilizers. Patients with less or equal to 10 CD34+cells/mcl on PB were considered poor mobilizers independently if they underwent collection or not. In our experience most children reach the target CD34+ cell dose after several procedures, even those considered as poor mobilizers, but in this study we tried to identify those who will likely benefit of new mobilization agents. All procedures were performed in the Transfusion Service at Hospital Infantil Universitario Niño Jesús (Madrid). Patients' diagnosis were as follows: central nervous system tumours 51(28%), Ewing sarcoma 42 (23%), neuroblastoma 35 (19%), rabdomyosarcoma 5 (3%), Wilms tumour 3 (2%), Osteosarcoma 6 (3%), Non-Hodgkin lymphoma 20 (11%), Hodgkin disease 12 (6%) and others 9 (5%). Disease status was: first complete remission 107 (59.1%), partial remission 44 (24.3%), disease progression 13 (7.7%), and complete remission after first relapse 17 (9.4%). Median age was 7 years (1-18), and median weight 23 kg (5-111). Mobilization regimens included: G-CSF 12mcgr/kg/12h (64.09%), 10mcgr/kg/24h (3.31%), 12mcgr/kg/24h (24.31%), 10mcgr/kg/12h (7.18%), others (1.1%). Contingency table was applied for categorical variables on the univariate analysis. Correlations were determined using logistic regression. *Results.* Median CD34+cells on PB before the collection was 40 (1-495). Twenty-six patients had less or equal than 10 CD34+ cells/mcl before the collection (14%). Of those, only 7 patients did not undergo HSC collection. Several variables were studied, but only radiotherapy was related on univariate analysis to poor mobilization (p=0.01). Other variables showed clear tendency but did not reach statistical signification: type of mobilization (p=0.14); age (p=0.15); diagnosis (p= 0.08); disease status ((p=0.08). We define a risk score giving one point each to radiotherapy, chemotherapy more than 6 cycles of chemotherapy before the mobilization, and age older than 9 year old. Those patients with 2 or 3 points have higher risk of mobilization failure (p=0.01). When these variables were included in a multivariate analysis, only prior radiotherapy HR 0.08 (0.18-0.35) p = 0.0009; and mobilization with 12 mcg/kg/day HR 0.29 (0.09-0.95) p=0.04, were related to poor mobilization. *Conclusion.* Prior use of radiotherapy is the main variable related to poor mobilization in children. No single variable is helpful to anticipate the need for using new mobilization agents in children. Only those patients with several risk factors may be candidates to be considered as potentially at risk for poor mobilization.

SIMULTANEOUS SESSION II

Therapy for relapsed/refractory Multiple Myeloma

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PHASE 2 RANDOMISED OPEN LABEL STUDY OF 2 MODALITIES OF POMALIDOMIDE (CC4047) PLUS LOW-DOSE DEXAMETHASONE IN PATIENTS WITH MULTIPLE MYELOMA, REFRACTORY TO BOTH LENALIDOMIDE AND BORTEZOMIB. IFM 2009-02

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Background. Patients with multiple myeloma (MM) who are refractory to bortezomib and lenalidomide (double refractory) have limited treatment options and less than a year of life expectancy. In prior phase 1 and 2 studies, pomalidomide was administered at 4mg, 21 days of each 28-days or at 2mg 28 days of each 28-days. Additional responses were achieved by patients escalated to 4mg and in combination to dexamethasone. *Aim.* We have designed a phase 2 multicenter, randomized, open-label study aimed to determine the efficacy and toxicity profile of 2 modalities of pomalidomide in double refractory patients. *Method.* The patients had symptomatic, progressive, measurable and double refractory MM. The primary objective was to determine response rate (PR and better) to pomalidomide and dexamethasone using IMWG response criteria. The response and FISH cytogenetic analysis were assessed centrally. Pomalidomide was given orally either 4 mg daily on days 1-21 of each 28-days (arm A) or continuously on days 1-28 of each 28-days (arm B). Dexamethasone was given orally at 40 mg weekly. All patients received prophylaxis against venous thromboembolism. *Results.* Eighty three patients (56 male and 27 female) were enrolled between August first and June first, 43 in arm A and 40 in arm B, respectively. The median age was 54 years (range, 36-78). The median time from diagnosis to enrolment was 55 months (range 11-227) in arm A and 76 (30-281) in arm B. All patients had loss of 17p and t(4;14) cytogenetic abnormalities studied on bone marrow plasma cells. As of August first, the median follow-up was 119 days. The median number of cycles administered was 4 in either arm. Overall, 40 and 36 patients were evaluable for response evaluation in arm A and B, respectively. In arm A, 12 (30%) patients had PR and better, including 3 VGPR, and 21 (52%) had stable disease. In arm B, 17 (47%) patients had PR and better, including 1 VGPR, and 15 (41%) had stable disease. The median duration of response was 77 and 89 days in arm A and B, respectively. Twenty three (57%) and 22 (61%) patients remained progression free, and 5 patients have died in either arm, respectively. Toxicity (at least possibly related to treatment) consisted primarily of myelosuppression in both arms. The occurrence of neuropathy was not observed nor worsening of pre-existing neuropathy. No thromboembolic events have occurred. *Conclusion.* Pomalidomide and dexamethasone is active and well tolerated in this heavily pre-treated population of lenalidomide and bortezomib-refractory MM patients. 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. Final results will be provided at EHA 2011.

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RESULTS OF AN INTERNATIONAL, RANDOMIZED PHASE II CLINICAL TRIAL OF BORTEZOMIB ± MAPATUMUMAB (TRAIL-R1 AGONIST MONOCLONAL ANTIBODY) FOR THE TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA (MM)

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Background. Development of new therapies for patients with relapsed/refractory MM remains an important clinical need. Monocolonal antibodies (mAb) have made an important impact in B-cell cancers especially when combined with chemotherapeutics. Unfortunately, currently there are no approved mAb for MM patients. Mapatumumab is a fully human mAb that targets and activates the TRAIL-R1 receptor. In preclinical MM models, enhanced efficacy of bortezomib was noted when combined with mapatumumab. *Aims.* Based on these preclinical observations, a randomized phase II clinical trial was designed to evaluate the clinical efficacy of mapatumumab when added to bortezomib. *Methods.* Informed consent was obtained from all patients participating in this study. Patients with relapsed or refractory MM, who had measurable M-protein in serum and/or urine and had failed up to 2 prior therapies, were eligible for participation in this study. Patients were excluded if they had received prior bortezomib. Response was evaluated using the Bladé criteria. Treatment: Patients were randomized to either Arm A (bortezomib 1.3 mg/m2 on days 1, 4, 8, 11 every 21 days) or Arm B10 (bortezomib + mapatumumab 10 mg/kg on day 1 every 21 days) or Arm B20 (bortezomib + mapatumumab 20 mg/kg on day 1 every 21 days). Patients received a maximum of 17 cycles (1 year) and treatment was discontinued at any time for progressive disease or unacceptable toxicity. Subjects with complete response (CR) were treated for an additional 2 cycles after documentation of CR (not to exceed 17) and then followed to progression. This trial is registered with clinicaltrials.gov, number NCT00315757. *Results.* A total of 104 subjects were randomly assigned to the treatment arms. The median age of patients was 61.7 and mean prior therapies 1.6. Toxicity: The most common toxicities observed were hematological and peripheral sensory neuropathy. In general, addition of mapatumumab did not alter or enhance the toxicity profile of bortezomib. Overall incidence of grade 3/4 toxicity in Arms A, B10 and B20 was 88.6% 69.7%, and 61.1%, respectively. Efficacy: The ORR was 51.4%, 30.3% and 52.8% and the median duration of response was 8.5, 9.3

Table 1.

	Arm A (n = 35)	Arm B10 (n = 33)	Arm B20 (n = 36)
CR	0	0	2
PR	18	10	17
CR + PR n(%)	18 (51.4)	10 (30.3) p = 0.09[a]	19 (52.8) p = 0.91[b]
Median PFS mo (95%CI)	9.7 (7.8, 10.0)	4.7 (2.5, 7.4) p = 0.29[a]	5.7 (5.2, 8.9) p = 0.21[b]
Median Duration of Response mo (range)	8.5 (6.9, 14.2)	9.3 (3.9, 16.1) p = 0.92[a]	7.6 (4.3, 8.6) p = 0.41[b]
[a]p value for comparison of Arm A with Arm B10 [b]p value for comparison of Arm A with Arm B20			

and 7.6 months, in arms A, B10 and B20, respectively (Table). *Summary/Conclusions.* Mapatumumab is a novel mAb that effectively engages TRAIL-R1. Encouraging preclinical observations led to a well designed randomized phase II clinical trial to determine safety and effectiveness of targeting TRAIL-R1 in MM. Our studies demonstrate that mapatumumab was well tolerated with no significant toxicity when added to bortezomib. However, in patients with rel/ref MM, mapatumumab failed to improve meaningful clinical endpoints. As TRAIL-R1 remains an important therapeutic target in cancer and mapatumumab is actively investigated in other malignant disorders, our data provide valuable insight into the tolerability and expected toxicity of two different doses of this novel drug. Detailed safety and efficacy analyses will be presented at the meeting.

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ELOTUZUMAB IN COMBINATION WITH LENALIDOMIDE AND LOW-DOSE DEXAMETHASONE IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA: A RANDOMIZED PHASE 2 STUDY

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Background. Elotuzumab is a humanized monoclonal IgG1 antibody targeting human CS1, a cell surface glycoprotein. CS1 is highly and uniformly expressed on multiple myeloma (MM) cells, with limited expression on natural killer (NK) cells and CD8+ cells and little to no expression in most other normal tissues. Preclinical data indicate that the mechanism of action is primarily through NK-mediated antibody-dependent cellular cytotoxicity. In a MM xenograft mouse model, the antitumor activity of elotuzumab was enhanced by the addition of lenalidomide, significantly reducing tumor volume compared with either agent alone. A phase 1 study of elotuzumab plus lenalidomide and low-dose dexamethasone demonstrated an 82% response rate but did not identify a maximum tolerated dose in patients with relapsed/refractory MM. Responses proved durable; median time to progression not reached at a median follow-up of 12.7 months. *Aims.* To assess the efficacy and safety of elotuzumab in combination with lenalidomide and low-dose dexamethasone and determine the optimal dose of elotuzumab (10 mg/kg vs 20 mg/kg). *Methods.* Patients with relapsed/refractory MM who had received 1-3 prior therapies (excluding lenalidomide) were randomized to elotuzumab 10 mg/kg or 20 mg/kg IV (days 1, 8, 15, and 22 every 28 days in the 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 potential elotuzumab infusion-related adverse events (AEs) consisted of methylprednisolone (50 mg IV), diphenhydramine (25-50 mg PO or IV) or equivalent, ranitidine (50 mg IV) or equivalent, and acetaminophen (650-1000 mg PO). Treatment continued until disease progression or unacceptable toxicity. All patients provided informed consent. Objective responses were assessed according to IMWG criteria. *Results.* Among 63 enrolled patients (median age 63 years; range, 39-82), 57% had ≥2 prior therapies, 54% had prior bortezomib, 59% had prior thalidomide, and 52% had a β2 microglobulin level ≥3.5mg/L. In total, 81% of patients had ≥ partial response (PR) including 37% ≥ very good PR. Overall response rates (ORR; defined as ≥PR) were 90% in the 10 mg/kg group (n=31) and 72% in the 20 mg/kg group (n=32). Results were similar irrespective of prior thalidomide, prior bortezomib, number of previous therapies, and β2 microglobulin. Median time to response was 30 days (range, 21-100). After a median follow-up of 4.9 months, median progression-free survival had not been reached; 9 (14%) patients have progressed. The most common grade 3/4 treatment-emergent AEs were neutropenia (14%), lymphopenia (14%), and thrombocytopenia (13%). The most common infusion-related AEs were grade 1/2 nausea (16%), dizziness (13%), headache (13%), and pyrexia (10%). One patient had a grade 3 infusion reaction AE (rash). No patient withdrew due to an infusion reaction. *Summary/Conclusions.* Elotuzumab plus lenalidomide/dexamethasone resulted in rapid and high ORR in patients with previously-treated MM. The most common grade 3/4 treatment-emergent AEs were cytopenias. With premedication the incidence and severity of infusion reactions were low. Further clinical study of this combination using 10 mg/kg elotuzumab is warranted; a phase 3 trial is planned in 2011 (NCT01239797). Updated results will be presented at the meeting.

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BORTEZOMIB(VELCADE®)-THALIDOMIDE-DEXAMETHASONE (VTD) IS SUPERIOR TO THALIDOMIDE-DEXAMETHASONE (TD) IN PATIENTS WITH MULTIPLE MYELOMA (MM) PROGRESSING OR RELAPSING AFTER AUTOLOGOUS TRANSPLANTATION

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In 2006, the EBMT and the IFM initiated a prospective, randomized, parallel-group, open-label phase III, multicenter study, comparing VTD (arm A) with TD (arm B) for MM patients in first progression/relapse after at least one autologous transplantation. TTP was the primary end point. Treatment was: bortezomib 1.3 mg/m2 as an i.v bolus on days 1, 4, 8 and 11, followed by a 10-day rest period (days 12 to 21), for 8 cycles (6 months) and then on days 1, 8, 15 and 22, followed by a 20-day rest period (days 23 to 42), for 4 cycles (6 months). In both arms, thalidomide was administered at 200 mg/day for 1 year and dexamethasone at 40 mg/day orally for 4 days every 3 weeks for 1 year. Response was assessed by EBMT criteria. Adverse events were graded by the NCI-CTCAE, Version 3.0. *Results.* On 01/07/10, a first interim analysis based on 246 patients and 134 events was performed. The trial was then stopped because of superiority of VTD over TD. We report an updated analysis as of 02/12/10. 267 patients (135 in arm A, 132 in arm B) had been enrolled in the study and 157 events had been observed. The median age was 61 years (range 29-76) The stage according to the ISS was I in 56 %, II in 27 %, III in 17 %. The number of previous autologous transplants was one in 71 vs 69 patients and two or more in 64 vs 63 patients, in arms A and B respectively. The median follow-up was 27 months. The median TTP was 19.5 vs 13.8 months respectively in arms A and B, with a cumulative incidence of relapse/progression at 2 years of 56% vs 71% (p=0.0011). The median PFS was 18.6 vs 12.7 months with a cumulative incidence at 2 years of 37% vs 23% (A vs B, p=0.0011). The OS in the first two years was 72% vs 68% (p=0.18). The probability of achieving CR and CR+PR

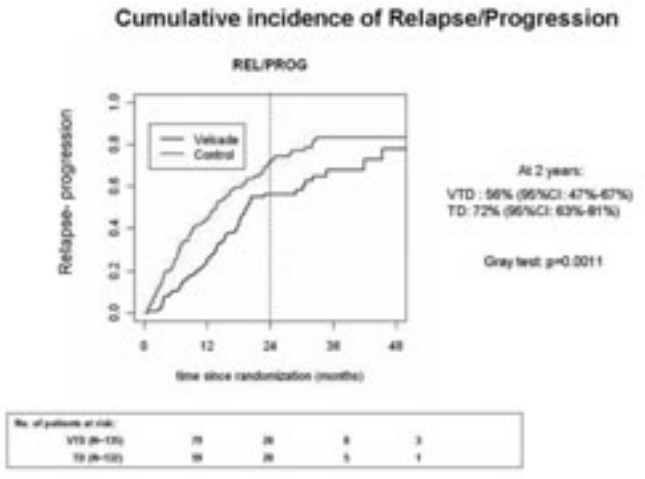

Figure 1.

during the first year was 32% vs 12% and 90% vs 69% with VTD and TD (p=0.0001, and p=0.0001). In the VTD and TD arms, the mean number of treatment cycles for the first 8 cycles were 6.25 vs 6.88 and for the 12 cycles, 7.56 vs 9.93 respectively. Treatment was discontinued due to toxicity in 48 patients (VTD= 36, TD=12). 33 patients died during the treatment period (VTD= 14, TD= 19).The incidence of thrombo-embolic events >= grade 3 was similar in the two arms (6.6% vs 5.2%, p=ns, VTD vs TD) while >= grade 3 thrombocytopenia was higher with VTD (16% vs 7%, p= 0.025). *Conclusion.* VTD resulted in significantly longer TTP and PFS in patients relapsing after ASCT with an acceptable toxicity. Protocol EU-DRACT number: 2005-001628-35.

1009

LENALIDOMIDE AND DEXAMETHASONE (LEN + DEX) TREATMENT IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) PATIENTS DOES NOT INCREASE THE RISK OF SECOND PRIMARY MALIGNANCIES (SPM): ANALYSIS OF MM-009/010

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Background. Multiple myeloma (MM) is an incurable malignancy characterized by multiple relapses, and eventually, refractory disease and death. In a pooled update of the phase 3 MM-009/010 trials comparing Len + Dex vs placebo plus dexamethasone (PBO + Dex), Dimopoulos *et al.* (2009) reported that overall survival (OS) was significantly longer in patients treated with Len + Dex vs PBO + Dex (median of 38.0 vs 31.6 months, respectively; P = 0.045). However, with longer survival a greater number of SPMs may be observed. Cancer registry data (2003-2007) indicate that the incidence of all invasive cancers increases from 0.8 per 100 person-years in persons aged 55-59 to 2.2 per 100 person-years among persons 85+, respectively (Surveillance, Epidemiology, and End Results [SEER] 2010; myelodysplastic syndromes [MDS] not included). Moreover, this incidence may be higher in patients with MM as result of immune deficiency and underlying genetic predisposition. *Aim.* This post hoc analysis of pooled MM-009/010 data was conducted to compare incidence rates (IRs) of SPMs between treatment arms and to compare these IRs with IRs of invasive cancers among similarly aged persons in the general population. *Methods.* Potential SPMs were identified by review of medDRA terms under the "Neoplasm" System Organ Class. SPM incidence rates per 100 person-years were evaluated during active, double-blind treatment. *Results.* The overall incidence of SPMs was low. There were 2 cases of MDS in the Len + Dex arm, and no cases of acute myeloid leukemia or B-cell malignancies in either arm. Non-melanoma skin cancers were noted in 14 Len + Dex patients and 2 PBO + Dex patients. Seven cases of solid tumors (5 with Len + Dex and 2 with PBO + Dex) occurred during double-blind treatment. IRs were similar between treatment arms. With an additional 1.5 average person-years during the extended follow-up phase, only one new SPM was identified in the Len + Dex arm, likely reflecting limitations in SPM ascertainment as only survival information was collected during this study phase. *Conclusions.* With a median follow-up of 48 months, a significant OS benefit for Len + Dex treatment in RRMM patients was observed. The number of SPMs was low and IRs during active treatment (when medical surveillance could be expected to be most equivalent) did not differ between treatment arms. Importantly, IRs in both arms were comparable to the expected background incidence of invasive cancer among older individuals. The low number and type of SPMs seen did not change the benefit-risk profile for lenalidomide in RRMM patients, especially given the survival advantage seen with this therapy. The role of lenalidomide in the RRMM setting is confirmed as an effective treatment option.

Table 1.

Variable	Len + Dex (n = 353)	PBO + Dex (n = 350)
Double-Blind Treatment		
Person-years	471.01	222.65
Cases of invasive malignancies*	5	2
IR per 100 person-years (95% CI)	1.06 (0.39-2.35)	0.90 (0.15-2.97)

*MDS and non-melanoma skin cancers not included

Chronic myeloid leukemia - Clinical 2

1010

A NEW PROGNOSTIC SCORE (EUTOS SCORE) PREDICTING CYTOGENETIC RESPONSE AND PROGRESSION-FREE SURVIVAL IN 2060 PATIENTS WITH CHRONIC MYELOID LEUKEMIA ON IMATINIB TREATMENT

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Background. The outcome of chronic myeloid leukemia (CML) has been profoundly changed by tyrosine kinase inhibitors (TKI), but the prognosis of CML is still based on prognostic scores developed in the chemotherapy and interferon era. *Aims.* We analyzed for prognosis a multicentric multinational series of 2060 Ph+ BCR-ABL + CML patients who were treated front line with Imatinib or an Imatinib-based regime. *Methods.* First, we examined the relationships between the time of achieving a complete cytogenetic response (CCgR) during the first 18 months of therapy, the probability of achieving a CCgR later on, and the risk of progression to accelerated phase (AP) or blast phase (BP) after 18 months. *Results.* It was found that the most powerful predictor was the Cg status at 18 months, since only 31% of the pts who were not yet in CCgR at 18 mo, achieved a CCgR later on, and 23% of them progressed after 18 months. The whole series was then divided in a learning sample and a validation sample. The analysis of the learning sample, by logistic regression and chi-squared tests, identified spleen size (assessed by manual palpation and measured in cm below costal margin) and blood basophils percentage as the most significant prognostic variables. Using spleen size and basophils (7 x basophils + 4 x spleen size), a risk score could be assigned to all patients, and by the minimal p-value approach a "high risk"and a "low risk"group were identified (score more than 87 vs equal / less than 87). The score was validated in the validation sample. The positive predictive value of the new score was 34%, the sensitivity was 21%, and the specificity was 92%. Progression-free survival at 5 years was 90% (95% CI 88-92%) for high risk patients and 82% (95% CI 73-89%) for low risk patients. *Conclusions.* The power and the efficacy of TKIs are such that it is increasingly difficult to elaborate a prognostic system, but the new EUTOS score marks a significant improvement over prior, Sokal and Euro scores, and can be easily applied in clinical practice, until new biologic and molecular factors will be identified and shown to predict better the outcome and to select the treatment.

1011

EFFICACY AND SAFETY OF DASATINIB COMPARED WITH IMATINIB IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): MINIMUM 24-MONTH FOLLOW-UP FROM THE DASISION TRIAL

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Background. In the phase 3 DASISION trial, first-line dasatinib showed higher and faster rates of complete cytogenetic response (CCyR) and major molecular response (MMR) compared with imatinib in patients with newly diagnosed CML-CP (Shah, Blood 2010; 116: abs 206). *Aims.* Assess 2-year efficacy and safety in the DASISION trial. *Methods.* After informed consent, patients were randomized 1:1 to dasatinib 100 mg once daily (QD) (n=259) or imatinib 400 mg QD (n=260). Primary endpoint was confirmed CCyR (cCCyR) rate (CCyR on two consecutive assessments) by 12 months. *Results.* Minimum 24-month follow-up will be presented. After 19.7 months' median follow-up (range 0.1-31.4), 81% and 80% of dasatinib-treated and imatinib-treated patients remained on therapy. 18-month cumulative response rates for dasatinib vs imatinib were: cCCyR 78% vs 70% (P=0.0366), CCyR 84% vs 78% (P=0.0932); and MMR 56% vs 37% (P<0.0001). MMR was more frequent with dasatinib in all Euro risk groups. BCR-ABL transcript levels ≤0.0032% (International Scale) occurred in 13% with dasatinib and 7% with imatinib (P=0.0119). Median time to CCyR or MMR calculated using competing risk analysis was shorter with dasatinib vs imatinib (CCyR: 3.2 vs 6.0 months; MMR: 15 months vs not yet reached). Transformation to accelerated/blast phase (AP/BP) occurred in six (2.3%) dasatinib-treated vs nine (3.5%) imatinib-treated patients; one additional patient transformed 183 days after discontinuing dasatinib; no patient achieving MMR transformed. Protocol-defined progression (death from any cause, transformation to AP/BP, increasing WBCs, loss of complete hematologic response or major cytogenetic response) occurred in 15 patients (5.8%) in each arm. For dasatinib vs imatinib, 18-month overall survival rates were 96% vs 98%, progression-free survival rates (no transformation to AP/BP or loss of response) were 95% vs 94%, failure-free survival rates (ELN 2006 criteria) were 93% vs 90%, and maximum clinical benefit rates (no progression, failure, or intolerance) were 87% vs 86%. Longer follow-up is needed and 5-year follow-up is planned. Drug-related nonhematologic adverse events (AEs; any grade) in ≥10% of patients (dasatinib vs imatinib) were fluid retention (23% vs 43%; including superficial edema: 10% vs 36%, pleural effusion: 12% vs 0%), diarrhea (18% vs 19%), nausea (9% vs 21%), vomiting (5% vs 10%), myalgia (22% vs 38%), fatigue (8% vs 11%), headache (12% vs 10%), and rash (11% vs 17%); grade 3/4 rates for these AEs were ≤1%. Only dasatinib-treated patients experienced pleural effusion (2% grade 1, 9% grade 2, <1% grade 3), which did not seem to impact efficacy. Grade 3/4 cytopenia rates with dasatinib vs imatinib were: anemia, 11% vs 7%; neutropenia, 22% vs 20%; and thrombocytopenia, 19% vs 10%. Most cytopenias (75%) occurred within 4 months of treatment. Grade 3/4 lab abnormality rates were ≤3% in both arms, except hypophosphatemia (dasatinib 5%, imatinib 24%). For dasatinib vs imatinib, 56% vs 39% had dose interruption, 25% vs 14% had dose reduction, and 6% vs 4% discontinued due to AEs. *Conclusions.* After 18 months, dasatinib continues to show significantly higher cCCyR and MMR rates over imatinib and remains generally well tolerated, supporting first-line dasatinib use in newly diagnosed CML-CP.

1012

TREATMENT OF CHRONIC PHASE (CP) CHRONIC MYELOID LEUKEMIA (CML) PATIENTS WHO HARBOR THE BCR-ABL T315I MUTATION WITH SUBCUTANEOUS OMACETAXINE RESULTS IN IMPROVED SURVIVAL COMPARED TO HISTORICAL DATA

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Background. Point mutations in the ABL kinase domain are emerging as the most frequent cause of drug resistance in CML with the specific T315I point mutation conferring the highest level of resistance to all approved TKIs. The T315I mutation is associated with disease progression and a poor prognosis. In the largest retrospective study of the natural history of T315I+ CML patients in CP, the median survival was only 22.4 months with a survival rate of 71% at one year from time of mutation detection (Nicolini *et al.*, 2009). New therapies, particularly ones that are independent of Bcr-Abl inhibitions are needed to provide a solution for this "Achilles Heel"in the TKI armamentarium and prolong survival in T315I patients. *Aim.* To evaluate the safety and efficacy of subcutaneously (SC) administered omacetaxine in imatinib-resistant T315I+ CML-CP patients. *Methods.* 62 adult CML-CP patients, who signed an informed consent, harboured the T315I mutation as confirmed by direct sequencing or DHPLC in a central laboratory, and demonstrated hematologic or cytogenetic resistance to imatinib therapy were enrolled in this study making it the largest prospective study in this patient population worldwide. Induction treatment con-

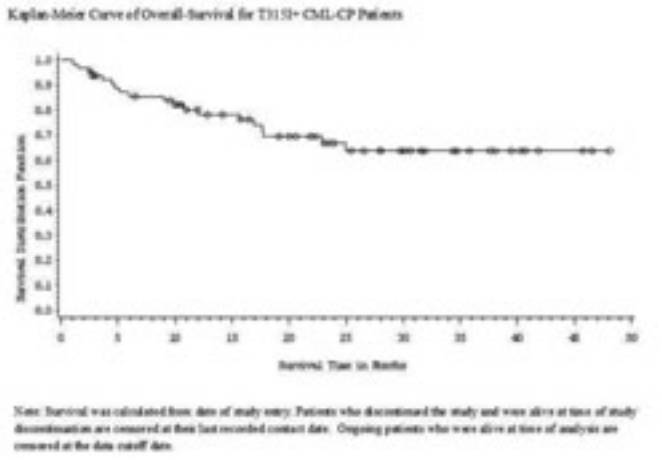

Figure 1. KM Curve of overall-survival.

sisted of 1.25 mg/m2 SC omacetaxine bid for 14-days every 28 days until hematologic improvement, followed by maintenance schedule of 1.25 mg/m2 SC omacetaxine bid for 7-days every 28 days and adjusted for tolerance. The primary efficacy endpoints were the achievement of complete hematologic response (CHR) and major cytogenetic response (MCyR) and a key secondary endpoint was median overall survival. *Results.* For the 62 CML-CP Patients enrolled the median age at entry was 59 yrs (26-83) with 69% male and a median disease duration of 51 months (13-234). All patients failed prior imatinib therapy, and 75% failed two or more prior TKIs (24% failed 3 or more TKIs). The median follow-up time was 19.1 months (1-48). CHR was achieved in 47 (76%) patients with a median duration of 8.9 months (1.5-43.6+) and MCyR was achieved in 24% patients (11 complete and 4 partial) with a median duration of 6.5 months (2.1-29+). At the latest follow-up the median overall survival time was not reached and the survival-rate at 24 months was 65.2% (Figure 1). Grade 3/4 related events occurred in 52/62 (84%) of patients. The most commonly reported events were thrombocytopenia (72%), anemia (44%) and neutropenia (36%). Non-hematologic toxicities were generally grade 1/2 with the most frequently reported; diarrhea (36%), pyrexia (27%), fatigue (25%), asthenia (25%), and nausea (24%). Treatment delays occurred in approximately 75% of the patients with median duration of 11 days (3-81). The primary causes of delay were; thrombocytopenia, neutropenia, pancytopenia and patient availability. Nine deaths occurred during the study. Three of these were considered to have a possible relationship to omacetaxine: sepsis, pancytopenia, and sudden death with unknown cause. *Summary.* Omacetaxine administration produces durable hematologic and cytogenetic responses with a safety profile primarily comprising of hematologic toxicities. The overall survival of T315I+ CML-CP patients treated with omacetaxine exceeds the survival reported in the literature.

1013

KIR2DS1 GENOTYPE IS ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO CML AND POOR RESPONSE TO IMATINIB

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Background. Killer immunoglobulin-like receptors (KIR), expressed on the surface of natural killer (NK) cells, play an important role in determining NK function and cytotoxicity. NK cells are important in tumour-cell killing and the inherited KIR-repertoire has been shown to influence tumour susceptibility. *Aims.* As NK cells can kill chronic myeloid leukaemia (CML) cells including the leukaemia-initiating-cell (LIC), we investigated if KIR-genotype influenced susceptibility to CML development and response of CML patients to treatment with imatinib. *Methods and Results.* Comparison of 190 CML patients in 1st chronic-phase (CP) to 161 healthy-controls revealed over-representation of KIR2DS1 in patients, 59% (121/190) vs 47.3% (69/161); OR 1.607 (P=0.03). We then investigated if KIR2DS1 genotype in CML patients predicts for response to imatinib treatment. NK cells are expanded in CML patients on tyrosine kinase inhibitors (TKI). We evaluated the impact of KIR genotype on the outcome of 166 patients with CML-CP receiving first-line imatinib treatment. KIR2DS1 positive patients had significantly lower 2-year probabilities of CCyR (82.3% and 65.6%, p=0.03), PFS (98.4% vs 91.0%, p=0.01 respectively) and OS (100% vs 92.6% p=0.03 respectively) than KIR2DS1 negative patients. Multivariate analysis revealed KIR2DS1 genotype (RR=0.66, p=0.03) and Sokal-risk-score (low RR=1, intermediate RR=0.65, p=0.04 and high RR=0.59, p=0.034) to be the only independent predictors for CCyR. Furthermore, KIR2DS1 was the only independent predictor for both PFS (P=0.02) and OS (P=0.03). The association between KIR2DS1 and outcome was validated in 174 CML-CP patients treated in the multi-center SPIRIT-1 trial. KIR2DS1+ patients (n=66) had a lower probability of achieving CCyR and lower PFS and OS than KIR2DS1- patients (n=106) (76.9% vs 87.9%, p=0.004, 85.3% vs 98.1% p=0.01 and 94.4% vs 100%, p=0.02 respectively). Again on multivariate analysis KIR2DS1 remained the only independent predictor for all three outcomes. Because KIR2DL1 interaction with group 2 HLA-C molecules on target cells could theoretically inhibit an activating signal mediated by KIR2DS1, we hypothesized that any effect of KIR2DS1 would be greatest among individuals who are missing group 2 HLA-C ligand for KIR2DL1. We determined the various combinatorial frequencies of KIR2DS1 with group 2 HLA-C alleles in the discovery plus the validation patients (n = 340). The impact of KIR2DS1 on CCyR was more significant when the ligand for the corresponding inhibitory receptor, KIR2DL1 was absent; the 2-yr CCyR rate for KIR2DS1-/KIR2DL1+/C2+, KIR2DS1+/KIR2DL1+/C2+ and KIRDS1+/KIR2DL1+/C2- patients were 98.6%, 94.7%, and 64.1% respectively (P=0.00006). The mechanism by which KIR2DS1 increases susceptibility and affects outcome in patients with CML-CP on imatinib is unclear. KIR2DS1+ve NK-cells secrete TGF-beta following ligand interaction, which can inhibits Akt signalling, a suppressor of FOXO3a, in CML-LIC. This may represent an important mechanism for imatinib resistance. Alternatively, KIR2DS1 may be simply a surrogate marker for genes directly involved in CML pathogenesis. *Conclusion.* In conclusion, our data demonstrate that KIR2DS1 is over-represented in patients with CML and may predict response to imatinib and identify those patients at greater risk of treatment failure.

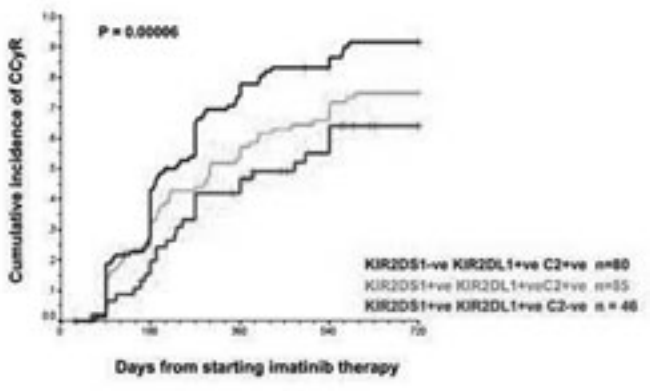

Figure 1. Association between KIR2DS1/KIR2DL1 and HLA-C geno.

1014

CESSATION OF DASATINIB OR NILOTINIB THERAPY IN CHRONIC-PHASE CHRONIC MYELOID LEUKAEMIA PATIENTS WITH SUSTAINED COMPLETE MOLECULAR RESPONSES

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Background. Despite the outstanding efficacy of tyrosine kinase inhibitors (TKI) in chronic myeloid leukaemia (CML), the curative potential of these drugs remains uncertain. Recent results from the STop IMatinib trial suggest that imatinib may be safely discontinued in pa-

tients with long-lasting complete molecular responses (CMR) (Mahon *et al.* Lancet Oncol. 2010). *Aims.* We asked whether second generation (2G)-TKI could ever be stopped in CML patients intolerant or resistant to imatinib who had achieved durable CMR. The primary objective was to evaluate the risk of loosing major molecular responses (MMR: BCR-ABL/ABL internationally standardized (IS) ratio ≤ 0.1%) and the key secondary objective to measure treatment-free survival. *Methods.* Patients aged at least 18 years with chronic phase (CP)-CML with prior imatinib treatment were proposed dasatinib or nilotinib discontinuation provided that (1) no prior progression to accelerated phase or blast crisis occurred (2) CMR was achieved and sustained on continuing therapy. CMR was defined by RQ-PCR, using a detection threshold of BCR-ABL of at least 4.5 Log (CMR4.5). After 2G-TKI discontinuation, BCR-ABL transcripts were quantified monthly during the first year and every 2 to 3 months thereafter. Dasatinib or nilotinib re-introduction was triggered by the loss of MMR which defined relapse. *Results.* As of February, 2011, 17 patients entered the study after informed consent. The results presented here focus on a subgroup of 12 patients with a minimum follow-up 6 months (median 12, range, 7-18). These were 7 females and 5 males, with a median age of 59 years (range, 34-81). The Sokal risk group was low in 8/12 (68%), intermediate in 1/12 (8%), high in 1/12 (8%) and unknown in 2/12 (16%). Imatinib was discontinued owing to intolerance (n=11) or resistance (n=1) and replaced by dasatinib (n=8) or nilotinib (n=4) after a median duration of therapy of 50 months (range, 3-92). At start of 2G-TKI, 1 patient had a complete hematologic response only, 2 had a partial cytogenetic response, 1 had a complete cytogenetic response but lacked MMR, 3 had a MMR without CMR and 5 had a CMR. The median time on 2G-TKI therapy prior to discontinuation was 33 months (range, 21-56). The median duration of sustained CMR was 29 months (range, 21-39). Subsequently, 30% (4/12) of patients lost MMR by 6 months. MMR was rapidly regained upon early 2G-TKI re-introduction. Treatment was also restarted in 1 patient without MMR loss but showing CMR loss on 2 consecutive assessments. Seven patients remained off therapy at the last follow-up after a median of 11 months (range, 7-18), with either a stable CMR or weakly detectable BCR-ABL transcripts on one or more occasions. Treatment-free survival rate by 6 months was estimated to 58.3 % (95%CI: 21.7-31.3). *Conclusion.* 2G-TKI may be safely discontinued in CML patients with stable CMR4.5. Importantly, a very low level of detectable residual disease after 2G-TKI withdrawal may be compatible with treatment-free survival. A longer follow-up is required to ascertain whether CML will recur. Our study provides a reasonable basis for subsequent prospective clinical trials. Updated results will be presented.

Acute myeloid leukemia - Clinical 2

1015

RISK OF HEMATOLOGICAL MALIGNANCIES AMONG FIRST-DEGREE RELATIVES OF PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) AND MYELODYSPLASTIC SYNDROMES (MDS) - A POPULATION-BASED STUDY IN SWEDEN

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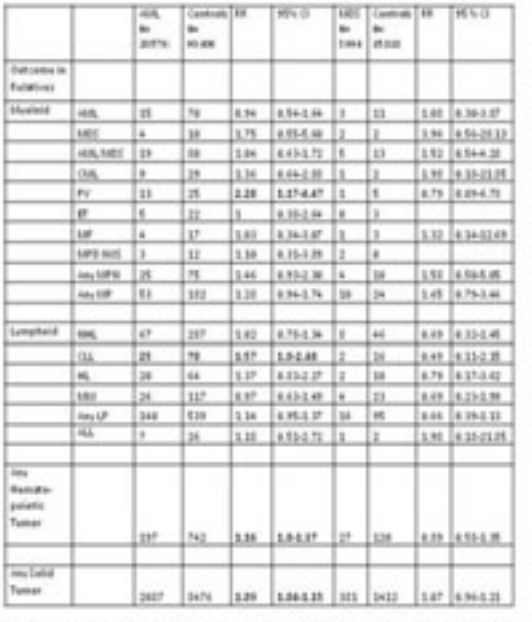
Background. Apart from rare pedigrees with multiple cases of AML there is limited data on the extent of familial aggregation in AML and MDS in the population. In a comprehensive population-based study, we estimated risk of AML, MDS, other hematological malignancies and solid tumors combined among first-degree relatives of AML and MDS patients compared to first-degree relatives of matched controls. *Design and Methods.* Swedish population-based registry data were used to evaluate outcomes in 20,579 first-degree relatives of 6,962 AML patients (diagnosed 1958-2004; median age 64 years) and 3,994 first-degree relatives of 1,388 MDS patients (diagnosed 1993-2004; median age 76 years) compared with 90,406 first-degree relatives of 37,384 and 15,818 first-degree relatives of 6,489 population-based matched controls for AML and MDS patients, respectively. Using a marginal survival model, we calculated relative risks (RR) and 95% confidence intervals (CI) as measures of familial aggregation. *Results.* AML and/or MDS did not aggregate significantly in relatives of patients of AML, MDS or in the combined group (Table). The risk of polycythemia vera (PV) was significantly higher in relatives of AML patients compared to controls but the increased risk of myeloproliferative neoplasms as a group did not reach statistical significance. Lymphoid malignancies showed some increased risk in relatives of AML patients (with a borderline significant increased risk of chronic lymphocytic leukaemia; CLL) but not in relatives of MDS patients. The global risks for any hematopoietic or solid tumor were significantly but modestly increased among relatives of AML patients. When analysing risks in relatives of younger patients (≤ 20 years at diagnosis) with AML a significantly increased risk (RR 7.53; CI 1.25-45.13) of AML/MDS and a 3.01-fold RR (CI 1.09-8.31) for all myeloid malignancies combined was ob-

Table 1.

Risk of Myeloid, Lymphoid, and Solid Malignancies in Relatives of AML and MDS cases



CML chronic myeloid leukaemia, PV polycythemia vera, ET essential thrombocythemia, MF myelofibrosis, MPD NOS unspecified myeloproliferative disorder, MPN myeloproliferative neoplasm (PV_ET_MF_MPD NOS), Myeloproliferative tumors (MP: AML, MDS, CML, MPN)

NHL Non-Hodgkin lymphoma, CLL chronic lymphocytic leukaemia, HL Hodgkin lymphoma, MM multiple myeloma, LP lymphoproliferative tumor (NHL, CLL, HL, MM), ALL acute lymphoblastic leukaemia

served. Among lymphoid malignancies, there was a significantly increased risk of multiple myeloma (RR 5.02; CI 1.25-20.11) but the numbers are small. All hematopoietic cancers combined were significantly increased (RR 1.85; CI 1.07-3.18) as well as all solid tumors combined (RR 1.27; CI 1.05-1.52). In general, despite the small sample size of younger patients, the relative risks were higher in this group than among all AML patients. *Conclusions.* The lack of familial aggregation of AML or MDS is striking and in sharp contrast to findings in patients with other myelo- and lymphoproliferative disorders. However, relatives of young patients do seem to be at increased risk of AML/MDS and other hematopoetic malignancies suggesting that they share a genetic susceptibility. Interestingly there is an increased risk of PV and small but significantly increased risks of any hematopoietic or solid cancer among relatives to AML patients. The results are important since many patients worry about a potentially increased risk of their family members to develop AML or MDS and many clinicians are of the opinion that there is a small but significant familial pathogenetic component. The increased risk of PV, any hematopoietic, any solid tumor and CLL among relatives of AML patients may point to the existence of broadly shared germ line susceptibility genes and/or environmental factors.

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INTEGRATIVE PROGNOSTIC RISK SCORE IN ACUTE MYELOID LEUKEMIA PATIENTS WITH NORMAL KARYOTYPE AGED 18-60 YEARS

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Background. Prognostic risk factors have been described in AML including cytogenetics, WBC count, and response to induction chemotherapy and have been used extensively to stratify treatment. Patients with AML and normal karyotype (CN-AML) have usually been classified as intermediate risk. Many efforts have been made to identify genetic mutations (e.g. FLT3, NPM1, CEBPA, MLL, NRAS, IDH1/2, and WT1) that allow further sub-classification of CN-AML patients and possibly risk-directed therapeutic intervention. In addition to mutations, modulated expression of genes important for proliferation, survival, and differentiation have also been shown to be predictive for CN-AML patient outcome (e.g. MN1, BAALC, ERG, ID1, WT1). This molecular heterogeneity of CN-AML is not fully reflected in current classification systems. *Aims.* To integrate available clinical and molecular information for CN-AML patients into one risk score. *Patients and Methods.* 275 CN-AML patients from multicenter treatment trials AML SHG Hannover 0199 and 0295 were evaluated for mutations/polymorphisms in NPM1, FLT3, CEBPA, MLL, NRAS, IDH1/2 and WT1. Transcript levels were quantified for BAALC, ERG, EVI1, ID1, MN1, PRAME, and WT1. As an external control, 131 CN-AML patients from HOVON/SAKK protocols were analyzed for all molecular markers included in the integrative prognostic risk score (IPRS). *Results.* The IPRS was modelled in 181 CN-AML patients to represent patients with low, intermediate, and high risk of death. Complete remission rate (CR, P=.005), relapse-free survival (RFS, P<.001), and overall survival (OS, P<.001) were significantly different for the three risk groups. In two independent validation cohorts of 94 and 131 patients, the IPRS predicted different OS (P<.001) and RFS (P<.001). The value of allogeneic stem cell transplantation in first CR was evaluated in all 225 evaluable patients from the AML SHG trials. High-risk group patients with related donor had longer OS (P=.016) and RFS (P=.026) compared to patients without related donor. In contrast, intermediate-risk group patients with related donor had shorter OS (P=.003) and RFS (P=.05). Donor availability had no impact on the outcome of patients in the low-risk group. *Conclusion.* The IPRS may improve consolidation treatment stratification in CN-AML patients. This weighted prognostic risk score of clinical and molecular prognostic markers in younger CN-AML patients may become useful for outcome prediction of currently available consolidation treatment options. This score may be expanded when new markers are discovered, and it may be used to evaluate the efficacy of novel drug treatments in specific subsets of AML patients.

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ASXL1 MUTATIONS IN ACUTE MYELOID LEUKEMIA: RESULTS ON 799 PATIENTS TREATED WITHIN THE AML STUDY GROUP (AMLSG)

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Background. The *ASXL1* (*Additional Sex Comb-Like 1*) gene on chromosome 20q11.1 encodes a protein believed to be involved in chromatin modification and to act as a co-activator for the retinoic acid receptor. *ASXL1* mutations occur with high incidence in CMML (~40%) and less frequently in AML, CML, MDS and myeloproliferative neoplasia. They cluster in exon 12 and are mainly frameshift mutations creating a premature stop codon. A recent AML study reported on unfavorable impact of *ASXL1* mutations on induction success and overall survival (OS) (Chou *et al.*, Blood 2010;116:4086-94). *Aims.* To analyze the incidence, clinical, cytogenetic and molecular features of *ASXL1* mutations in younger (18-60 years) AML patients and to assess their prognostic relevance. *Methods.* Diagnostic samples from 799 patients who were intensively treated on AMLSG trials [AML HD98A (n=729), APL HD95 (n=70)] were analyzed for the presence of *ASXL1* mutations. Hot spots of *ASXL1* exon 12 were screened for mutations using GeneScan fragment analysis of PCR products followed by direct sequencing. Patients were also assessed for the presence of *NPM1*, *FLT3* (ITD, TKD), *CEBPA*, *IDH1/2*, *RUNX1* and *TET2* mutations by standard PCR-based methods. *Results. ASXL1* mutations were detected in 54 (6.8%) of 799 pts. All mutations were heterozygous. The most frequent mutation (38/54; 70%) was an insertion of guanine (c.1934dupG; p.G646WfsX12), which is concordant with the study by Chou *et al.*; all other frameshift mutations also created a premature stop codon. *ASXL1* mutations were found to be more frequent in older patients (P=.001), in males (P=.02), and in secondary AML (P<.001). Patients with *ASXL1* mutations showed lower values for WBC (P=.02), bone marrow blasts (P=.03) and LDH (P=.02). *ASXL1* mutations were not detected in AML with inv(16), and appeared to be less frequent in AML with t(15;17) or t(8;21). *ASXL1* mutations were more frequently associated with *RUNX1* (P<.001) and *IDH* (P=.002) mutations, but inversely correlated with *NPM1* mutations (P<.001). The median follow-up for survival was 6.5 years. Patients with an *ASXL1* mutation tended to have an inferior complete remission rate compared with patients exhibiting *ASXL1* wildtype (61% vs 72%; P=.09); the same was true for the subset of cytogenetically normal (CN) AML (56% vs 74%; P=.06). *ASXL1* mutations did not impact on relapse-free-survival in the entire AML cohort, and in CN-AML. However, in the entire cohort there was a trend towards a shorter OS in patients with an *ASXL1* mutation compared to those with *ASXL1* wildtype (P=.06; 5-year OS rates, 29% vs 41%). The adverse impact of *ASXL1* mutations on OS was more pronounced in CN-AML (P=.05; 5-year OS rates, 24% vs 41%). *Conclusions. ASXL1* mutations are detected in almost 7% of younger AML patients. They are associated with *RUNX1* and *IDH* mutations, and are less frequent in patients with *NPM1* mutation. The potential unfavorable prognostic impact of *ASXL1* mutations in AML still requires confirmation in further studies. Due to the involvement of the ASXL1 protein in epigenetic regulation and retinoic acid pathway *ASXL1* mutations should be evaluated in the context of epigenetic or ATRA-containing therapeutic approaches in AML.

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PRE-TREATMENT ASSESSMENT IN ELDERLY AML PATIENTS: ACCURACY OF PROGNOSTIC PREDICTIONS BY THE TREATING HEMATOLOGIST IN 343 PATIENTS FROM THE AML 60PLUS TRIAL

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Background. Most patients diagnosed with acute myeloid leukemia (AML) are older than 60 years. Response rates and long-term survival in this large group of AML patients are worse than in younger patients. In order to facilitate the process of decision making between intensive curative and palliative treatment options, a clinical and/or geriatric assessment is of particular importance in this patient group. Several scores focusing on laboratory organ function, vital signs or comorbidities have been proposed. Obstacles to a widely accepted standard clinical use are issues such as prospective validity, generalizability, and questions on which assessment system to use or which scores to combine. We prospectively evaluated the value of an simple prognostic judgment which relied on the clinical experience of the treating physician and is very easy to obtain. *Aims.* To prospectively assess the prognostic value of a clinical judgment score obtained at initial AML diagnosis by the treating hematologist in a large cohort of elderly patients treated in the AML60plus trial. *Methods.* All patients included in the AML60plus trial were categorized into three prognostic groups by the treating hematologist. Prognostic assessment was performed at initial diagnosis before the start of treatment. On the basis of comorbidities and the general clinical impression, the treating physician had to make a judgment regarding the probability of disease cure and survival in relation to the age-specific standard patient. The corresponding categories were "standard"(0), "reduced probability of cure"(-1), and "increased probability of cure"(+1). All patients received intensive induction and consolidation treatment according to the 60plus trial protocol. The potential influence of the clinical assessment scores on overall survival (OS) was assessed in univariate and multivariate analyses. *Results.* Pre-treatment assessments were available for 343 patients. Percentages of patients assigned to the clinical prognostic groups -1, 0, and +1 were 18%, 69%, and 13%, respectively. Survival analyses showed significant differences between the three prognostic groups with a median OS of 5.6 months, 10.7 months, and 14.8 months, respectively. In order to adjust for the influence of established biologic prognostic factors, we performed a multivariate Cox regression model for OS including the variables age, cytogenetic risk, WBC, LDH, NPM, FLT3-ITD, de-novo versus secondary AML, and ECOG status. Even after adjustment for these variables, the clinical judgment "increased probability of cure"remained of significant influence on OS, translating into a hazard ratio of 0.47 for death of any cause compared to the average prognosis. *Summary/Conclusions.* These are the first published prospective data demonstrating the additional prognostic value of clinical judgment in AML. The fact that the clinical prognostic score kept its significant influence on OS even after accounting for established biologic factors emphasizes the importance of clinical assessment and its ability to grasp integrated clinical prognostic information beyond clearly defined biologic parameters. Based on these results, physicians should be encouraged to include their clinical judgment in the shared decision making process for intensive curative versus palliative treatment options in elderly AML patients.

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A PHASE II OPEN-LABEL, AC220 MONOTHERAPY EFFICACY (ACE) STUDY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) WITH FLT3-ITD ACTIVATING MUTATIONS: INTERIM RESULTS

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Background. FLT3-ITD mutations in AML are associated with poor survival and high relapse rates after standard chemotherapy. Quizartinib (AC220) is a potent, narrow-spectrum, oral FLT3 tyrosine kinase inhibitor that showed preliminary activity in FLT3-ITD+ patients (pts) during Phase I testing. *Aims.* This is an ongoing, Phase II study to determine the efficacy of quizartinib monotherapy in pts with relapsed/refractory FLT3-ITD+ AML. *Methods.* A planned interim analysis was performed for the first 62 pts: 25 pts in Cohort 1 (≥60 yrs and relapsed/refractory to 1st-line chemotherapy) and 37 pts in Cohort 2 (≥18 yrs and relapsed/refractory to 2nd-line chemotherapy or HSCT). All pts gave informed consent. *Results.* 62 pts (29 female, 33 male) with a median age of 59 yrs (21-86) received quizartinib. The most common drug-related AEs were nausea, QTc prolongation, vomiting, fatigue, dysgeusia, anorexia, febrile neutropenia, diarrhea, and dyspepsia. Drug-related SAEs in >15% of pts were febrile neutropenia and asymptomatic Grade 3 QTc prolongation. QTc prolongation occurred in 21 pts (Grade 3 in 12 pts). The incidence of QTc prolongation was decreased by reducing AC220 starting dose from 200 to 135 mg/day (males) and 90 mg/day (females). 4 pts discontinued due to AEs other than AML or progressive disease (febrile neutropenia, hyperbilirubinemia, bacterial sepsis, and dehydration; all considered not drug-related except Grade 4 hyperbilirubinemia). 14 pts (23%) experienced fatal AEs; none were considered drug-related. 53/62 (85%) pts were evaluable for efficacy (FLT3-ITD+ by central laboratory, received at least 1 cycle of quizartinib, and no major efficacy-related protocol deviations). The composite CR (CRc=CR+CRp+CRi) rate was 43% (23/53: 1 CRp, 22 CRi) and PR rate was 28% (15/53). Cohort 1 had a CRc rate of 36% (8/22), and Cohort 2 had a CRc rate of 48% (15/31). Starting dose did not affect response. Of the pts refractory to any prior therapy, 56% (15/27) had CRc and 22% (6/27) had PR in response to quizartinib treatment. Median duration of CRc has not yet been reached in Cohort 1 and was 10.6 wks in Cohort 2. Overall, 9% (2/22) of pts in Cohort 1 and 39% (12/31) of pts in Cohort 2 were bridged to HSCT and censored for duration of response at that time. Median overall survival was 20.0 wks in Cohort 1 and 24.4 wks in Cohort 2. *Conclusions.* These preliminary data suggest that quizartinib achieves clinically meaningful reductions in marrow blasts in a substantial proportion of pts with both refractory and relapsed FLT3-ITD+ AML, and many of these pts were successfully bridged to HSCT. These encouraging efficacy results and an acceptable safety profile in this high risk population support continued clinical evaluation.

Myeloproliferative disorders - Clinical

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A RANDOMIZED STUDY OF JAK INHIBITOR RUXOLITINIB (INC424) VS BEST AVAILABLE THERAPY IN PRIMARY MYELOFIBROSIS (MF), POST-POLYCYTHEMIA VERA-MF OR POST-ESSENTIAL THROMBOCYTHEMIA MF

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Background. Dysregulated JAK-STAT signaling is a key feature of myelofibrosis (MF), a myeloproliferative neoplasm associated with splenomegaly, constitutional symptoms and shortened survival. Overall median survival is 2.25-4 years for high- and intermediate risk-2 patients, respectively as defined by IWG-MRT criteria (Cervantes *et al.*, Blood 2009). Approximately half of MF patients carry a gain-of-function mutation in the Janus kinase (JAK) 2 gene (JAK2V617F); dysregulation of the JAK pathway occurs regardless of JAK2V617F status and contributes to disease pathophysiology. There are currently no effective drug therapies for MF. Ruxolitinib is a selective JAK1 and JAK2 inhibitor with clinical activity in MF. *Aims.* COMFORT-II, a randomized (2:1), open-label, phase 3 study measured the efficacy, safety, and tolerability of ruxolitinib given twice daily compared to best available therapy (BAT), which could include no therapy or other agents, in adult patients with high- or intermediate risk-2 primary MF, post polycythemia vera MF or post essential thrombocythemia MF, with palpable splenomegaly. The primary efficacy endpoint is the proportion of subjects achieving ≥ 35% reduction in spleen volume from baseline to week 48 as determined by magnetic resonance imaging (MRI) or computed tomography (CT) and analyzed using the Cochran-Mantel-Haenszel test stratified by baseline risk category. For the 219 subjects enrolled, the power of two-sided CMH test with alpha level of 0.05 would be 93.7% assuming that the ratio of subjects with baseline prognostic category of intermediate- vs. high-risk was 1:1, response rate for intermediate-risk for active and control treatment groups was 40% and 15%, respectively, and for high-risk 30% and 5%, respectively. The key secondary endpoint is the proportion of subjects achieving a ≥ 35% reduction of spleen volume (by MRI or CT) from baseline to week 24, while other secondary endpoints include duration of maintenance of reduction in spleen volume, time to achieve a first ≥ 35% reduction, progression-free survival, leukemia-free survival, overall survival and change in bone marrow histomorphology. Patients were enrolled at 56 sites in Europe and the UK from July 2009 until January 2010. Primary analysis will occur in March 2011. *Results.* A total of 219 patients were randomized; 146 to ruxolitinib and 73 to BAT. Results for the primary and secondary endpoints will be reported. A summary of the most common adverse events in the ruxolitinib vs BAT arms will be reported as will the rate of treatment discontinuation. *Summary/Conclusions.* The results of COMFORT-II will provide important information that may result in a new standard of care for a large number of patients with myelofibrosis. A companion study, COMFORT-I, has met its primary and symptom assessment secondary endpoints. COMFORT-I enrolled 309 patients with similar eligibility criteria from the US, Canada, and Australia, and compared ruxolitinib to placebo with a primary endpoint of response defined as the percentage of patients achieving 35% or greater reduction in spleen volume at 24 weeks. It will be important to compare the results of these 2 studies that differ in their comparator arm and the timing of response assessment.

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EZH2 MUTATIONAL STATUS PREDICTS POOR SURVIVAL IN MYELOFIBROSIS

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Background. Inactivating mutations of EZH2 (chr7q36) have been described in patients with myeloproliferative neoplasms (MPN), mainly primary myelofibrosis (PMF), myelodysplastic-MDS/MPN neoplasms (Ernst *et al.*, Nat Gen 2010) and MDS (Nikoloski *et al.*, Nat Gen 2010). EZH2 encodes a histone H3 Lys27 methyltransferase that is the catalytic unit of the polycomb repressor complex-2. EZH2 functions as a transcriptional repressor involved in gene silencing, and may behave as tumor suppressor or oncogene depending on the cellular context. *Aims*. To define the epidemiology and clinical correlates of EZH2 mutations in a large population of MF patients. *Methods*. The study population included 518 patients with MF: 370 PMF, 84 post-polycythemia vera myelofibrosis (PPV-MF), 64 post-essential thrombocythemia myelofibrosis (PET-MF). Sixty-two percent of the patients were JAK2V617F-positive, 4% were MPLW515L/K mutated. High-resolution melting (HRM) analysis was used to screen DNA in whole genome amplified samples from granulocytes. Products showing abnormal melt patterns were directly sequenced from genomic DNA. Whenever possible, sequencing of buccal swab samples was performed concurrently to rule out rare SNPs. Mutational status was correlated with baseline hematological and clinical characteristics. *Results.* Overall, 23 different EZH2 mutations were detected in 30 patients (6%); 23 patients had PMF (6% of all PMF) and 7 had PPV/PET-MF (5%). Nine mutations were located in SET domain, 5 in CXC domain, 6 and 4 in the D1 and D2 domain, respectively. Most exonic mutations were heterozygous missense changes caused by single nucleotide substitution, while 3 (10%) were homozygous; one patient presented two mutations. Four intronic mutations were identified, all in putative splicing sites. Forty-three percent of EZH2 mutated patients were JAK2 V617F-positive, none was MPL mutated; median V617F allele burden was 47±30% in EZH2/JAK2V617Fpos vs 53±25% in EZH2 wild-type/JAK2V617Fpos. No mutation in IDH1/2 was found in EZH2 mutated patients, while 50% of them harbored ASXL1 mutation. There were more EZH2 mutated patients in IWG-MRT high-risk category (19.6%) than low-risk (5.4%) (P=0.03). EZH2-mutated patients had significantly higher leukocyte (P=.02) or >1% blast cell count (P=.003) at diagnosis. There was no correlation of EZH2 mutation with age, gender, other hematologic characteristics at diagnosis including splenomegaly, constitutional symptoms and rate of leukemic transformation; the latter occurred in 81 patients, 28.6% and 17.8% of the EZH2-mutated or wild-type, respectively. After a median follow-up of 38 months, 128 patients (25.9%) died. Survival was influenced by IWG-MRT risk categories, age, leukocytosis, hemoglobin, platelet and blast count, constitutional symptoms, leukemia transformation, low JAK2V617F allele burden and EZH2 mutation. The overall median survival (OS) was significantly reduced in EZH2 mutated patients (25 mo, range 0-183) compared to wild-type (39 mo, 0-340; P=.001). In multivariate analysis OS was predicted by IWG-MRT high-risk category (P<.0001), a <25% JAK2V617F allele burden (P=0.04), and EZH2 mutated status (P=0.002). *Conclusions.* EZH2 mutations occur in 6% of patients with primary and secondary forms of myelofibrosis and are associated with shorter survival independent of other known risk factors including IWG-MRT score. (PG, FB, JS, NCPC, AMV equally contributed) (Supported by AIRC, Milano to AGIMM; Leukaemia and Lymphoma Research to N.C.P.C.)

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PHASE 2 STUDY OF SB1518, A NOVEL ORAL JAK2 INHIBITOR, IN PATIENTS WITH PRIMARY, POST-POLYCYTHEMIA VERA, AND POST-ESSENTIAL THROMBOCYTHEMIA MYELOFIBROSIS

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Background. The $JAK2^{V617F}$ mutant enzyme has been linked to the pathogenesis of myeloproliferative neoplasms. SB1518 is a potent inhibitor of JAK2 and its $JAK2^{V617F}$ mutant. In previously reported Phase 1 studies, patients with myelofibrosis (MF) who received SB1518 achieved steady-state plasma levels above the IC50 for JAK2 at all doses (100-600 mg/d). Target inhibition, measured by phosphorylation of the STAT3, STAT5, and JAK proteins in PBMCs and whole blood, was also shown at all doses. 400 mg/d was chosen as the recommended dose (RD) for Phase 2 study. *Aim.* To assess the spleen response rate, defined as a ≥35% reduction in MRI-measured spleen volume between baseline and Week 24. *Methods.* Qualifying patients had primary, post-ET, or post-PV MF, palpable splenomegaly ≥5 cm below the left costal margin, and were not suitable for standard therapy. Based on minimal myelosuppression in the Phase 1 trial, no minimal hematology values were required for study inclusion. Each patient received daily oral SB1518 in ongoing 28 day cycles. Disease symptoms were evaluated using the MF-SAF. *Results.* Patients: In this ongoing trial, thirty-four MF patients (median age 58.5; range, 44-84 years) were consented and enrolled, of which 25 (74%) were men. Median baseline platelet count was 120,000/μL (range, 15,000-859,000/μL). Fifteen patients (44%) had platelet counts <100,000/μL, including seven <50,000/μL. Twenty-eight patients (82%) were $JAK2^{V617F}$ mutation-positive. Median time on study is 6.0+ months (range, 0.5 to 11.1 months). Safety: Seven patients (21%) discontinued SB1518 due to AEs. Seven patients required dose reduction, all within the first 6 months. The most common treatment-related AEs were gastrointestinal, which were generally low grade and easily managed. GI AE's >Grade 2 included only Grade 3 diarrhea in 2 patients (6%). Efficacy: SB1518 produced meaningful reductions in splenomegaly. Thirty patients (88%) showed reductions in palpable splenomegaly; 15 (44%) showed decreases of ≥50% and seven (21%) showed a reduction of 100%. At Week 24, 23 patients (68%) showed spleen reduction (3% to 50% by MRI volumetric assessment). Nine patients (26%) had reduction in splenomegaly by ≥35% reduction. Splenic reduction of 50% by PE correlated with a 25% reduction in spleen volume by MRI. Twelve patients (35%) had reduction in splenomegaly by >25%. Spleen response rates were as high among patients with low baseline platelet counts as those with normal baseline counts. Responses were durable; response duration among those achieving an IWG MRT response ranged from 1 to 164+ days (median, not reached). Two patients met IWG-MRT criteria for clinical improvement in hemoglobin and 1 for platelet count. At the 6 month visit, a significant reduction (>2 point improvement) was observed for MF associated symptoms, including abdominal pain, cough, and night sweats. *Conclusions.* SB1518 shows promising efficacy in alleviating MF-associated splenomegaly and constitutional symptoms at a dose that induces minimal myelosuppression. Once-daily dosing is well tolerated, with manageable GI toxicity as the main side effect. Given the lack of myelosuppression with SB1518, this JAK2 inhibitor is of particular importance for MF patients with impaired hematopoiesis.

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A PHASE II STUDY OF VORINOSTAT (MK-0683) IN PATIENTS WITH POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

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Background. Conventional agents used in the management of polycythemia vera (PV) and essential thrombocythemia (ET) include hydroxyurea and in younger patients alpha-interferon or anagrelide as useful alternatives. Since all of these agents have side effects besides potential leukemogenic potential for hydroxyurea several clinical trials have been conducted to find alternative and better drug formulations. Histone deacetylase inhibition (HDACi) has been shown to impair the autonomous proliferation of haematopoietic cells of PV and ET patients carrying the JAK2 V617F mutation. *Aims*. The present study evaluates the efficacy and safety of vorinostat in the treatment of PV and ET in a non-randomized, open-label ongoing phase II study. *Methods*. Fifty-nine pts. (17 ET, 39 PV) from whom informed consent was obtained from Denmark, Sweden, Holland and the UK were included and given 400 mg of vorinostat daily for 6 months. *Results*. We report preliminary data for 59 pts, 70 % PV (m:f=53%/47%), 30 % ET (m:f=38%/62%) with a median follow-up from start of vorinostat of 8 weeks (range=0-36, IQR=4,28). Median age at inclusion was 64 years (29-82) (PV=65 (29-82), ET = 62 (50-77)). Period between diagnosis and inclusion was 2.8 years (0-27,4) (PV= 3,1 (0-27,4), ET=2,2 (0,01-22,6)). Treatment prior to vorinostat included hydroxyurea (53%) (PV=43%, ET=75%), interferon -alpha (8%) (PV=11%, ET=0%), anagrelide (12%) (PV=6%, ET=27%) and busulfan (4%) (PV=3%, ET=8%). Of the 14 patients (8 PV, 6 ET) evaluable for clinicohaematological response after visit 11 (completion of protocol), 4 achieved complete response (CR), 6 achieved partial response (PR) and 4 achieved no response (NR) identifying a response-rate of over 70%. Adverse effects (AE's) reported at visit 11 were all grade 1 and included constipation (1 pt), weight loss (1 pt.), fatigue (1 pt.), hyperglycemia (1 pt.) and mucositis (1 pt.) The most common AE's during the treatment period were gastrointestinal (anorexia, nausea, vomiting, diarrhea) typically grade 1/2, manageable and improving within months. Seventy-eight percent experienced hair loss, 1 pt. grade 3. Fourteen percent experienced renal toxicity (grade 1/2) and 7% liver toxicity of unknown grade. Other grade 3/4 non-hematologic toxicities were anorexia (14%), nausea (7%), diarrhea (7%), fatigue (7%), dry mouth (7%). Fifty-seven percent required at least one dose reduction. An additional 11 pts. dropped out before visit 11 due to AE's (no: 4), serious AE's (no: 4) and unknown (no: 3). Serious AE's included 1 pt. with a deep vein thrombosis, 2 pts. with thrombocytopenia (1 grade 3, 1 unknown) and 1 pt. due to renal toxicity. Of the 11 pts. 2 achieved CR, 2 achieved PR, 6 achieved NR as best response before dropping out of protocol. Further up-dating will be at EHA. *Conclusion and perspectives*. Vorinostat is effective in PV and ET patients. Clinical responses are achieved in a high proportion of patients adhering to therapy. After months of treatment vorinostat is generally well-tolerated with minimal AE's and hematologic toxicity. An early drop-out rate of 44% draws attention to side effects which in future trials may be diminished by dose reduction or combination therapy (eg. hydroxyurea, interferon-alpha or JAK2-inhibitors).

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ASXL1 MUTATIONS IN PATIENTS WITH MYELOFIBROSIS. EPIDEMIOLOGY AND CLINICAL CORRELATIONS

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Background. Additional sex comb-like 1 (ASXL1) gene (chr 20q11.1) belongs to the Enhancer of Trithorax and Polycomb gene family. It functions as dual transcriptional activator/suppressor including repression of retinoic acid receptor-mediated transcription. Mutations in ASXL1 were recently demonstrated in a spectrum of chronic- and blast-phase myeloproliferative neoplasms (MPN). *Aims.* The current study seeks to determine ASXL1 mutational frequency and clinical correlates in a large series of patients with myelofibrosis (MF). *Methods.* We investigated ASXL1 mutational status of 230 patients with MF including 166 PMF, 36 PPV and 28 PET. Somatic mutations of ASXL1 were identified by sequencing exon 12 of whole-genome amplified DNA isolated from granulocytes; all mutations were validated by re-sequencing genomic DNA from the archival sample. Mutational status was correlated with clinical and biological features. *Results.* At the time of writing, results were fully available for 117 of 230 patients; details of the whole series will be presented at the meeting. A total of 21 different mutations were identified in 36 patients (31%); 24 out of 64 with PMF (38%) and 12 out of 53 (23%) with PPV/PET-MF. All mutations were heterozygous deletions, missense or nonsense mutations presumed to truncate the plant homeodomain finger domain. Sixty-four percent of ASXL1 mutated patients were JAK2 V617F-positive and one patient was MPLW515L mutated. The frequency of ASXL1 mutations was similar in JAK2V617Fpos (30%) and JAK2V617Fneg (32%) cases. Median V617F allele burden was 48.6±26.7% in ASXL1/JAK2V617Fpos vs 57.9±24.4% in ASXL1wild-type/JAK2V617Fpos patients. Screening of EZH2 and IDH1/2 mutations in the same cohort showed that ASXL1, EZH2 and IDH1/2 are not mutually exclusive events. In fact, mutations of EZH2 and ASXL1 were simultaneously present in 6 cases. IDH1/2 mutations were found in 3 cases, two of whom showed concomitant ASXL1 mutation. ASXL1/IDH1-2 mutations were detected at chronic phase in both patients who later developed acute leukemia. Seven patients showed ASXL1 mutation as the sole molecular abnormality. There was no significant difference between mutated and unmutated patients as concerned age, sex distribution, clinical characteristics, leukemia transformation and overall survival. However, ASXL1 mutations were found to be preferentially associated with an abnormal karyotype; the latter occurred in 10 of 17 mutated patients (59%) compared to 7 of 29 unmutated patients (24%) (P=0.02). *Conclusions.* Preliminary data in 117 patients with MF analyzed for ASXL1 mutations discovered a high mutational rate in both primary and post-PV/post-ET MF. ASXL1 mutations did not associate with an unique phenotype nor deserved a detrimental effect on survival. However, the high frequency (32%) detected in JAK2V617F-negative subjects suggests that ASXL1 genotyping may be of help in the routine diagnostic path in MF patients. (A project of AGIMM supported by AIRC, Italy)

Myelodysplastic syndromes - Biology

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WHOLE EXOME ANALYSIS OF MYELODYSPLASTIC SYNDROMES

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Background. Myelodysplastic syndromes (MDS) are heterogeneous groups of myeloid neoplasms characterized by multi-lineage cytopenias of varying degrees and transition to acute myeloid leukemia (AML). At present, no curative therapeutics for MDS has been established except for allogeneic hematopoietic stem-cell transplantation, which is not applicable to the majority of the MDS patients due to their higher ages. Thus, to improve the outcome of MDS, it is essential to develop novel therapeutic agents with both high efficacy and low toxicity, and to this goal, the discovery of the key molecules for MDS pathogenesis is of particular importance. To date, a number of gene mutations have been identified and implicated in the pathogenesis of MDS, including NRAS, TP53, RUNX1, c-CBL, TET2, ASXL1, and more recently, IDH1, IDH2 and EZH2. However, in view of therapeutic targets, our current knowledge of disease causing mutations in MDS is still incomplete. Recently, high-throughput sequencing technologies have been shown to be effective for the identification of disease-related gene and been successfully used to determine the genetic basis of some neoplastic disorders, such as AML and diffuse large B-cell lymphoma. More recently, the resequencing technology targeted for all protein-coding subsequences (i.e., whole exome analysis) has enabled cost-effective comprehensive mutation analysis of coding sequences. *Aims.* In this study, to obtain a complete registry of genetic lesions in MDS and to identify novel therapeutic targets, we performed whole exome analysis for novel mutations using high-throughput sequencing technologies, combined with large-scale screening of mutations in candidate genes using barcode-labeled DNA for a panel of ~180 MDS samples. *Methods.* Whole exome analysis was performed for 20 MDS samples, where entire exon sequences were enriched by using SureSelect Human All Exon kit (Agilent Technologies) and were subjected to resequencing analysis using Genome Analizer IIx (Illumina). *Results.* More than 60% of mapped reads contained exon sequences. > 80% of exons were sequenced at the depth of >20 with average fold-coverage of >50 times. Given that the constitutive genomic DNA was difficult to obtain in MDS patients, paired CD3-positive T cells were used as a normal control. By comparing sequences in tumors and paired T cells, nearly 200 somatic mutations and 10 insertions-deletions were detected in the whole exome analysis. Novel gene targets were also explored by resequencing barcord-labeled DNAs from 180 MDS specimens, which targeted 80 candidate genes for MDS. A number of mutations were identified, including those in IDH2, ASXL1, TET2, EZH2 and novel target genes such as PHF6, which has been reported to be mutated in T-ALL previously, and other genes which have not been reported to mutated in human cancers. Nearly 3 mutations per sample were detected and multistep model of oncogenesis in MDS were revealed. *Summary/Conclusions.* Our results suggested that target-capture resequencing technology is a powerful method to identify new gene mutations that are implicated in the pathogenesis of MDS.

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THE CLONAL ADVANTAGE OF DEL(5Q) MDS STEM CELLS IS MEDIATED BY INCREASED ADHESION TO THE MICROENVIRONMENT

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Background. The myelodysplastic syndromes (MDS) constitute a heterogenous group of clonal disorders of hematopoietic stem cells (HSC) leading to ineffective hematopoiesis in one or more lineages in the bone marrow. The most frequent cytogenetic entity is the 5q-syndrome, characterized by loss of the 5q31-33 region. Lenalidomide has emerged as a very effective therapy for this subgroup of patients. Its mechanism of action, however, has hitherto remained elusive. Interestingly, the more primitive hematopoietic compartment seems to possess a clonal advantage where del(5q) HSC are able to outcompete non-5q HSC. We have previously demonstrated that lenalidomide is able to abrogate this clonal advantage and found that lenalidomide restored expression of the matricellular protein SPARC, a gene located within the commonly deleted region on chromosome 5q. We hypothesized that the decreased expression of SPARC in del(5q) HSC leads to increased adhesion of HSC to their respective niche cells, translating to increased rates of proliferation, partly explaining the competitive advantage against non-del(5q) HSC. *Aims.* We conducted a study to analyze the effect of lenalidomide on the HSC/progenitor compartment in del(5q) MDS in order to test whether an hematopoietic stem cell (HSC)-intrinsic decrease of SPARC explains the why and how a clone of cells inherently defective at spawning functioning cellular descendants is not selected against, but rather exhibits a clonal advantage. *Methods.* We analyzed cell cycle distribution, frequency of apoptosis, and expression of adhesion markers on normal and del(5q) HSPC by multi-parameter flow cytometry. We analyzed the adhesion of normal and del(5q) HSPC to defined matrix components of the microenvironment such as fibronectin and VCAM-1. We overexpressed SPARC by lentiviral transduction in HSPC and analyzed the effect on engraftment in NSG-mice. *Results.* Multiparameter flow cytometry revealed a slight increase in proliferation of del(5q) versus normal HSC. Patients treated with lenalidomide exhibited complex changes in their expression of adhesion markers. In functional adhesion studies we observed that HSPC from del(5q) patients exhibited stronger adhesion than normal bone marrow cells to fibronectin and VCAM-1. Recombinant SPARC protein abrogated adhesion to VCAM-1 specifically in a subset of patients, while having no significant effect on normal HSPC. Overexpression of SPARC led to severely reduced engraftment in NSG-mice. *Summary/Conclusion.* These studies suggest that decreased expression of SPARC leads to increased adhesion of del(5q) HSC/progenitor cells to defined components of the microenvironment and may explain why del(5q) HSC are able to outcompete the remaining healthy HSC. Our studies implicate that lenalidomide is able to abrogate this clonal advantage partly via its increase in SPARC expression with a consecutive decrease in adhesion.

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GENE MUTATIONS OF THE TELOMERASE COMPLEX IN PATIENTS PRESENTING WITH REFRACTORY CYTOPENIA OF CHILDHOOD (RCC) - DO WE NEED TO KNOW?

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Refractory Cytopenia of Childhood (RCC), the most common form of myelodysplastic syndrome (MDS) in childhood, frequently presents with a hypocellular bone marrow. Thus, inherited bone marrow failure (IBMF) disorders are an important differential diagnosis. In particular, dyskeratosis congenita (DC), one of the most common types of IBMF, has to be taken into account. Because RCC and DC cannot be distinguished by hemato-morphological features, accurate clinical examination is mandatory. However, subtle clinical characteristics associated with DC may be overlooked or absent. Recently, our group reported on two patients with mutations in the gene of the human telomerase RNA component (TERC) who had been diagnosed with RCC in the absence of obvious clinical signs of DC. We hypothesized that a number of children presenting with hypocellular RCC may in fact suffer from DC identifiable by mutational analysis of the genes of the telomerase complex - namely, DKC1, TINF2, NOLA2, NOLA3, TERC and TERT. We therefore performed mutational screening for these 6 genes in 100 consecutive German patients enrolled in the prospective study EWOG-MDS-98 with primary hypocellular RCC without chromosomal aberrations or myelofibrosis. The two patients with known TERC mutations were included in this series. Including the 2 patients with TERC mutation previously published, we uncovered six patients with mutations in the 6 genes studied. One boy carried a mutation in DKC1, two patients had a TINF2 mutation and another patient harboured a mutation in TERT. No further TERC mutations were identified. All mutations detected were heterozygous, and, with the exception of one child with a TINF2 mutation, all aberrations proved to be of germline origin. Five of the six patients carried known mutations, while the patient with TERT mutation exhibited a novel alteration. None of these patients had clinical signs of DC at time of diagnosis of RCC. The boy with DKC1 mutation had stable low blood counts over several years and died of pulmonary fibrosis. Both children with TINF2 mutation underwent HSCT; the child with the somatic mutation in hematopoietic cells remains in stable condition, while the other patient died from severe skin and pulmonary GvHD. One of the two children with TERC mutation died of CMV reactivation after HSCT, while the other child with TERC mutation and the patient with TERT alteration showed decreasing blood counts in the absence of HSCT. In summary, 6% of phenotypically normal children with RCC were shown to carry germline or somatic mutations in one of 6 genes of the telomerase complex. At least one of the identified individuals had an unusually complicated course after HSCT. In view of the excellent outcome of HSCT in children with RCC, we suggest that it is important to identify patients with telomerase complex mutations. We currently perform telomere length analyses on granulocytes from the 100 patients to determine whether there is a significant difference in telomere length between RCC cases with or without telomerase complex mutations. Telomere length may provide a diagnostic tool for rapidly identifying RCC patients with mutations in one of these genes.

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ABCB7 PLAYS AN ESSENTIAL ROLE IN THE PATHOGENESIS OF ACQUIRED REFRACTORY ANEMIA WITH RING SIDEROBLASTS

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Background. Refractory anaemia with ring sideroblasts (RARS) is characterized by anaemia, erythroid apoptosis, and mitochondrial ferritin (FTMT) accumulation. To dissect the molecular mechanisms underlying the RARS phenotype, we recently described the gene expression profiles of erythroblasts generated from normal (NBM) and RARS marrow $CD34^+$ cells. The expression of ABCB7, an mitochondrial iron transporter, was severely down-regulated in RARS progenitors, and further decreased in cells undergoing erythroid differentiation. Importantly, ABCB7 expression was inversely correlated with the percentage of marrow ring sideroblasts. *Aim.* To test the hypothesis that ABCB7 has en essential role in the molecular pathogenesis of RARS. *Methods and Results.* During erythroid differentiation of K562 cells induced by hemin treatment, up-regulation of ABCB7 by lentiviral transduction, facilitated the expression of γ-globin and glycophorin A, indicating that ABCB7 potentiated erythroid differentiation. To study whether up-regulation of ABCB7 could rescue the erythropoiesis of RARS progenitors, bone marrow $CD34^+$ cells from RARS patients were transduced with lentiviral vector expressing ABCB7-YFP or mock vector. Colony forming cell assay was initiated from day 3 cultures and evaluated day 14 after transduction. ABCB7 over-expression, verified by qRT-PCR and YFP expressing colonies, significantly increased erythroid colony growth in 4/4 patients (Fig 1). In addition, up-regulation of ABCB7 reduced the expression of FTMT. Down-regulation of ABCB7 in K562 cells by lentiviral shRE1 vector, induced striking up-regulation of ALAS2 and accumulation of FTMT. Moreover, erythroid differentiation assessed by γ-globin expression was reduced during hemin-induced differentiation. Subsequently, normal bone marrow $CD34^+$ cells were transduced with the shRE1 vector and cell survival and colony forming capacity were assessed. The number of erythroid colonies was dramatically decreased, and erythroid cell survival was reduced when erythropoietin was added to induce terminal erythroid maturation. Interestingly, FTMT expression considerably increased in the ABCB7 down-regulated cells (Fig 2). *Summary.* Our results show that reduced

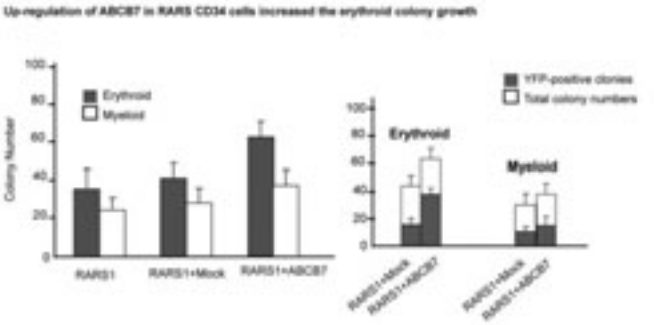

Figure 1.

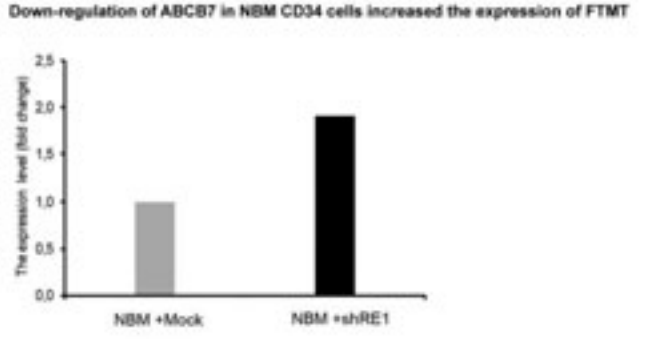

Figure 2.

ABCB7 expression plays a key role in mediating the erythroid failure, aberrant ALAS2 and FTMT expression and mitochondrial iron accumulation in acquired RARS. Moreover, up-regulation of ABCB7 restores RARS erythropoiesis and reverts ALAS2 and FTMT expression towards the normal range. In the absence of ABCB7 mutations or hypermethylation, upstream events are currently being investigated.

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EXOME SEQUENCING IDENTIFIES THE MPL GENE AS A CAUSE OF FAMILIAL APLASTIC ANAEMIA

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Background. The primary cause of many paediatric diseases remains elusive; however in some cases they may represent recessive single gene disorders. The problem is therefore how to identify a gene defect in a particular individual. To this end, exome sequencing has become the method of choice. One problem with this methodology is that the discriminatory power often lies in comparing unrelated individuals with the same disease to identify a common causative variant. This depends on the disease being genetically homogenous, but this will not always be the case. An example is aplastic anaemia (AA) which can be "labelled" as idiopathic, acquired or constitutional. Although mutations have been described in some genes, these only account for a small proportion of cases of AA. *Aims.* From our collection of uncharacterised AA patients we selected a consanguineous family with severe AA in two siblings. In order to characterize this family one of the affected sibs had the entire exome sequenced. The disease causing gene identified in this family would then be screened in other uncharacterized AA patients. *Methods.* Genomic DNA from an affected individual was hybridised to a NImblegen exome library before being sequenced on a GAIIx genome analyzer. Sequencing data were processed and by comparing to the reference human genome sequence and the 1000 genomes project, unique homozygous changes were identified. Biologically relevant changes were confirmed by Sanger sequencing. After confirming segregation in the rest of the family, the *MPL* gene was screened for mutations in 33 index cases with AA (<13 years) using denaturing HPLC. Any abnormal traces were confirmed by direct sequencing. *Results.* A homozygous mutation c.1248 G>A, p.Trp416Stop in the thrombopoietin receptor gene, *MPL*, was identified by exome sequencing and was shown to segregate with the disease (patient 1). This is a novel mutation occurring in a gene that has been previously associated with congenital amegakaryocytic thrombocytopenia (CAMT) - a rare autosomal recessive bone marrow failure syndrome characterised by early onset of isolated hypomegakaryocytic thrombocytopenia that often evolves to affect all three marrow lineages. We then identified two further novel mutations in two different patients from our screen of 33 index cases with AA (patients 2 and 3, Table 1). Interestingly, in patient 3 with the Pro394Ser mutation, the mutant T allele is present at a reduced level compared with what would be expected for a normal heterozygote (26% rather than 50%). This suggests the possibility of a mosaic for this mutation which has not been previously described in *MPL*. *Summary.* A novel homozygous nonsense mutation was identified in *MPL* by exome sequencing in a patient with familial severe aplastic anaemia. Screening of 33 uncharacterized AA patients revealed two additional novel mutations, one of which was recurrent and appeared to present as a mosaic. This study demonstrates that in a subgroup of patients with AA the disease is due to biallelic mutations in the *MPL* gene.

Table 1. Coding changes identified in MPL in AA.

Patient	Sex	Age at sampling (diagnosis)	Coding change	Protein change	Exon	Status	Family segregation
1	F	3 years (3 years)	c.1248 G>A	Trp416Stop	8	homozygous	yes
2	M	6 years (18 mo)	c.1180 C>T	Pro394Ser	8	homozygous	yes
3	F	7 years (7 years)	c.1180 C>T	Pro394Ser	8	heterozygous	n/a
3	F	7 years (7 years)	c.314 T>A	Phe105Tyr	3	heterozygous	n/a

n/a = family samples unavailable, mo = months

Hematopoiesis, stem cells and microenvironment

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COMBINING CELLULAR BARCODING AND MULTIPLEX DEEP SEQUENCING FOR HIGH-RESOLUTION QUANTITATIVE CLONAL ANALYSIS IN THE HEMATOPOIETIC SYSTEM

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Background. Accurate assessment of clonality is important in a variety of hematopoietic stem cell research areas, including cell expansion, aging, gene therapy, cancer progression and treatment. However, detailed studies of stem cell clonality are hindered by the absence of methods with sufficient sensitivity and resolution to measure clonal contributions of both major and minor clones. Recently, we have designed and validated a novel cellular barcoding technique for clonal analysis of complex cell populations *in vitro* and *in vivo* (Gerrits *et al.*, 2010). We have demonstrated that cellular barcoding combined with Sanger sequencing-based detection technique allows direct and unbiased assessment of clonality. *Aim.* Now, we coupled the barcoding approach with a high-throughput sequencing detection system and tested if such set-up will allow detailed quantitative analysis in heterogeneous populations of hematopoietic cells. *Methods and Results.* First, we developed a multiplexing protocol which allows simultaneous analysis of up to 200 DNA samples containing barcoded cell populations in a single Solexa sequencing run. We applied this protocol to analyze clonal dynamics in cultures of primary mouse bone marrow cells transduced with barcoded vectors. A high number of sequence reads (4000 to 2000000 reads per sample) allowed detailed and quantitative assessment of clonal fluctuations of over 100 barcodes in these cultures over time. Next, we transplanted barcoded murine bone marrow cells into irradiated recipients in a limiting dilution setting and followed the changes in barcodes presented in various blood lineages at different time points post-transplantation. This allowed us to trace behavior of both individual stem cells in monoclonally-repopulated animals and analyze minor and major clones in polyclonal mice. *Summary/Conclusions.* These data confirm that cellular barcoding in combination with high-throughput sequencing is an effective tool for the study of cell population dynamics. This approach permits a quantitative, high-resolution assessment of clonality and offers an unprecedented sensitivity in the ability to analyze heterogeneous cell populations. In the future, we anticipate that this method can be used for detailed monitoring of clonal changes in gene therapy protocols.

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FORCED EXPRESSION OF THE HISTONE H3K36 DEMETHYLASE FBXL10/KDM2B MAINTAINS THE SELF-RENEWAL CAPACITY OF MOUSE HEMATOPOIETIC STEM CELLS

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Post-translational covalent modifications of histone N-terminal tails are central to the epigenetic regulation of transcription, replication, and repair. Histone methylation marks contribute to transcriptional activation or repression depending upon the types and sites of modified residues and the degree of methylation. The methylation status of histones changes dramatically depending on cellular context and defines cell type-specific gene expression profiles. Histone demethylases have recently been implicated in this process. However, their roles in the regulation of hematopoietic stem cells (HSCs) remain poorly understood. To explore the relevance of histone demethylases in HSCs, we profiled the expression of 26 histone demethylase genes in mouse hematopoietic cells and found that *F-box and leucine-rich repeat protein 10* (*Fbxl10*, also known as *Kdm2b* or *Jhdm1b*) is highly expressed in $CD34^-c\text{-}Kit^+Sca\text{-}1^+$Lineage marker$^-$ ($CD34^-$KSL) HSCs and $CD34^+$KSL multipotent progenitors, but is markedly down-regulated during differentiation in bone marrow. *Fbxl10* belongs to the jumonji C domain-containing histone demethylases and is a demethylase specific to histone H3 mono/di-methylated at lysine 36 (H3K36me1/me2). Fbxl10 promotes the proliferation and functions as a physiological inhibitor of senescence in mouse embryonic fibroblasts through repression of *p15Ink4b* and *p16Ink4a*. Based on these data, we hypothesized that *Fbxl10* plays a role in the maintenance of HSCs and investigated the role of Fbxl10 in HSCs by conducting a gain-of-function analysis. $CD34^-$KSL HSCs were transduced with an empty control or an *Fbxl10* retrovirus and cultured in the presence of SCF and TPO. At day 14 of culture, although *Fbxl10*-transduced $CD34^-$KSL HSCs gave no apparent growth advantage to cells, the percentage of KSL cells was 2-fold higher in the *Fbxl10* culture than in the control culture. The total number of colony-forming cells (CFCs) derived from *Fbxl10*-transduced $CD34^-$KSL HSCs was slightly increased in the 3.5-day culture compared to the control, but increased up to 2-fold in the 10-day culture. Morphological evaluation of the colonies revealed that the control and *Fbxl10* cultures retained comparable numbers of colony-forming unit-neutrophil/macrophage/Erythroblast/Megakaryocyte (CFU-nmEM) with the potential for differentiation into multiple myeloid lineages at day 3.5 of culture while after 10-day culture, the *Fbxl10* culture retained more CFU-nmEM than the control culture. These data demonstrated that forced expression of *Fbxl10* in $CD34^-$KSL HSCs expands CFCs with multi-lineage differentiation potential during *ex vivo* culture. Forced expression of *Fbxl10* significantly repressed the expression of *p15Ink4b*, *p16Ink4a*, *p18Ink4c*, and *p57Kip2* in KSL cells expressing *Fbxl10*. Chromatin immunoprecipitation assays confirmed the direct binding of flag-tagged Fbxl10 to the *p15Ink4b*, *p19Arf*, *p16Ink4a*, and *p18Ink4c* promoters and a significant decrease in H3K36me2 levels and a moderate increase in H2Aub levels at these promoters and gene bodies on forced expression of *Fbxl10* in $Lineage^-c\text{-}Kit^+$ cells. Competitive repopulation assays demonstrated that *Fbxl10*-transduced $CD34^-$KSL HSCs prevents exhaustion of the long-term repopulating potential of HSCs following serial transplantation. We conclude that the histone H3K36 demethylase, *Fbxl10*, is a novel epigenetic regulator in HSCs, which plays an important role in maintenance of self-renewal capacity and multipotency of HSCs.

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ESSENTIAL ROLE OF TIP49 IN HEMATOPOIETIC STEM CELLS

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Background. Tip48 and Tip49 are members of a conserved protein family with homology to bacterial ATP-dependent DNA helicase RuvB. In eukaryotes, Tip48 and Tip49 perform essential functions as components of several multi-protein complexes, involved in regulation of transcription, DNA repair and telomere maintenance. In mammalian cells Tip48/49 regulate the function of several key regulators of cell proliferation, such as Myc, E2F, β-catenin, p53 and likely C/EBPα. Their function in mouse development and hematopoiesis has not been yet investigated. *Aims.* The aim of this study is to evaluate the importance of Tip49 protein *in vivo* by conditionally deleting tip49 gene in mice. The role of Tip49 is addressed during mouse development as well as in adult hematopoietic tissues, specifically in hematopoietic stem cells (HSCs). *Methods.* A conditional allele of Tip49 gene in mice has been generated using a standard Cre-LoxP approach. The floxed gene was then bred into homozygocity (tip49 fl/fl). In order to generate tip49-null allele, mice carrying the conditional alleles were bred to a general Cre deleter line. To specifically delete Tip49 in adult mice, tip49 fl/fl mice were crossed to Mx1-Cre interferon-inducible line to efficiently delete tip49 in bone marrow, spleen and liver. *Results.* General deletion of Tip49 results in early embryonic lethality at periimplantation stage, while inducible deletion of tip49 in hematopoietic system also results in rapid lethality associated with anemia and pancytopenia. Bone marrows of tip49-deficient mice manifested a rapid drop in total cell number, associated with a decrease in progenitor cells proliferation and disappearance of HSCs. The effects of tip49 gene deletion were hematopoietic cell autonomous, as Tip49 deletion in chimeric bone marrows led to an early apoptotic cell death exclusively in mutant long-term HSCs (Lin-Sca-1+c-kit+CD150+) and not in the wild type competitor HSCs. *Summary.* These data demonstrate the essential role of transcriptional regulator Tip49 in adult hematopoietic stem cells, and suggests that modulation of its activity may represent a way to impinge on HSC functions.

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PLEIOTROPHIN IS A SECRETED NICHE FACTOR WHICH REGULATES ENGRAFTMENT OF HEMATOPOIETIC STEM CELLS

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Hematopoietc stem cells (HSC) are maintained at a quiescent state during steady-state hematopoiesis. HSC are charachterised by their ability to respond quickly to hematopoietic stress *in vivo*, as well as in culture, by increasing proliferation and inducing early lineage commitment. It is likely that secreted niche factors are involved in this transition from quiescence towards activation. We here show that knockdown of the secreted factor pleiotrophin (Ptn-KD) in stromal cells increases proliferation as well as production of hematopoietic progenitors and HSC activity in co-cultures with lineage-negative (Lin-) hematopoietic cells. Moreover, engraftment of cells co-cultured with Ptn-KD stromal cells is associated with increased numbers of Cd34- Lin- Sca+ Kit+ (LSK) cells and dominant myeloid regeneration. Despite clear effects of Ptn deficiency in co-cultures, steady-state hematopoiesis is not altered in Ptn knockout (Ptn-/-) mice. This suggests that Ptn May be involved in limiting HSC activation, but is probably not involved in quiescence. Indeed, engraftment of wild-type HSC in lethally irradiated Ptn-/- mice mirrors the cultures on Ptn-KD stromal cells in that engraftment is increased in serial transplantations with progressive myeloid skewing and accumulation of CD34- LSK donor cells. On a molecular level, steady state Ptn-/- LSK cells express decreased levels of cyclin D1, but an increased expression of the myeloid master- regulator C/EBPalpha. In contrast, the observed increase in hematopoietic regeneration in co-cultures on PtnKD stromal cells is associated with UPregulation of cyclin D1 (Ccnd1), whereas again C/EBPalpha was also increased. Thus, the difference between steady-state quiescence and activation of engraftment appears to lie in strict regulation of cyclin D1 through Ptn during early HSC activation. Interestingly, neither in steady state Ptn-/- cells, nor in wild-type LSK cells co-cultured on Ptn-KD stroma, did the regulation of cyclin D1 through Ptn depend on changes in transcript or protein levels of b-catenin. This finding indicates that regulation of HSC activation and engraftment through Ptn is independent of canonical Wnt signaling. In conclusion, our results point to different regulatory mechanisms in normal hemostasis and in hematopoietic regeneration. Moreover, our results support the hypothesis that Ptn secreted by the microenvironment is an important regulator of cyclin D1-dependent hematopoietic regeneration.

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ENERGY METABOLISM OF GLUCOSE AND ATP AFFECTS THE GROWTH AND DIFFERENTIATION OF HEMATOPOIETIC STEM/PROGENTOR CELLS

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Background and Aims. Recently, it has been reported that undifferentiated state of hematopoietic stem cells is regulated by their environmental factors, such as oxygen concentration. Furthermore, we have clarified that a NAD dependent histone deacetylase, Sirt1, is involved in the fate of hematopoietic stem cell as energy sensor. In this study, we examine the effects of energy metabolism on the proliferation and differentiation of hematopoietic stem/progenitor cells. *Methods and Results.* To clarify the relation of energy metabolism and cell cycle of hematopoietic stem/progenitor cells, murine bone marrow derived lineage(-), Sca-1(+), c-Kit(high) (KSL) cells were double stained with Pyronin Y and Hoechst, and were divided into cells in G0, G1, and S/G2/M periods. The intracellular ATP concentrations rose as the cell cycles shift from G0, G1 to S/G2/M (0.25, 0.45, 0.60pg/cell, respectively). We also visualized intracellular NADH with 460nm excitation. Though NADH could be detected at a low level in cells in G0, potent fluorescence was observed in cells in S/G2/M. From these data, it was supposed that energy metabolism such as ATP or NADH production may be activated when a hematopoietic stem cell enters into cell cycle. Next, we cultured KSL cells in the medium containing 0, 50, 100, 150 or 200 mg/dL of glucose, supplemented with TPO, SCF, Flt-3L. Cell proliferations were promoted glucose-dose dependently. On the other hand, the residual KSL population was the highest in cells cultured with 50 mg/dL of glucose and the lowest in cells cultured with 200mg/dL. Next, we performed paired daughter cell colony assays. Murine bone marrow KSL cells were clonally sorted into 96 well plates and cultured with medium containing 50 or 200 mg/dL of glucose. When a sorted cell divided into two daughter cell pair, which were then separated by micromanipulation and transferred into methylcellulose medium containing 200mg/dL of glucose. We evaluated the period from sorting to the first division of each cell, and the colonies from each daughter cell pair were evaluated 8 days after manipulation. The periods of division were shorter in KSL cells cultured with 200mg/dL of glucose. If a stem cell is divided symmetrically into two stem cells, which means selfrenewal, the daughter cell pair will form mix/mix colony pair. Forty-four percent of daughter cell pairs from KSL cells cultured with 50mg/dL of glucose formed mix/mix colony pairs, in contrast to 22% in KSL cells cultured with 200mg/dL. These data demonstrated that low glucose concentration leads cell cycle suppression and promotes selfrenewal of hematopoietic stem/progenitor cells. We also performed the same paired daughter cell assays using KSL cells cultured with nicotinamide(NA), the inhibitor of Sirt1. NA-supplement cancelled completely the cell cycle suppression and partially mix/mix colony pair formation in KSL cells cultured in low dose glucose, suggesting that Sirt1 may be involved in the cell cycle suppression and the promotion of selfrenewal of hematopoietic stem/progenitor cells in low dose glucose environment. *Conclusions.* In hematopoietic stem/progenitor cells, the environmental glucose concentration and the intracellular energy metabolism are involved in the maintenance of stemness and the regulation of proliferation.

Granulocytes and signaling

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IDENTIFICATION OF A NOVEL MODE OF KINASE INHIBITOR RESISTANCE: AN F604S EXCHANGE IN FIP1L1-PDGFRA MODULATES FIP1L1-PDGFRA PROTEIN STABILITY IN A SHP-2 AND SRC-DEPENDENT MANNER

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FIP1L1-PDGFR alpha is a constitutively activated protein kinase which is associated with chronic eosinophilic leukemia (CEL). Imatinib is clinically active in FIP1L1-PDGFRA positive disease. However, clinical resistance to imatinib has been observed in FIP1L1-PDGFRA positive leukemia and was shown to occur due to secondary mutations in the PDGFR alpha kinase domain. Using a screening strategy to identify imatinib resistant mutations, we generated imatinib resistant single cell clones expressing FIP1L1-PDGFRA. Analysis of the PDGFRA kinase domain in these cell clones revealed a spectrum of resistance mutations including the clinically reported exchange T674I. Interestingly, one of the most abundant mutations was a Phe to Ser exchange at position 604 (F604S), which occurred alone or in combination with other exchanges. Surprisingly, FIP1L1-PDGFRA/F604S (F604S FP) did not increase the biochemical or cellular IC50 value to imatinib when compared to wild-type FIP1L1PDGFR(wt). However, F604S FP transformed Ba/F3, NIH3T3 and mouse bone marrow more efficiently compared to wt. Immunoprecipitation and immunoblotting indicated greatly increased amounts of F604S FP protein compared to wt in the cells. Pulse chase analysis revealed that F604S FP is strongly stabilized compared to wt. SRC coimmunoprecipitated with FIP1L1-PDGFRA but not with F604S FP. Co-expression of SRC in 293T cells augmented degradation of wt-FIP1L1-PDGFRA, but not F604S FP, indicating that SRC is a negative regulator of FIP1L1-PDGFRA protein stability. Accordingly, both the SRC inhibitor PD166326 and SRC siRNA mimicked the F604S phenotype and resulted in stabilization of the wt protein. Importantly, phosphatase inhibitor treatment of F604S FP led to destabilization and SRC recruitment indicating that phosphatases might be responsible for the enhanced stability of F604S FP. In fact, coimmunuprecipitaion experiments identified the phosphatase SHP2 as a specific binding partner of F604S and mapping experiments revealed that the phosphatase domain of SHP-2 directly interacted with F604S FP but not with wt- FIP1L1-PDGFRA. Together, these results suggest that stabilization of F604S FP is due to dephosphorylation by SHP-2 leading to lower activation of the SRC and Cbl mediated ubiquitination machinery. Therefore this work identified a novel class of resistance mutations in FIP1L1-PDGFRA, that do not act by impeding drug binding to the target, but increase target protein stability and abundance by interfering with SRC- mediated degradation.

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TARGETING THE CDC2-C/EBPA PATHWAY INDUCES DIFFERENTIATION OF HUMAN ACUTE MYELOID LEUKEMIAS WITH FLT3ITD ACTIVATING MUTATIONS

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Activating mutations in FLT3 (fms-like tyrosine kinase 3) receptor, such as FLT3ITD, are among the most prevalent mutations in acute myeloid leukemias. The oncogenic role of FLT3 mutants has been attributed to the abnormal activation of several downstream signaling pathways, such as STAT3, STAT5, AKT, or ERK1/2. We have previously demonstrated that ERK1/2 kinase can phosphorylate C/EBPA on serine 21, and consequently inactivate its function. C/EBPA is a transcription factor playing a critical role in granulocytic differentiation and often inactivated in various subtypes of leukemia by multiple mechanisms. Among FLT3ITD patients, only 39% demonstrated activation of MEK1, and thus the ERK1/2 pathway, yet we show here that C/EBPA can be still phosphorylated on serine 21. We identified cdc2 (also known as CDK1) as a novel FLT3ITD activated kinase and we determined that FLT3ITD mutant receptors superactivate cdc2 via upregulation of cyclins A and B. Furthermore, we demonstrate that cdc2 directly phosphorylates C/EBPA on serine 21 (*in vitro* and *in vivo*), which inhibits its differentiation-inducing function. Importantly, we found that pharmacological and genetic (knock-down) inhibition of cdc2 activity relieves the differentiation block in FLT3ITD cell lines. Next, we investigated the effect of cdc2 inhibitor, NU6102, in primary FLT3ITD leukemia patient samples collected at diagnosis. As expected, cdc2 inhibition led to a remarkable hypophosphorylation of C/EBPA and a dose-dependent decrease in immature myeloid surface marker expression, such as CD133 and CD38, as well as increase in CD15 expression. Moreover, the treatment of FLT3ITD carrying specimens with NU6102 for 7 days was accompanied by morphological changes suggesting granulocytic differentiation. Altogether, our data indicate that FLT3ITD mutants superactivate cdc2, which leads to phosphorylation of C/EBPA at serine 21 resulting in a differentiation block. Clinical trials with cdc2 inhibitors are currently under way for various malignancies. Our data strongly suggest that targeting the cdc2 pathway might be applied for the treatment of FLT3ITD mutant leukemias, especially of those resistant to FLT3 inhibitor therapies.

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COMPARATIVE GENOME-SCALE RNA INTERFERENCE SCREENS IDENTIFY THE MLL-FUSION ASSOCIATED GENE AF4 AS A REGULATOR OF CD133 TRANSCRIPTION

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Background. The AC133 epitope expressed on the pentaspan transmembrane glycoprotein CD133 was first discovered as a cell-surface marker of hematopoietic progenitor cells. Since then, it has been used as a marker for cancer stem cells from various tissue types, including blood. Although numerous studies have reported that DNA methylation of the CD133 promoter regulates its transcription regulation, the specific transcription factors involved remains poorly understood. *Aim.* To identify factors involved in regulating CD133 transcription. *Methods.* We performed a pooled short-hairpin RNA interference screen targeting >11,000 human genes in the CD133 endogenously expressing human epithelial colorectal adenocarcinoma Caco-2 cell line and in an engineered human embryonic kidney (HEK) 293 line exogenously expressing CD133. shRNA knockdowns that resulted in decreased cell-surface CD133 expression as determined by fluoresent-activated cell sorting were considered hits and their identities were deconvoluted using custom microarrays. To identify genes involved in endogenous CD133 transcription, we focused on shRNA hits specifically required for CD133 expression in Caco-2 cells, but not in the HEK 293/CD133 line. *Results.* We identified the transcription activator AF4 as a regulatory of CD133 transcription, as gene knockdown of AF4 results in a dramatic reduction in CD133 transcript levels. It has been well established that AF4 is associated as a MLL-fusion in acute lymphoblastic leukemia (ALL). Consistent with our findings, MLL-AF4 has been demonstrated to interact with the CD133 promoter in the ALL cell line SEM. Furthermore, MLL-AF4/AF4 was shown to be involved in a protein complex involved in transcription elongation. When we performed gene targeted knockdown of complex members, we observed a significant decrease in CD133 transcript, suggesting that MLL-AF4 and AF4 functions in this protein complex to regulate CD133 transcription elongation. *Summary.* Our study provides mechanistic insight into the transcriptional regulation of CD133 in MLL-AF4 expressing ALL cell lines and in non-leukemia cell lines.

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DIFFERENTIAL STIMULATION OF CEBPA TARGET GENES DURING ATRA-INDUCED GRANULOCYTIC VERSUS CDDO-INDUCED GRANULOMONOCYTIC DIFFERENTIATION

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Background. Induction of differentiation may be a powerful means to treat human acute myeloid leukemia (AML). However, while all-trans retinoic acid (ATRA), which induces granulocytic differentiation, has been applied successfully in patients with acute promyelocytic leukemia, the clinical benefit in the other AML subgroups of patients has been much less promising. *Aims.* We have previously shown that 2-cyano-3,12-dioxooleana-1,9-dien-28-oic-acid (CDDO) induces granulomonocytic differentiation, and this was at least in part mediated by translational activation of the transcription factor CCAAT Enhancer Binding

Protein alpha (CEBPA) (Koschmieder *et al.*, Blood 2007). Here, we investigated similarities and differences between ATRA- and CDDO-induced effects. *Methods.* HL60, 32D, or 293T cells were used for analysis of RNA expression by RT-PCR, CEBPA transactivation potential using luciferase assays, and CEBPA target identification by chromatin immunoprecipitation (ChIP)-chip array techniques. *Results.* Both ATRA (1 μM) and CDDO (0.5 μM) induced Id1 and Id2 mRNA in HL60 cells, although Id1 induction was less pronounced with CDDO. Since Id1 and Id2 are CEBP target genes, we analyzed CEBPA binding to Id1 and Id2 target genes and found that CEBPA bound to Id1 and Id2 promoters as well as the Id1 enhancer. CEBPA stimulated luciferase activity of the Id1 enhancer but not the Id1 promoter. CEBPA, CEBPB, and CEBPD stimulated Id1 enhancer activity to different extents. Moreover, while ATRA and CDDO only weakly stimulated Id1 enhancer activity in parental 32D cells, concomitant transfection of CEBPA, CEBPB, or CEBPD additively increased this activity. ChIP-Chip array analysis of CEBPA binding in HL60 cells demonstrated remarkable similarities of known and novel CEBPA target genes induced by both ATRA and CDDO, including increased binding to Myd88, Vva1, IL3, Ly6e, Rgs2, Csf3, IL8 and decreased binding to Csf1 and EpoR. *Conclusions.* The differentiation inducers ATRA and CDDO stimulate similar pathways of granulocytic differentiation involving CEBPA and its target genes. In addition, our ChIP-chip array approach identified novel targets of CEBPA which may enhance our understanding of cell fate decisions during granulocytic versus monocytic differentiation.

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FAS GENE EXPRESSION IS EPIGENETICALLY REGULATED AND PREDICTS THE RESPONSIVENESS TO AZACITIDINE IN HIGH-RISK MYELODYSPLASTIC SYNDROMES

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Background. Low risk myelodysplastic syndromes (MDS) CD34+ cells exhibit high level of the death receptor Fas at their surface and abnormal Fas-dependent apoptosis. Fas expression decreases when the disease progresses to acute myeloid leukemia (AML). Based on recent evidence of higher DNA methylation level in high-risk MDS, we explore the epigenetic regulation of FAS gene during MDS evolution to AML. *Aims.* This study aims at investigating the regulation of Fas and FasL in MDS/AML cells. *Methods.* We quantify FAS gene expression by RT-qPCR in bone marrow mononuclear cells (BMMNC) from 136 patients (86 MDS, 30 AML) and in 20 controls, including 61 patients treated with azacitidine according to the FDA/EMEA schedule. Response is scored according to IWG2006 criteria for MDS and to Cheson for AML. DNA methylation on bisulfite DNA and ChIP assays for histone modifications (H3K9/14ac, H3K4me2, H3K9me2, H3K27me3) are performed in HL60 cell line, SW480 colon carcinoma cell line and in bone marrow-derived CD34+ MDS/AML cells before and during azacitidine treatment. *Results.* We observe a significant decrease in FAS mRNA in AML compared to MDS (P<0.001). Fas decrement significantly correlated with disease progression but its expression, at diagnosis, had no impact either on overall survival or leukemia-free survival. FAS promoter was hypomethylated with an open state of the chromatin while it is hypermethylated in the Fas-negative SW480 cell line. In BM CD34+ cells, the methylation of 3 CpG dinucleotides is significantly increased in 14 AML compared to 18 MDS. In 4 AML patients, DNA methylation decreases from 28% [range: 20 - 37] to 14% [range: 11 - 15] and active marks of the chromatin are enriched, mainly the H3K4me2 after 6 cycles of azacitidine. *In vivo* azacitidine removes chromatin repression at FAS promoter and increases Fas protein expression in correlation with clinical response (Chi-square test P= 0.002). Multivariate analysis showed that high Fas expression on CD45lo/CD34+ cells at diagnosis was predictive of failure of azacitidine treatment (Hazard ratio 3.6 [95%CI: 1.1 - 11.6], P=0.032), independently of IPSS, age and previous treatment. *Conclusions.* An epigenetic mechanism is responsible for the down-regulation of Fas in AML, which is corrected by azacitidine in responder patients. Our data show that FAS gene reactivation could predict the responsiveness to azacitidine and that its expression, at diagnosis, is a biomarker of the response to azacitidine.

Stem cell transplantation - Clinical 2

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SINGLE AND DOUBLE CORD BLOOD TRANSPLANTATION FOR ADULT WITH ACUTE MYELOID LEUKAEMIA: A SURVEY ON BEHALF OF EUROCORD AND ALWP-EBMT

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Background. Patients with high-risk acute myeloid leukemia (AML) have few chances of cure without allogeneic stem cell transplantation (HSCT). HSCT can be used in first remission for pts with poor-risk cytogenetics, as rescue for pts refractory to chemotherapy, at first relapse or in second and subsequent remission. UCB is an established stem cell source for HSCT. *Methods.* We retrospectively analyzed 604 adult (>18y) with de novo AML who received UCBT as first transplant. *Results.* 229 patients were transplanted in first complete remission (CR1, 228 in second or third CR and 147 in advanced disease. Patients were transplanted from 2000-2010 in 131 EBMT centers. Median age was 41 years, 18% of the patients received a previous autologous transplant. Based on available cytogenetic and molecular markers at diagnosis (n=339) 56% were in intermediate risk and 31% in unfavorable risk group. Grafts were composed of 1 (sUCBT) (n=361) or 2 (dUCBT) (n=243) CB units, 39% of CB units were identical to recipient or had 1 HLA disparity (antigen level for HLA-A and B allelic level for DRB1) while 61% had 2-3 HLA disparities. At infusion median TNC cell dose was 3.1x10^7/kg (2.4x10^7/kg with sUCBT and 3.7x10^7/kg with dUCBT) and median CD34+, 1.2x10^5/kg (1x10^5/kg with sUCBT and 1.3x10^5/kg with dUCBT). Fifty-one percent of pts received a myeloablative conditioning regimen (MAC) and 49% a reduced intensity regimen (RIC). The most common regimens used were busulphan+fludarabine+thiotepa for MAC and cyclophosphamide+fludarabine+TBI2Gy for RIC. GVHD prophylaxis consisted of CSA±MMF in 58% of pts and CSA±steroids in 32%. Median follow-up was 18 months and it was 23 months for sUCBT and 15 months for dUCBT. Cumulative incidence (CI) of neutrophil recovery, acute GVHD (II-IV) and 1y TRM was 80±2%, 26±3% and 21±3%, respectively. CI of 2y relapse was 38±3% (27% CR1, 29% CR2 and CR3, 56% advanced, p=0.001). Relapse incidence was 31% for those patients transplanted with MAC (n=291) and 30% with RIC (n=282). The 2y probability of leukemia-free-survival (LFS) was 33±2% (45% CR1, 41% CR2, 16% advanced, p <0.001). In patients given a MAC, 2y LFS was 50% for CR1, 27% for CR2 or CR3 and 17% for more advanced phase of the disease whereas it was 35%, 44% and 18% for RIC respectively. Among patients transplanted in CR1 within intermediate risk group, 2 y LFS was 46±6% and it was 33±8% for those within unfavorable risk group, while for those transplanted in CR2 it was 38±6% and 30±9%, respectively. In multivariate analysis, disease status at transplant (remission vs advanced) was the main factor associated with improved LFS (HR 2.12, 95%CI 1.3-3.15, p<0.001). Causes of death were infections or other transplant-related events (n=195) or disease progression (n=149). *Conclusion.* In conclusion, this large series of patients shows that UCBT is an option treatment for adults with high risk AML after a myeloablative or reduced conditioning regimen without a suitable HLA matched donor.

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COMBINED MISSING SELF MODEL AND LIGAND-LIGAND MODEL CAN PREDICT HIGHER RELAPSE RATE AFTER HLA-MISMATCHED TRANSPLANTATION WITHOUT T CELLS DEPLETION *IN VITRO*

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Background. HLA-mismatched/haploidentical stem cell transplantation (SCT) is a feasible therapeutic option for advanced hemato-

logic malignancies patients 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 leukemic patients. *Aims.* The goal of this study was to explore the predictive roles of missing self model in our HLA-mismatched/haploidentical transplantation without T-cell-depletion *in vitro*, and to develop a simple algorithm on the basis of recipients and donor HLA-C and HLA-Bw4 gene content that can be used today to identify HLA-mismatched donors who will provide the most protection against relapse in T cell-replete transplants. *Methods.* We studied the HLA genotype of 153 donor-recipient pairs, who underwent unmanipulated HLA-mismatched/ haploidentical transplantation without T cells depletion *in vitro*. To apply the missing ligand model, the first step was to divide our donor-recipient pairs into 2 groups according to the number of KIR ligand in donor and recipient, 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. *Results.* Among the 153 pairs of donor-recipients, 110 and 43 recipients received HLA-mismatched/haploidentical transplants from "with missing self"and "without missing self" donors, respectively. Using Ligand-ligand model, 119 and 34 recipients received haploidentical transplantation from "KIR ligand matched"and "KIR ligand mismatched"donors, respectively. In contrast to Perugia's KIR ligand-ligand mismatched model or Handgretinger's KIR missing self model between donor-recipient pairs, we found that the cumulative incidence of 7-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=0.00746) or "without KIR ligand mismatched"(p=0.01194) donors, respectively. When combined the above predictive model together, patients were subgrouped as receiving graft from "without missing self and without KIR ligand mismatch"(best, n=43), "with missing self and without KIR ligand mismatch"(better, n=76), and "with missing self and with KIR ligand mismatch"(neutral, n=34), respectively. We found the 7-year disease-free survival (DFS), overall survival (OS), and relapse rate were best predicted by the combination of missing self and KIR ligand mismatch between recipients and donors pairs (HR 1.517(1.081-2.128), p=0.016 for DFS; HR 1.518(1.077-2.142), P=0.017 for OS; HR 2.46(1.424-4.205), P=0.001 for relapse, figure1).

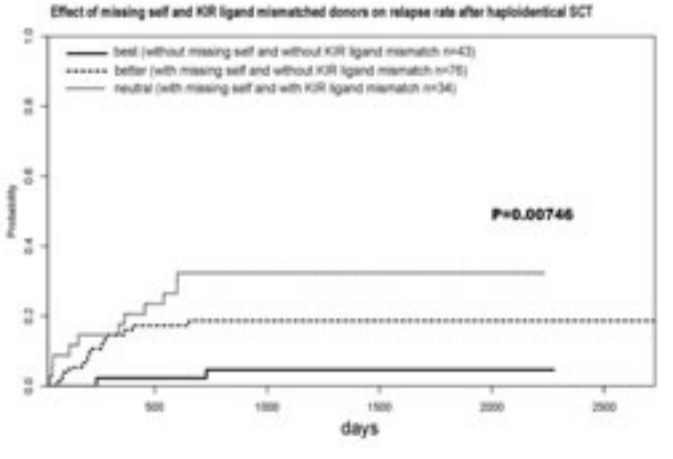

Figure 1. Seven years relapse rate after haploidentical SCT.

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 a predominant role in this model. Meanwhile, we developed a simple algorithm on the basis of recipients and donor HLA-C and HLA-Bw4 gene content that can be used today to identify HLA-mismatched donors who will provide the most protection against relapse in T cell-replete transplants.

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BIRTH ORDER IS NOT A MAJOR FACTOR INFLUENCING TRANSPLANT OUTCOME IN HLA-IDENTICAL SIBLING SCT - AN ANALYSIS ON BEHALF OF THE CIBMTR

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Background. Recent single-center studies have found a birth order effect in HLA-identical sibling myeloablative stem cell transplantation (SCT): superior outcome (e. g. overall survival (OS), less relapse, lower relapse mortality) when the recipient is older than the donor. The proposed mechanism is microchimerism (MC) due to fetomaternal and transmaternal sibling cell trafficking as the donor is exposed to recipient antigens in utero. *Aim.* The aim of this study was to validate single-center data in a multicenter patient cohort. *Methods.* This is a retrospective analysis from the CIBMTR dataset. Patients with a diagnosis of a hematologic malignancy (AML, ALL, MDS, CML), at any age (adults and pediatric patients), receiving an allogeneic SCT from HLA-identical sibling donors (recipient and donor of different ages at the time of transplant, but no more than 15 years apart) since 1990 up to December 2007 were included. Outcome was analysed in terms of OS, relapse rate and relapse mortality, leukemia free survival (LFS), treatment related mortality (TRM), acute and chronic graft versus host disease (GvHD). *Results.* A total of 11877 patients (6089 where the recipient was older; 5788 where the donor was older) have been identified. The median age of the patient was 35 years (range 2-75y.) in the recipient older group compared to the donor older donor with 31 years (range < 1-72y.; p< 0,0001). In univariate analysis, recipient older pairs have a higher TRM which was confirmed in multivariate analysis. In multivariate analysis for survival, there was an interaction between age and birth order. A better survival was observed in recipient older pairs in the youngest recipient age group compared to a superior OS in donor older pairs when the recipient was 40-49 years. Further, there was no main effect on LFS, relapse rate, acute or chronic GVHD. Subset analysis limited to early stage myeloid diseases, peripheral blood T replete grafts, and myeloablative conditioning showed better survival in the donor older group (RR 0.78, 95% CI 0.64-0.96, p=0.02). *Conclusions.* The hypothesized positive effect of having the recipient older than the donor compared to pairs where the donor is older than the recipient was not observed in this study, except in the very youngest recipients.

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FASTER REGISTRATION ON INTERNATIONAL DONOR REGISTRIES AND SHORTER TIME TO ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION AFTER HAVING FOUND A DONOR CONFERS BETTER OUTCOME IN ACUTE LEUKEMIA PATIENTS

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Background. A patient has 30% chance to find an HLA identical sibling donor (SD) and approximately 40% chance to find a suitable unrelated donor (UD), 40% of registered patients relapse or die before finding a donor. *Aims.* We evaluated the outcome in acute leukemia (AL) patients, whether they had an HLA identical SD, an UD or no donor (ND) after registration on France Greffe de Moelle (FGM) registry, either transplanted later or not. Secondary objectives were to evaluate the impact of intervals diagnosis-allo-HSCT, donor finding-allo-HSCT, registration-allo-HSCT, on OS and EFS. *Methods.* We analyzed 251 AL patients, 117 (47%) males and 134 females, median age at diagnosis 40 years [16-66], 177 (71%) AML and 75 ALL. Seventy six (30%) patients had an available SD and received allo-HSCT within a median time of 3.5 months (0.5-43) and 38 (15%) had SD but were not transplanted due to early relapse and/or death. For patients with no available SD, a registration on FGM registry was done, 137 patients were registered after a median interval of 2.3 months (0.4-135) from diagnosis, 33 (13%) patients did not find any donor and they received

Figure 1.

the standard of care; 104 (41%) patients found an UD or UCB unit after a median time of 1.6 months (0.3-26): 86 with UD of which only 60 have been transplanted within a median time of 2.3 months (0.4-14), 18 with UCB of which only 17 were transplanted. Among transplanted patients, 113 (74%) were in CR, 40 in <CR. Fifty (33%) received PBSC, 86 (57%) received BM and 17 (10%) UCB units. For conditioning, 56 (37%) were RIC and 96 standard. For HLA, there were 45 HLA 10/10, 14 HLA 9/10, 1 HLA 8/10 and for UCB 14 HLA 4/6 and 3 HLA 5/6. *Results.* After a median follow-up of 25 months (0.2- 234), the median OS was 78 months (51-133) for transplanted patients with SD (3years OS: 68%), it was 33 months (27-47) for transplanted patients with UD (3years OS:44%), 21 months (15-37) for not transplanted patients with available SD or UD (3years OS:34%) and it was 31 months (23-221) for patients with ND (3years OS:45%). Median EFS for the same groups was 38 months (23-133), 24 months (17-36), 15 months (11-24) and 23 months (14-48) respectively. In multivariate analysis, 3 significant factors affected OS: disease status (<CR) HR= 2.8 [1.5-5.3] p<0.001; long interval diagnosis-registration HR= 2 [1.2-3.6] p=0.001 and conditioning (standard) HR=0.27 [0.1-0.8] p=0.02. *Conclusion.* The interval diagnosis-registration appeared as major factor affecting survival in UD allo-HSCT settings.

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UNRELATED CORD BLOOD TRANSPLANTATION (UCBT) FOR CHILDREN WITH ACUTE MYELOID LEUKAEMIA (AML): AN ANALYSIS OF EUROCORD, ALWP AND PDWP OF EBMT

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Background. In 2003, Eurocord group has described outcomes after UCBT in 95 children with AML. *Aims.* Better define the role and to identify prognostic factors of UCBT in childhood with AML. *Methods.* Retrospective analyses of 390 children with AML who received single UCBT in EBMT centres. *Results.* Median age and median weight at UCBT was 6 years and 22 Kg. Transplantation were performed from 1994 to 2010: 37% during CR1, 42% in CR2 and 21% in more advanced disease. On the basis of cytogenetic and molecular characteristics, 253 children (65%) were stratified into 3 groups: 35% in the unfavourable, 22% in the intermediate and 8% in the favourable. The majority of grafts were HLA 5/6 (44%) or 4/6 (37%). Median number of infused total nucleated (TNC) and CD34+ cells were 4.95x107/kg and 1.9 x105/kg, respectively. The majority of patients (86%) received myeloablative conditioning regimen with TBI (25%) or busulphan (60%). ATG was used in 80% of cases. Median follow-up time was 24 months. Median time to achieve neutrophil and platelet recoveries was 24 and 42 days. Cumulative incidence (CI) of ANC recovery was 85%; in a multivariate model it was favourably associated with a higher TNC dose (> median, HR: 1.40, p=.008) and transplantation in CR1 (HR: 1.39, p=.015). At day 100, CI of grade II-IV acute GvHD was 34% (11% grade III, 5% grade IV). At 2y CI of NRM was 24%. Multivariate analysis showed that: TNC dose (HR: 0.58, p=.024) and disease status (CR1 vs others) at time of UCBT (HR: 0.55, p=.026) were associated with decreased NRM. There was a trend toward a decreased NRM in patients given a 6/6 or 5/6 HLA graft (p=.06). CI of relapse at 2 years was 17% in CR1, 26% in CR2 and 44% for more advanced disease. Estimated 2 y-LFS was 63% in CR1, 43% in CR2 and 22% in more advanced patients. LFS of 49 children transplanted in CR1 with unfavourable disease was 70±7 %, not statistically different from the overall CR1 group. For those transplanted in CR2, 2y-LFS was 71±9%, 33±9 % and 40±7% in the favorable, intermediate and unfavorable subgroup. Multivariate analysis in CR2 cohort identified 2 significant prognostic factors: favorable disease (HR:3,74, p=.005) and previous CR1 duration longer than 7 months (the threshold of the first quartile, HR:1,85, p=.03). *Conclusions.* We conclude that UCB is an attractive stem cell source in childhood AML when no HLA-identical donor is available. Cell dose remains an important factor for engraftment and NRM. Results are very encouraging for unfavorable diseases in CR1.

Progress in the treatment of non-Hodgkin Lymphomas

1045

DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) IN VERY ELDERLY PATIENTS (OLDER THAN 80 YEARS): PROMISING RESULTS IN THE ERA OF CHEMOIMMUNOTHERAPY DESPITE LOWER DOSE INTENSITY

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Background. The optimal treatment of very elderly patients with DLBCL has not been been well established. Many patients have received chemotherapy (CIT) without anthracyclines with rather poor results. The combination of Rituximab with CHOP has greatly improved the prognosis of DLBCL. The administration of Rituximab in very elderly patients might permit dose reductions in the chemotherapy regimen without significant loss in efficacy. *Aims.* To describe the clinical characteristics, the actually delivered doses of chemoimmunotherapy (CIT) drugs in routine clinical practice and the final outcome of very elderly patients with DLBCL in the era of CIT. *Patients and Methods.* Among 579 patients with DLBCL treated in 5 medical Centers in the era of CIT, 56 were older than 80 years (10%). We studied the clinical and laboratory characteristics of these patients (individual factors of IPI, gender, B-symptoms, anemia, lymphocytopenia, albumin), Progression Free Survival (PFS) and Overall Survival (OS) in comparison with 523 patients younger than 80 years, who were treated during the same period. In addition, we analyzed the relative dose intensity (RDI) of the 4 drugs (except of corticosteroids), which was actually delivered to the very elderly patients, using as reference the doses and time intervals of standard R-CHOP-21. *Results.* The median age of the very elderly patients was 82.5 years (80-91). The frequency of adverse prognostic factors (IPI, R-IPI, low serum albumin, severe lymphocytopenia) was significantly higher in very elderly patients, but the difference was mainly due to the favorable prognostic profile of the younger patient group (<60 years). In contrast, the characteristics of patients aged 61-79 and ≥80 years were similar. The 5-year PFS was 81%, 68% and 62% (p=0.0005) in the group of ≤60, 61-79 and ≥80 year-old patients respectively (p=0.12 for 61-79 vs. ≥80). The corresponding 5-year OS was 90%, 65% and 55% (p=0.0001, but p=0.048 for 61-79 vs. ≥80). In multivariate analysis of PFS, age ≥80 years had no independent prognostic significance when IPI and lyphocytopenia were taken into account. On the contrary, it was an independent prognostic factor for OS with a relative risk 2.9 (p<0.001) and 2.1 (p=0.01) compared to patients <80 years or 61-79 years respectively. Among patients ≥80 years-old, who received >1 cycle of anthracycline-based CIT, the median RDI and IQR were: Rituximab 85% (78-99), cyclophosphamide 76% (67-86), anthracycline 60% (50-69), vincristine 64% (50-76). Moreover, 6/56 patients did not receive anthracyclines and 2 received only 1 cycle. *Summary/Conclusions.* CIT provides prolonged survival in >50% of DLBCL patients ≥80 years-old. These results are very encouraging even though RDI for chemotherapeutic drugs was low in the everyday clinical practice. Age ≥80 years was not an independent prognostic factor for PFS but only for OS. These observations suggest that the majority of very elderly patients with DLBCL should not be treated with palliative approaches. Instead they should receive as complete R-CHOP-like CIT as possible with curative intent.

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TREATMENT OF SPLENIC MARGINAL ZONE LYMPHOMA WITH RITUXIMAB MONOTHERAPY VS SPLENECTOMY

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Background. Treatment of splenic marginal zone lymphomas (SMZL) is not standardized. Splenectomy has traditionally been considered as the treatment of choice. Recent data indicate that rituximab is highly effective and that it could be considered as initial therapy for SMZL. *Aims.* To compare the efficacy of rituximab monotherapy versus splenectomy in SMZL patients. *Methods.* The studied population included 52 patients with SMZL who were diagnosed and prospectively treated in our Departments between September 2003 and July 2010 with rituximab monotherapy (induction phase at a dose of 375 mg/m^2 per week for 6 weeks and maintenance phase every 2 months for one year) and 26 patients who were diagnosed prior to rituximab period and who were faced by splenectomy only. Rituximab treated patients were evaluated for response 2 months after induction and 2 months after the end of maintenance phase. Demographic features, clinical and laboratory characteristics, type of response, response duration, overall survival, progression-free survival and cause of death were analyzed in all patients. *Results.* Patients characteristics were similar between the two treatment groups. The overall response rate (ORR) to rituximab after the end of induction phase was 92% (46% CR, 19% CRu, 13% PR, 13% CHR). The median time to hematologic and clinical response was 4 and 5 weeks respectively. 31/52 patients have already completed the maintenance phase, 29 of them (81%) sustained their initial response, while 5 improved their response and one progressed. 85% of splenectomized patients achieved clinical and haematological remission. The 5-year OS and PFS for rituximab treated and splenectomised patients was 97±3% and 75±9% (p value=0.01) respectively and 58±10% and 56±11% (p value=0.49) respectively (Table 1). Rituximab seems to confer a survival advantage over splenectomy, although so far no difference was noticed in the 5-year PFS time. One toxic death was recorded in the splenectomy arm. In the rituximab arm grade 2 neutropenia was noticed in one patient, grade 3 thrombocytopenia in another, while one patient could not complete therapy due to severe hypotension. *Conclusions.* Rituximab is a highly effective and well tolerated therapy and it can substitute splenectomy as the treatment of choice for SMZL.

Table 1. Rituximab vs splenectomy in SMZL pts.

Treatment	Pts (#)	ORR (%)	CR (%)	PR (%)	CHR (%)	5-year PFS (%)	5-year OS (%)
Rituximab	52	92	46	13	13	58	97
Splenectomy	26	85	0	0	85	56	75

ORR Overall Response Rate, CR: Complete Response, PR: Partial Response, CHR: Complete Haematological Response, PFS: Progression Free Survival, OS: Overall Survival

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INFLUENCE OF THE TYPE OF IMMUNOSUPPRESSIVE THERAPY IN THE DEVELOPMENT OF PTLD FOLLOWING ORTHOTOPIC LIVER TRANSPLANT: A SINGLE CENTER, LONG-TERM SURVEY ON 1,649 PATIENTS

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Background. Post-transplant lymphoproliferative disorder (PTLD) is a serious complication in solid organ transplant recipients. The incidence is quite variable, and the role of the immunosuppressive treatments on the risk of PTLD development remains to be elucidated. *Aims.* To evaluate the frequency and the risk factors of PTLD in a large series of Orthotopic Liver Transplant (OLT). *Patients and Methods.* Data have been collected on 1,803 OLT performed in 1,649 patients at the Liver Transplant Center in Torino, Italy, during the period 1990 - 2008. OLT was performed in patients aged up to 65 yr. old, with 60 pediatric patients (age at OLT < 12 ys) and two patients aged 65 to 68 yr. The most common indications for OLT were viral cirrhosis, biliary disease and alcoholic cirrhosis. Cyclosporine A (CsA) has been used as primary immunosuppression in most patients; in the last few years, tacrolimus has been increasingly employed as primary immunosuppressive drug; steroids are usually associated to Calcineurin-inhibitors. Overall, 1,189 (73.8%) patients received CsA in prevalence, 423 (26.2) had tacrolimus in prevalence. PTLD were diagnosed by histology and immunophenotipical analysis on biopsy specimens. Several parameters were evaluated for possible association with PTLD occurrence, including age, sex, liver disease and HCV state, presence of hepatocellular carcinoma, time elapsed from OLT to PTLD, CsA vs tacrolimus use, other drugs for graft rejection. The cumulative incidence of PTLD was determined using the Fine and Gray competing risk regression model. Treatment for PTLD included: i. reduction or discontinuation of the immunosuppression; ii. chemotherapy and/or radiotherapy (15 cases); Rituximab was delivered to 11 patients (combined with chemotherapy in 6). *Results.* At a median follow-up of 5.8 yrs. (range 0.1-17.5)., 1,298 (78.7%) patients are alive, with a 5-yr Overall Survival projection of 79.5 %. So far, 20 PTLD have been recorded, with a cumulative incidence of 0.94, 1.57 and 3.03% at 5, 10 and 15 yrs, respectively. Median time of PTLD occurrence was 32 mos. (range 2-155) since OLT. On competing risk multivariate analysis, the use of tacrolimus vs. CsA was the main factor associated with increased risk of PTLD (SDHR: 2.54, p=0.041). Despite the increased PTLD incidence, the overall risk of death was significantly lower with tacrolimus compared to CSA. An increased risk of PTLD was also observed in the 83 patients receiving OKT3, with a SDHR of 3.77, p=0.013. None of the other parameters had any significant impact on PTLD development. Treatment of PTLD resulted in good response in most patients and at a median follow up of 4.85 yrs., 17 out of 20 patients (85%) are alive. *Conclusions.* The overall incidence of PTLD in this large series of OLT is among the lowest reported so far in patients receiving solid organ transplant; the use of tacrolimus is shown as a significant risk factor for PTLD; nevertheless, the addition of tacrolimus significantly increased the life expectancy following OLT; the study confirms the improved outcome of PTLD and the availability of Rituximab is quite likely to have contributed to the prolonged survival observed.

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THE USE OF INTERIM 18FDG-PET IS NOT JUSTIFIED IN DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) DURING FIRST LINE IMMUNO-CHEMOTHERAPY

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Background. In diffuse-large-B-cell-lymphoma (DLBCL), the response to first line immuno-chemotherapy remains somewhat unpredictable. Interim 18FDG-PET (PET-int) analysis could fulfill this important goal, aiding early shift to intensified regimens. *Methods.* We prospectively evaluated the ability of PET-int carried out at mid-treatment of standard immuno-chemotherapy in predicting relapse in a series of 83 consecutive DLBCL. PET-int results were dichotomized as positive or negative using the recently validated five-point-scale scoring system. This

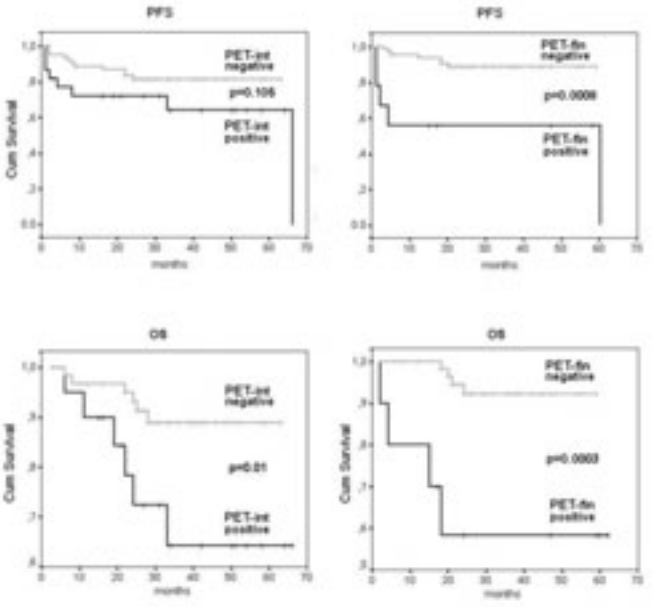

Figure 1. On the left: progression free survival (PFS) and.

exam was also compared with interim computerized tomography (CT-int) and final PET (PET-fin). End-points were: complete remission (CR); positive predictive value (PPV) of refractoriness and relapse; negative predictive value (NPV); overall survival (OS) and progression free survival (PFS). Observation time was fixed to 24 months unless a DLBCL-related event. *Results.* The PPV of PET-int was 63.6% and the NPV was 81.9%. Within PET-int positive patients who underwent targeted biopsy the incidence of faulty positive results due to inflammation was 57%. The achievement of CR was correlated with both PET-int and CT-int (p<.0001), but in multivariate analysis only CT-int was correlated with CR (p=.0002). CT-int and PET-fin were predictive of both OS and PFS, whereas PET-int was predictive only of OS (p=.018), but not of PFS (p=.105). In Cox-regression only PET-fin was predictive for both OS (p=.002) and PFS (p=.006). *Conclusions.* PET-int resulted unable to discriminate chemo sensitive patients who will later relapse. Therefore we think that the use of this expensive and radioactive tool is not justified as an interim analysis.

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CLINICAL SIGNIFICANCE OF METABOLIC TUMOR VOLUME BY 18F-FDG PET IN THE STAGE II AND III OF DIFFUSE LARGE B CELL LYMPHOMA

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Background. Aggressive Non Hodgkin's Lymphoma (NHL) has been staged according to the Ann-Arbor staging system, which originally designed for Hodgkin lymphoma (HL). Because of the heterogeneity and hematogenous spread pattern of dissemination in NHL in contrast to contiguous lymphatic spread with HL, Ann-Arbor staging system has a limited value in the context of assessment of accurate tumor burden in NHL. *Aims.* The objective of the present study is to investigate whether metabolic tumor volume (MTV) by PET can be a potential prognostic tool compared with Ann-Arbor stage in the patient with stage II and III nodal diffuse large B cell lymphoma (DLBCL). *Methods.* One hundred sixty patients with de novo nodal DLBCL whom underwent PET-CT at diagnosis were enrolled for the present study. All patients received 6 to 8 cycles of R-CHOP therapy by Coiffier *et al.* Median follow-up duration was 36 months. MTV was delineated on the PET images by a circle encompassing regions equal or greater than standard uptake volume (SUV) 2.5 in involved Lymph Node (LN) and each slice was determined by multiplying the area within the threshold margin by CT interval. The final MTV was calculated by adding all MTA of each slice using by fusion software (Syntegra, version 2.1E, Philips Co.). *Results.* The differences of several prognostic factors between stage II and III

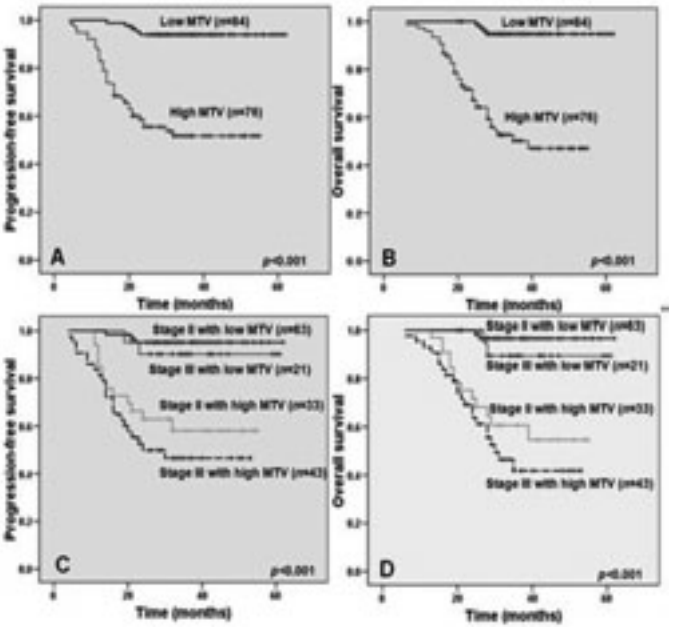

Figure 1. Comparisons of survival according to MTV and stage.

groups were not present. 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. 169.3cm3 of various values acquired a sensitivity of 91.7% and specificity of 65.3%. The outcomes were compared among the four subgroups based on tumor burden and stage II or III. High MTV group regardless of stage had lower PFS and OS pattern compared to low MTV group (PFS & OS in stage II with low MTV, 95.2% & 96.8%; in stage III with low MTV, 90.5% & 90.5% versus in stage II with high MTV, 60.6% & 60.6%; in stage III with high MTV 48.8% & 48.8%; p<0.001, p<0.001) whereas prognostic impact of stage in same MTV group was absent. (in the low MTV group, difference of PFS and OS according to stage, p=0.431, p=0.261; in the high MTV, p=0.277, p=0.28, Figure 1). Multivariate analysis using Cox proportional hazard model was performed for high MTV and stage III. In the analysis high MTV was an independent factor predicting an unfavorable outcome (PFS, HR=9.243, 95% CI=3.543-24.115, p<0.001; OS, HR=11.660, 95% CI=4.062-33.465, p<0.001) whereas stage III had not significant value (PFS, HR=1.513, 95% CI=0.792-2.888, p=0.210; OS, HR=1.556, 95% CI=0.806-3.003, p=0.188). *Conclusion.* The present study suggests that total tumor burden of lymphoma is a more important prognostic parameter rather than Ann-Arbor stage in DLBCL. Our data reflect that Ann-Arbor staging system has limited worth in DLBCL due to heterogenous spread pattern of NHL. Therefore, simply classifying for prognosis according to diaphragm would be not wise at least for DLBCL in rituximab era.

Chronic lymphocytic leukemia - Genes and microenvironment

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MISSENSE MUTATIONS LOCATED IN STRUCTURAL P53 DNA-BINDING MOTIFS ARE ASSOCIATED WITH EXTREMELY POOR SURVIVAL IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. There is a distinct connection between TP53 defects and poor prognosis in chronic lymphocytic leukemia (CLL). It remains unclear whether patients harboring TP53 mutations represent a homogenous prognostic group. p53 missense mutations involved in a direct or indirect contact with the target DNA, have been shown to be associated with a particularly poor survival in, e.g., breast cancer or diffuse large B-cell lymphoma. *Aims.* To analyze an impact of specific p53 mutations on prognosis of CLL patients. *Methods.* We evaluated the survival of CLL patients with p53 defects identified at our institution by p53 yeast functional assay (FASAY) and complementary I-FISH analysis detecting del(17p) from 2003-2010. *Results.* A defect of the TP53 gene was identified in 100 of 550 patients. p53 mutations (n=96 patients) were strongly associated with the deletion of 17p and the unmutated IgVH locus (both P<0.001). The patients who had p53 mutation but also the mutated IgVH gene (range of homology 92.4%-97.9%) manifested substantially better survival than p53-mutated patients with the unmutated IgVH (homology ≥98%) (P=0.018). We therefore omitted the small subgroup (n=11) of p53-affected patients with mutated IgVH gene from the subsequent analysis, as their survival data would be misleading. In line with this, only the wt-p53 patients harboring the unmutated IgVH gene were used as a control group in all subsequent survival evaluations. Survival assessed from the time of abnormality detection (or investigation showing wt-p53) was significantly reduced in patients with both missense (P<0.001) and non-missense p53 mutations (P=0.004) in comparison with wt-p53 patients. In addition, patients harboring missense mutation located in p53 DNA-binding motifs (DBM), structurally well-defined parts of the DNA-binding domain, manifested a clearly shorter median survival (12 months) compared to patients having missense mutations outside DBM (41 months; P=0.002) or non-missense alterations (36 months; P=0.005). The difference in survival was very similar in the analysis limited to patients harboring mutation accompanied by del(17p) (n=50). A subset of p53 mutations was identified already at diagnosis. The p53 DBM mutations (n=12) once again resulted in a very short survival of only 17 months (P<0.001; hazard ratio to p53-wt patients 20.8; 95% CI 8.82-48.82); patients with the remaining p53 mutations (n=16) manifested 51 month median survival (P<0.001; hazard ratio to p53-wt patients 5.3; 95% CI 2.41-11.69); a median survival of wt-p53 patients was 110 months. The patients with p53 DBM mutation at diagnosis also manifested very short median time to first therapy (TTFT) (1 month); the remaining p53-affected patients had TTFT 6 months and p53-wt patients 19 months. *Summary/Conclusions.* The substantially worse survival and the short TTFT suggest a strong mutated-p53 gain-of-function (GOF) phenotype in CLL patients with DBM mutations. The impact of p53 DBM mutations on prognosis and response to therapy should be analyzed in investigative clinical trials. CLL patients with p53 DBM mutations should be primary candidates for allogeneic stem cell transplantation as their long-term survival is improbable. Supported by grants NS9858-4/2009, NS10439-3/2009, and NS10448-3/2009 (IGA MH, CZ), Research Proposal MSM0021622430 (MEYS, CZ), and the European Research Initiative on CLL (ERIC).

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13Q14 DELETION LOAD AND SIZE BOTH CONTRIBUTE TO REFINE PROGNOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background. Deletion at 13q14 (del13q) is detected by fluorescence in-situ hybridization (FISH) in about 50% of CLL. Although CLL patients with del13q as the sole cytogenetic abnormality (del13q-only) usually have a good prognosis, more aggressive clinical courses are reported for del13q-only CLL bearing high percentages of deleted nuclei. Moreover, del13q of different sizes have been described, the prognostic significance of which is still unknown. *Aims.* to investigate the prognostic significance of del13q by a FISH approach in 342 del13q-only cases and in 265 consecutive unselected CLL cases. *Methods.* FISH at diagnosis was performed using the following probes: LSI-D13S319 (detecting the *DLEU2/MIR15A/MIR16-1* region), LSI-RB1, LSI-ATM, LSI-p53, CEP12. *Results.* Maximally selected log-rank statistics identified the 70% of deleted nuclei as the most appropriate cut-off capable of separating del13q-only cases into two subgroups with different time-to-first-treatment (TTT, p=0.0022). Del13q-only CLL with ≥70% of deleted nuclei showed a shorter TTT than <70% del13q-only cases (median TTT 77 versus 120 months, p=0.0001). One hundred and thirty-five of 342 del13q-only cases (39.5%) had 13q deletions that included the *RB1* locus in at least 5% of nuclei (delRB1). Genomic profiles using Affymetrix GeneChip Human SNP6 arrays in 67 cases showed that larger deletions involving the *RB1* locus occurred in a proportion of del13q-only cases (34.3%) and were always monoallelic (median size 2,380 Kb versus 1,200 Kb for del13q without delRB1). We classified del13q-only cases combining both deletion load and size: i) del13q-only cases bearing del13q in <70% of nuclei without delRB1 (del13q<70%, n=144); ii) del13q-only cases bearing del13q in <70% of nuclei with delRB1 (del13q<70%+delRB1, n=95); iii) del13q-only cases bearing del13q in ≥70% of nuclei without delRB1 (del13q≥70%, n=64); iv) del13q-only cases bearing del13q in ≥70% of nuclei with delRB1 (del13q≥70%+delRB1, n=39). The median TTT of del13q<70% cases (not reached) was significantly longer than the median TTT of del13q<70%+delRB1 (92 months, p=0.012), del13q≥70% (68 months, p<0.0001) and del13q≥70%+delRB1 cases (82 months, p=0.0025). The presence of delRB1 in del13q<70% CLL was associated with a hazard ratio (HR) for progressive disease of 1.91 (95% CI 1.18-3.08; p=0.008), whereas no additional prognostic information was provided in del13q≥70% cases (HR=0.87, 95% CI 0.50-1.51; p=0.63). In multivariate analysis, the presence of delRB1 increased the risk of progressive disease of del13q<70% cases (HR=1.69, p=0.036), independently of Rai staging and *IGHV* status. In 265 consecutive unselected CLL, the presence of del13q in <70% of nuclei in the absence of delRB1 identified patients with particularly stable and benign clinical courses (n=48; median TTT not reached). Conversely, del13q<70%+delRB1 cases (n=24), or patients characterized by a del13q in ≥70% of cells (with or without delRB1, n=25) or a normal karyotype (n=75) had shorter median TTT intervals (range 105-129 months, p<0.01 in all comparisons). Finally, patients bearing trisomy 12 (n=48), 11q deletion or 17p deletion (n=45) experienced the worst clinical course (p<0.0001). *Conclusions.* FISH analysis of both deletion load and size allowed to reveal the prognostic heterogeneity of del13q-only cases. A novel prognostic flowchart involving sequential hybridization with the LSI-D13S319 and LSI-RB1 probes can be proposed.

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FUNCTIONAL CD49D/CD38 MOLECULAR ASSOCIATION IS DETRIMENTAL FOR CLINICAL OUTCOME IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. CD49d and CD38 are independent negative prognosticators, whose expressions are often correlated on chronic lymphocytic leukemia (CLL) cells. We recently reported a functional link between CD49d and CD38 as component of a pro-survival circuitry operating in CD49d+CD38+ CLL. This network includes in sequence the CD38/CD31 pair, the CCL3/CCL4 chemokines with the respective receptors, and the CD49d/VCAM-1 axis. *Aims.* The nature of this circuit was studied by investigating the physical association between CD49d/CD38 and their functions on CLL cell membranes; the validity of the hypothesized prognostic relevance of CD49d/CD38 co-expression was tested in a wide cohort of CLL patients. *Methods.* The organization of CD49d and CD38 on the membrane of primary CLL cells was investigated by confocal microscopy and biochemical assays. Mec-1, a CD49d+CD38- cell line derived from CLL cells was used in a comparative way with a clone where CD38 expression was induced by transfection (Mec-1/CD38+). These cells were analysed in terms of adhesion potential. Data on CD49d and CD38 expression along with time-to-first-treatment (TTT) were available for 564 patients. *Results.* Co-capping and immunoprecipitation experiments in CD49d+/CD38+ CLL cells demonstrated a clear membrane relationship between CD49d and CD38. Co-localization between the molecules was maintained when CLL cells adhered and spread onto VCAM-1 and fibronectin, the CD49d ligands. This finding is an indication that the CD49d/CD38 association is also functional. This issue was answered by designing adhesion assays on VCAM-1-coated plates using the Mec-1 model. Mec-1/CD38+ showed a marked increase in VCAM-1 adhesion compared to Mec-1 (adherent cells relative to control=5.4 vs. 2.3, and 5.4 vs. 1.9 at 15 and 30 min, respectively; p<0.05). Phase-contrast microscopy highlighted the existence of significant differences in the morphology of adherent cells; indeed, Mec-1/CD38+ cells were characterized by a more complex uropod pattern than Mec-1, suggestive of cytoskeleton re-organization. The last tested issue dealt with the influence of CD49d and CD38 co-presence on the network of apoptosis. To this aim, we checked whether adhesion to VCAM-1 might influence the apoptosis promoted by serum deprivation in Mec-1 and Mec-1/CD38+ cells. After 4-5 days of culture on VCAM-1-coated wells without serum, the mean values of Mec-1/CD38+ viable cells (62%±4.8, day 4, and 55%±3.2, day 5) were significantly higher than those observed in the Mec-1 sample (38%±4.2, day 4, and 36%±2.2, day 5; p<0.001 and p=0.003 respectively). To evaluate the clinical relevance of these observations, we compared the TTT in 564 CLL patients (303 CD49d-/CD38-, 125 CD49d+/CD38+, 95 CD49d-/CD38+, and 41 CD49d+/CD38-). Patients CD49d+/CD38+ were characterized by TTT significantly shorter as compared with patients expressing only CD38 (p=0.01) or CD49d (p= 0.001), the longest TTT being however observed in CD49d/CD38 double negative cases. *Conclusions.* The association between CD49d and CD38 on the CLL membrane is not only physical, but also functional. The validity of this conclusion is corroborated at a clinical level: the analysis of a simultaneous CD49d/CD38 expression produce a significant refinement of the prognostic potential provided by any of the two factors independently analysed.

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INTERACTION BETWEEN ENDOTHELIUM AND CHRONIC LYMPHOCYTIC LEUKEMIA B-CELLS RESCUES FROM APOPTOSIS AND MODULATES GENE EXPRESSION PROFILE OF LEUKEMIC CELLS

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Background. Despite an apparent long life *in vivo*, CLL cells die rapidly *in vitro*. This observation suggests that the apoptotic resistance is not intrinsic to leukemia B cells but extrinsic factors are necessary for CLL prolonged survival. *Aims.* we investigated the interactions between endothelial cells and CLL cells, highlighting molecular networks involved in this cellular crosstalk. *Methods.* we co-cultured CLL cells on HUVEC endothelial monolayer (HC) or in medium alone (CLL only). Then, we detected CLL viability by flow cytometry and we performed whole-genome high density microarrays. *Results.* we found that endothelial cells protected CLL from spontaneous apoptosis. After 48h, increased number of alive CLL cells was present in HC condition (59.7 ± 4.2%) compared to CLL alone (22.9 ± 5.1%) ($p<0.0001$). Moreover, we found that spontaneous *in vitro* apoptosis was higher in unmutated IGHV CLL (UM-CLL) compared to mutated ones (M-CLL). In HC condition, similar survival was detected between M-CLL and UM-CLL, implying a 2.2-fold increase in relative viability in M-CLL and a 6.1-fold increase in UM-CLL. Moreover, the endothelial cell layer decreased the *in vitro* sensitivity of CLL cells to Fludarabine-induced apoptotic cell death. The mean viability of CLL cells treated with 10 μM Fludarabine was 19.8% (±4.4%) after 48 hours and 3.8% (±1.3%) after 72 hours. In HC with Fludarabine addition, the mean viability of CLL cells was 37.8% (±9.1%) after 48 hours and 14.3% (±3.2%) after 72 hours. Then, we compared gene expression profiles (GEP) between CLL cultured in contact with EC layer and CLL at baseline to unravel the transcriptional modifications induced by EC cells. Overall 1944 genes were found to be modulated ($FC\geq2$, $p<0.05$). CLL cells in HC condition showed a 22.6-fold increase of CCL2, able to recruit tumor-activated monocytes ($p=0.0032$) and a 6.5-fold increase of PDGFC, chemoattractant for mesenchymal stromal cells ($p=0.0051$). Other soluble factors up-regulated by EC/CLL contact were VEGFC (FC=9.4, $p=0.0061$), ANGTL4 (FC=8.6, $p=0.015$), EDN1 (FC=9.2, $p=0.0061$), AMOTL2 (FC=4.3, $p=0.019$) and THBS1 (FC=45.1, $p=0.0004$) as well as the metalloproteases MMP2 (FC=8.3, $p=0.02$) and MMP4 (FC=3.0, $p=0.039$). The GEP data were confirmed by evaluating the secreted levels of soluble factors in conditioned medium collected after 48h-HC culture. In addition, CLL cells on endothelial layer maintained or increased the expression levels of anti-apoptotic factors Bcl-2, Bcl2A1, BIRC3/c-IAP2 and BIRC5/Survivin compared to CLL cells at baseline. Of interest, the Ang2 tyrosine kinase receptor Tie2 mRNA was found to be increased in CLL cells in co-culture (FC=10.7, $p=0.017$). We confirmed GEP data by flow cytometry finding a 2-fold and a 4.3-fold increase of percentage of Tie2+CLL cells at 48h and 72h in HC. *Conclusion.* our results demonstrate a role of endothelial cells in CLL survival advantage and Fludarabine-resistance. The intimate contacts with EC seem to determine a microenvironmental-driven angiogenic switch of CLL phenotype, improve the secretion of cytokines involved in regulation of microenvironmental elements such as stromal cells and macrophages and increase the expression of anti-apoptotic molecules.

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ANALYSIS OF AUTOPHAGY IN B-CLL SAMPLES REVEALS INCREASED BASAL AUTOPHAGY WHEN COMPARED TO NORMAL B-CELLS

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Background. B-cell chronic lymphocytic leukaemia (B-CLL) is the most common leukaemia in the Western World. It is characterized by the accumulation of long-lived mature B cells. Autophagy is the process of cell component removal whereby cellular elements are encompassed in vesicles and transported to lysosomes for degradation. Autophagy has a role in both cell survival and apoptosis, and is involved in antigen presentation in mature B-cells. Deregulation of autophagy has been implicated in various cancers including leukaemia, although it has not been described yet in B-CLL. *Aims.* To determine if B-CLL patients have recurrent abnormalities in autophagic genes and if so, identify the affect of these abnormalities on the autophagy process. *Methods and Results.* We tested DNA samples from 212 patients with B-CLL using high-resolution SNP 1M-Duo arrays and analyzed the data using Nexus Biodiscovery software. The results were compared against the Database of Genomic Variants and data from the Wellcome Trust Case Control Consortium, as well as from germline samples in selected cases. Amongst the abnormalities noted were recurrent abnormalities involving important autophagy genes (ATG5, ATG6 and ATG7). Whole genome sequencing of two patients with B-CLL also detected mutations in a gene involved in the regulation of autophagy (NOD1). Next we investigated the autophagin flux in B-CLL using the Amnis Imagestream, which combines flow cytometry with cell imaging, allowing simultaneous assessment of morphological characteristics alongside fluorescence signals of large numbers of cells. This makes it an ideal instrument to calculate co-localisation of autophagosomal markers while identifying a subpopulation of cells by their surface markers. We analysed CD5+CD19+ B-CLL cells from eight patients (including one with an ATG5 deletion, 2 with TP53 abnormalities, one with a NOD1 mutation and 4 without genetic autophagic abnormalities) and CD19+ B-cells taken from age-matched controls. Cells were incubated under standard conditions for 2 hours either with or without lysosomal inhibitors prior to staining with antibodies for CD19, CD5, lysosome and LC3. We used the co-localization of LC3 and lysosome as a marker of autolysosomal formation and thereby autophagic flux. Autophagic flux was significantly higher in B-CLL samples when compared with normal B-cells, implying that B-CLL cells have a higher basal autophagy level than normal B-cells. Surprisingly, these increased levels were observed in the B-CLL samples both with and without abnormal autophagy genes. *Conclusion.* This is the first time that increased autophagy levels have been demonstrated in B-CLL. Investigations to determine whether this pathway might provide a mechanism of survival and perturbance of programmed cell death and therefore represent a new drug target in B-CLL are ongoing.

Myelodysplastic syndromes - Biology & clinical

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GENOMIC ALTERATIONS ASSOCIATED WITH BONE MARROW FAILURE AND PROGRESSION TO LEUKEMIA IN THE BONE MARROW OF DNA-REPAIR DEFICIENT MICE

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Background. Bone marrow (BM) failure may arise from (inherited) mutations or develop through sustained exposure to cytotoxic stresses. Secondary leukemia's arising from these BM failures are often refractory to current treatments. We focus on genotoxic-stress induced bone marrow failure, such as seen in Fanconi Anemia, Nijmegen breakage syndrome and therapy-related myelodysplastic syndrome/AML. These diseases have a high risk of progression to leukemia. We use a mouse model deficient for Ercc1, an enzyme involved in the repair of interstrand crosslinks (ICLs). In the absence of efficient repair, ICLs result in stalled replication forks and cause cell cycle arrest, mediated by activation of the cell-cycle gatekeepers p16/p19 and/or p53. We have previously shown that Ercc1-deficient mice have a strong reduction in hematopoietic stem- and early progenitor cells (LSK) throughout their lifespan of ~20 weeks. LSK levels are restored in mice deficient for both Ercc1 and p53. Ercc1-deficient mice do not develop leukemia during their lifespan, but when we transplanted Ercc1-deficient BM cells that were heterozygous for *Trp53* into lethally irradiated recipients more than 57% of the animals got leukemia and an additional 37% died prior to diagnosis. *Aims.* Our aim is to dissect the early and sequential molecular defects that occur in stem cells from BM failure to pre-leukemia and contribute to the development of overt leukemia. *Methods.* To monitor genetic changes in different maturation stages we determined gene expression changes in immature (LSK) and mature (BM) hematopoietic cells of 20-week old Ercc1-deficient and control mice. To determine genomic alterations associated with BM failure, we sequenced the exome of the BM 20-week old Ercc1-deficient animals and of Ercc1-deficient fetal liver cells, which contain normal LSK levels. *Results.* Ercc1-deficient BM cells show an up-regulation of the cell-cycle gatekeepers p16, p19 and the p53-targets *PUMA* and *NOXA*, which induce apoptosis. In contrast, none of these transcripts were up-regulated in purified LSK cells of Ercc1-deficient mice. Instead, LSK cells showed up-regulation of p21, which is involved in cell cycle arrest and plasminogen activator inhibitor -1 (*PAI-1*), which is a marker for senescence. In the sequences of two exomes of Ercc1-deficient BM we found 1170 and 780 exonic mutations, of which 360 were found in common. These mutations were acquired during the bone marrow failure, as they were not found at the fetal liver stage nor were they present in the mouse SNP database. We will next sequence the leukemia's arising from Ercc1-deficient BM that is heterozygous for *Trp53* to identify leukemia-associated genomic aberrations and compare them to genetic aberrations already present at the BM failure state. *Summary/Conclusions.* The gene expression response to ICL-damage in LSK cells is directed at survival by inducing cell cycle arrest/senescence without apoptosis, while in mature cells apoptotic genes are up-regulated. While BM failure is thought to prevent survival of cells with altered genomes, strikingly we found 360 common exonic mutations in two samples of Ercc1-deficient BM. Our mutational analysis is expected to elucidate critical players involved in early and late stages of leukemogenesis from DNA-damage induced BM failure.

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ACE-536, A MODIFIED TYPE IIB ACTIVIN RECEPTOR PROMOTES ERYTHROID DIFFERENTIATION IN AN EPO INDEPENDENT MANNER AND PREVENTS ANEMIA IN MYELODYSPLASTIC SYNDROME

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Myelodysplastic syndromes (MDS) are stem cell disorders characterized by impaired hematopoiesis and cytopenias. The majority of MDS patients present with anemia that is often refractory to treatment with erythropoietin (EPO). As an alternative approach to treating anemia in MDS patients, we have targeted the TGF β superfamily. This group of molecules has been implicated in erythropoiesis. We have developed a soluble receptor fusion protein consisting of a modified form of activin receptor IIB extracellular domain linked to a human Fc region (ACE-536) that generates a robust increase in RBC's in several animal models. The aim of this study is to investigate which erythrocyte precursors are affected by ACE-536 and to evaluate the efficacy of RAP-536 (murine analogue of ACE-536) in treating anemia in a murine model of MDS. Subcutaneous administration of ACE-536 (10mg/kg) to C57BL/6 mice resulted in a significant increase in hematocrit, hemoglobin and red blood cells as compared with the vehicle (VEH) group within 4 days. These effects were observed in the presence of an EPO neutralizing antibody suggesting that EPO is not directing the initial RBC response. BFU-E or CFU-E colony formation assays from bone marrow or spleen were also carried out at 48hrs following ACE-536 treatment and found that ACE-536 does not effect the erythroid progenitor population compared to VEH treatment. Differentiation profiling of bone marrow and splenic erythroblasts by FACS analysis following 72 hours after ACE-536 treatment revealed a decrease in basophilic erythroblasts and an increase in late stage poly-, ortho-chromatophilic and reticulocytes in bone marrow and spleen compared to VEH treated mice. The data demonstrate a novel mechanism of ACE-536 promoting maturation as contrasted to the noted effect of EPO which increases proliferation of BFU-E and CFU-E. To investigate the therapeutic potential of RAP-536 in a model of MDS, four month old NUP98-HOX13 transgenic mice (10/dose group) were treated with vehicle (VEH) or RAP-536 (10 mg/kg) twice per week. Wild-type littermates (10/dose group) were dosed with VEH or RAP-536 (10 mg/kg) and used as controls. Prior to the first dose (Day 0), the MDS mice had significantly decreased levels of RBC (-8.8%, P<0.05), hematocrit (-8.4%, P<0.05) compared to their wild-type control littermates. After 7 months of dosing, MDS mice treated with RAP-536 had increased RBC counts (+13.8%, P=0.09), hemoglobin (+19.8%, P<0.05) and hematocrit (+14.8%, P=0.05) in compared to VEH treated controls. These results demonstrate that ACE-536 may represent a novel therapy for severe anemia for patients with Myelodysplastic Syndrome.

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MORPHOLOGICAL DIFFERENTIATION OF HYPOCELLULAR REFRACTORY CYTOPENIA OF CHILDHOOD AND SEVERE APLASTIC ANEMIA AND CLINICAL OUTCOME

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Bone marrow failure syndromes of childhood comprise a heterogenous group of inherited and acquired hypocellular bone marrow conditions. Severe aplastic anaemia (SAA) and hypoplastic refractory cytopenia (RC), a subtype of childhood myelodysplastic syndrome (MDS) are the main and most difficult differential diagnoses. In the German childhood SAA 94 study the probability to develop clonal disease, particularly MDS and AML, was 23% after immunosuppressive therapy. To investigate whether the first morphological diagnosis has an impact on the outcome with respect to clonal disease, we established distinct morphological criteria to differentiate between SAA and RC. Only bone marrow trephines and smears with hematopoietic aplasia were diagnosed as SAA, whereas cases even with only small foci of MDS typical morphology. i.e. patchy erythropoiesis with defective maturation, in an otherwise adipocytic bone marrow where classified as RC. The participating centers of the European Working Group on Childhood myelodysplastic syndrome (EWOG-MDS) and German SAA 98 study established a central morphological review. Since introduction of the central review in 1998 the probability to develop clonal disease has continuously dropped to 3% (p<0.01). We performed a

double-blinded inter observer study of 100 different cases of SAA and RCC among 7 haematopathologists. Only in 4 out of 100 cases no agreement could be achieved whether to classify SAA or RC. The kappa-index was 0.79 indicating that the vast majority of SAA and RC cases can be reliably differentiated by morphological means only. Our results suggest that the main reason for development of MDS or AML after immunosuppressive therapy of SAA might be the initial morphological differentiation of SAA and RC in childhood and that a clear morphological differention reduces the secondary cases of MDS and AML after immunosuppressive therapy significantly.

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A PHASE IB STUDY OF PANOBINOSTAT IN COMBINATION WITH 5-AZACITIDINE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES, CHRONIC MYELOMONOCYTIC LEUKEMIA, OR ACUTE MYELOID LEUKEMIA

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Background. Panobinostat, a pan-deacetylase inhibitor (pan-DACi), has demonstrated anti-tumor activity in patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Preclinical studies suggest the combination of a hypomethylating agent such as 5-azacitidine (5-aza) and a pan-DACi may, at least in part through gene reactivation and apoptosis induction, improve outcomes in patients with MDS and AML. *Aims.* This phase I, open-label, multicenter, dose-escalation study will assess the maximum tolerated dose (MTD) and safety of panobinostat in combination with 5-aza in patients with MDS, chronic myelomonocytic leukemia (CMML), and AML. *Methods.* Inclusion criteria of this ongoing study are: adult patients with IPSS intermediate-2 or high-risk MDS, CMML, or AML with multi-lineage dysplasia and 20-30% marrow blasts not eligible for hematopoietic stem-cell transplantation. Oral panobinostat (20 mg/day starting dose) is administered during a 28-day cycle on Days 3, 5, 8, 10, 12, and 15 in combination with a fixed dose of 5-aza (75 mg/m^2 sc) on Days 1-7 of each cycle. Safety and tolerability are described as type, duration, frequency, relatedness, and severity of adverse events (AEs) according to CTCAE, v3.0. The adaptive Bayesian logistic regression model is used to guide panobinostat dose escalation with overdose control at all dose levels. The minimum exposure criteria for determining MTD is 6 scheduled doses of panobinostat and 7 scheduled doses of 5-aza during cycle 1. *Results.* 18 patients, median age 69 years (range 34-80), have been enrolled to date including 13 with MDS, 3 with AML, and 2 with CMML. Patients were evaluated at 3 different panobinostat doses: 6 (20 mg), 5 (30 mg), and 7 (40 mg). The AEs analysis is based on currently available data of all 18 patients. AEs of all grades, regardless of study drug relationship, included nausea (12 [67%]), vomiting and diarrhea (11 each [61%]), fatigue (10 [56%]), decreased appetite (9 [50%]), and asthenia (6 [33%]). Grade 3/4 treatment-related AEs included thrombocytopenia (4 [22%]) and febrile neutropenia (3 [17%]). Serious AEs, regardless of study drug relationship, included febrile neutropenia and asthenia (4 each [22%]). One dose-limiting toxicity (DLT) was observed (grade 4 febrile neutropenia) in the 20 mg panobinostat cohort and two DLTs (grade 3 hyperbilirubinemia; grade 3 nausea and asthenia) in the 40 mg cohort. *Summary/Conclusions.* Current data show that the addition of panobinostat to 5-aza therapy is safe with no unexpected toxicities. To date, the most common AEs are gastrointestinal events and fatigue. The most common grade 3/4 treatment-related AEs include febrile neutropenia and thrombocytopenia, with one DLT observed in the 20 mg cohort and two in the 40 mg cohort. Based on the occurrence of two DLTs and one withdrawal of consent following grade 3 fatigue in the 40 mg cohort, the decision was made to enroll additional patients at a lower dose level to further assess safety and tolerability at 30 mg. In parallel, the ongoing patients at 40 mg are monitored for long term tolerability. Updated data, including preliminary efficacy data, will be presented at the meeting.

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COMORBIDITIES INFLUENCE PROGNOSIS IN MDS HIGH-RISK PATIENTS TREATED WITH 5-AZACITIDINE

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Backgrounds. Myelodysplastic syndromes (MDS) are affecting mainly elderly patients, and age is considered per se a negative prognostic factor. Most patients have comorbidities that presumably impact negatively overall survival and quality of life, and may influence response to therapy. It was recently demonstrated that MDS patients aged > 75 yrs treated with azacitidine have a significantly longer overall survival respect to best supportive care treated patients. *Aims.* We wanted to analyze whether the presence of comorbidities could have an impact on survival, response and management of azacitidine treatment in MDS patients, in clinical practice. We also evaluated patient outcome, type of response according to IWG criteria 2006, as well as adverse events and cause of death. *Methods.* We analyzed 103 MDS patients (IPSS: 30% INT-1, 49% INT-2 and 21% High) treated with Azacitidine 75 mg/mq/day sc for 7 days every 28. Mean number of cycles was 9 (range:2-42). Mean age was 69 yrs (50-82); 30% of patients were >= 75 yrs and 39% of the latter >= 80 yrs; 71% of patients were male. Patients were evaluated by three different geriatric score: Charlson comorbidity index (CCI) (54 % of patients scored 0, 37% of patients 1 or 2, and 9% of patients >= 3), the Cumulative Illness Rating Scale (CIRS) (37% of patients scored 0, 37 % of patients 1 and 26% of patients >= 2) and the Adult Comorbidity Evaluation-27 (ACE-27) (41% of patients scored none, 29% of patients mild, 23 % of patients moderate and 7% severe). The OS of patients treated with azacitidine was compared with that of a diagnosis- and age- matched untreated control group of patients (n= 246) (Italian registry of MDS -AISSM) in whom comorbidities had been evaluated by CIRS score. *Results.* Median overall survival (OS) of our patient cohort was 22 months. Median OS in patients < 75yrs (25 months) and >= 75 yrs (15 months) was not significantly different (p value > 0.160). No correlation was present between comorbidity scores, age and hematological response. OS was strictly depending on scores. OS in patients with higher CCI, CIRS, and the ACE-27 was respectively 6.5 months, 10 months and 8 months vs OS in patients with lower CCI, CIRS and ACE-27 was respectively 20, 22 and 22 months. Overall response rate (HI, CR and PR) was 49%, stable disease was obtained in 37 % of MDS patients. IWG responses did not correlate with age, sex and comorbidity scores. Hematological or non hematological adverse events grade III and IV were presented by 34% and 36% of patients, respectively. Adverse events were uniformly distributed independently from age. *Conclusions.* MDS patients with comorbidities may be treated with success with azacitidine, without any substantial increase in AE and with improvement of OS respect to untreated patients. Nevertheless, comorbidities per se negatively influence OS. Evaluation of comorbidities with validated indexes is an useful and easily applicable tool to refine prognostic evaluation and should be include routinely in patient assessment.

Myeloproliferative disorders - Biology

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DNMT3A MUTATIONS IN MYELOPROLIFERATIVE NEOPLASMS

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Background. An increasing number of gene mutations is being identified in the 'classic' myeloproliferative neoplasms (MPN) essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). For instance, use of array-based techniques led to the identification of mutations in candidate genes involved in epigenetic regulation such as *TET2*, *ASXL1*, and *EZH2*. Most recently, a whole-genome sequencing study in acute myeloid leukemia (AML) uncovered recurrent mutations in 22% of AML patients in another epigenetic regulator, the DNA methyltransferase 3A gene *DNMT3A* (Ley *et al.*, N Engl J Med 2010; 363: 2424-2433). *Aim.* To explore mutations of *DNMT3A* in a series of 115 well-characterized chronic- and blast-phase MPN cases: ET, n=30; PV, n=30; PMF, n=16; secondary MF (SMF), n=4; AML secondary to MPN (sAML), n=35. *Methods.* All coding exons of *DNMT3A* (2-23) were analyzed using direct DNA sequencing. Mutation data on *JAK2* V617F, *MPL* W515L, *CBL* (exon 8-9), *TET2* (exon 3-11), *ASXL1* (exon 12), *EZH2* (exon 2-20), and *IDH1/2* (exons 4) as well as 250K SNP-array profiling data were available in all cases. *Results.* In total, 12 heterozygous *DNMT3A* sequence variants were identified resulting in an overall frequency of 10% (12/115). *DNMT3A* alterations were most frequently detected in SMF (50%, 2/4) and sAML (17%, 6/35), followed by PV (7%, 2/30), PMF (6%, 1/16), and ET (3%, 1/30); they consisted of 8 nucleotide substitutions and 4 frameshift deletions (P264fs, W305fs, R488fs, and D768fs); 2 nucleotide substitutions resulted in direct stop codons (E523* and E477*), whereas 6 represented missense alterations; of these, the amino acid residue R882 was recurrently affected in 4 cases (R882H, n=3; R882C n=1). Somatic origin was confirmed in 5 cases including 2 known (E477* and R882H) and 3 novel (W305fs, R488fs, and E523*) *DNMT3A* mutations. Since presence of germline frameshift deletions is unlikely, tumor-specific origin remained uncertain in 2 missense alterations (N501S and W860R) due to lack of control DNA. Furthermore, none of the *DNMT3A* altered cases harbored *MPL*, *CBL*, *TET2* or *EZH2* mutations. In contrast, *DNMT3A* alterations occurred concurrently with *JAK2* (48%, 7/12), *IDH1/2* (33%, 4/12), and *ASXL1* (8%, 1/12) gene mutations. In SNP array analysis, no distinct patterns of concurrent genomic aberrations could be identified. In terms of clinical data, none of the ET/PV cases showed features of an aggressive clinical course, while outcome of sAML was poor. However, longer observation time and systematic collection of clinical data will be necessary to clarify whether mutated *DNMT3A* is of clinical relevance in chronic- and blast-phase MPN. *Conclusions.* Our data underscore the increasing complexity of MPN pathogenesis due to the growing number of genetic lesions being identified. Based on our data, one might speculate that *DNMT3A* mutations represent an important mechanism for advanced phase disease in a subset of MPN cases. Genotyping of single colonies and functional studies will be necessary to further elucidate the pathogenic interplay between *DNMT3A* and other mutations such as *JAK2*, *IDH1/2*, and *ASXL1* in terms of fibrotic and leukemic transformation.

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ROS1, THE ORTHOLOG OF DROSOPHILA SEVENLESS, REPRESENTS A NEW MOLECULAR DEFECT IN CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML)

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Background. Chronic myelomonocytic leukemia (CMML) is a clonal disorder sharing features of both myelodysplastic syndromes and chronic myeloproliferative neoplasms. Although rare aberrations are reported in CMML, the molecular defects underlying the disease are largely unknow. c-ROS codes for an orphan tyrosine kinase which is abnormally expressed and translocated in brain tumors. The involvement of ROS1 in MPN has never been described. Aims of the study were to evaluate the involvement of c-Ros in the pathogenesis of chronic myelomonocytic leukemia (CMML) and to establish the biological consequences of c-Ros activation. *Methods.* c-Ros expression was evaluated by RQ-PCR in 258 samples. 119 CMML patients were included (76 BM, 71 PB and 4 CD34+ purified samples), 25 samples from PMF (9 BM and 16 PB), 7 ET, 5 PV, 5 JMML, 7 AML and 9 MDS. As control, 23 BM, 14 PB and 7 CD34+ specimens from healthy donors and 5 from patients presenting reactive monocytosis were included. The protein amount and localization was analyzed by WB and immunofluorescence. To assess the causes of ROS1 overexpression, sequence analysis of ROS1 promoter and TK regions were performed. Since ROS1 ligand is still unknown, in order to establish the biological effects of c-ROS overexpression and activation, a chimeric receptor with the transmembrane and intracellular domains of ROS1 and the extracellular domain of EGFR was generated with the specific aim of activating ROS1 with EGF.The chimeric receptor was then transfected in NIH3T3 and HEK293T cells. Transfected and control cells were then stimulated with EGF and proliferation, cell adhesion and apoptosis evaluated.The activation of SOS/Grb/Akt/Erk pathways was investigated. *Results.* We found that ROS1 is quite undetectable in healthy subjects but it is overexpressed in 70% of CMML ($p<0,0001$) in both BM and PB cells with a median value of 2-DeltaDeltaCt of 13391±61895 (range 0-524288) in BM and 2031±4706 in PB. In CD34+ cells from healthy subjects c-ROS is undetectable while in CD34+ cells from CMML patients the mean value is 21136± 6273 (range 15287-29193). Sequence analysis revealed the absence of mutations of c-Ros promoter. SNPs analysis exclude the presence of duplications or deletions of the gene. The EGF induced activation of ROS1 significantly increased the proliferation rate, reduced cell adhesion and reduced apoptosis. In addition it was shown that the biological effects observed after ROS1 activation are mediated in both, cell lines and CMML cells, by SOS/Grb2/Akt/Erk activation. *Conclusion.* This study demonstrates that ROS1 is abnormally expressed in patients with CMML. The abnormal activation of ROS1 is responsible for loss of adhesion, increased proliferation and reduction of apoptosis. ROS1 is responsible for these effects in CMLL by activating Akt and Erk pathway. ROS1 is a TK which may represent a new target for molecular therapies leading us to test the *in vitro* effects of specific ROS1 inhibitors in CMML. daniela.cilloni@unito.it

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IMPACT OF PEG-INTERFERON-ALPHA-2A THERAPY ON THE EVOLUTION OF JAK2 AND TET2 MUTATED CLONES IN POLYCYTHEMIA VERA

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Background. In a phase 2 trial of peg-IFNa-2a, we observed a substantial decrease of JAK2V617F allele burden in granulocytes from 29 patients treated with IFNa, including complete molecular remissions (CMR) in 7/29. Clinical significance of such decrease in JAKV617F allele burden remains unclear. In addition, some studies showed that several mutated clones might coexist in a single patient. We previously reported parallel evolution of JAK2V617F and TET2 mutation during IFNa therapy in a single patient with both mutations. In this patient, JAK2V617F was no longer detectable after IFNa, whereas TET2 mutation was unchanged. In contrast, opposite results (disappearance of TET2 mutation, no change in JAK2V617F allele burden) were recently reported in a single patient treated with IFNa in another clinical trial (Quintas-Cardama, ASH 2010). In order to confirm those findings, we studied the evolution of TET2 mutations during IFNa therapy in a series of 5 patients of our cohort carrying a TET2 mutation, including 4 with both JAK2 and TET2 mutations. *Methods.* We searched for JAK2 and TET2 mutations by genotyping from granulocytic DNA before and after IFNa therapy. Four patients presented both JAK2 and TET2 mutations (including 1 frameshift, and 3 different missense mutations), and one patient had only a TET2 missense mutation (patient #3, Table). Mean duration of IFNa therapy was 47.6 months (range: 41 - 60 months). JAK2V617F allele burden was measured with a quantitative allele specific assay, while mutant TET2 allele burden was estimated

Table 1.

Patients	JAK2V617F baseline	JAK2V617F After IFNa	JAK2V617F % evolution	TET2 mutation baseline	TET2 mutation after IFNa
1	20%	0	-100%	20%	unchanged
2	40%	0	-100%	50%	unchanged
3	Wild-type	NA	NA	50%	unchanged
4	20%	5%	-75%	45%	unchanged
5	100%	45%	-55%	60%	unchanged

from the sequencing analysis. *Results.* All the patients achieved clinical hematological response with IFNa therapy. Respective evolution of JAK2V617F and TET2 mutations are summarized in the Table. Median decrease in JAK2V617F allele burden was 100% (Q1 - Q3: 75% - 100%), and mean decrease was 86% (range: 55 - 100%). These long-term results show that IFNa therapy can induce sustained molecular responses regarding JAK2V617F mutation. In contrast, mutant TET2 allele burden estimated from sequencing analysis was unchanged after IFNa therapy in the 5 patients. *Conclusion.* In this series of PV patients with prolonged exposure to IFNa, we found that IFNa treatment was able to durably reduce the JAKV617F allele burden in PV, including long-term persisting complete molecular remissions. In contrast, TET2 mutated allele burden appeared unchanged after IFNa therapy, even after prolonged exposure to the drug (up to 60+ months). Our result suggest that, although inducing clear clinical responses, and marked decrease in JAK2 mutated clone, IFNa may not be able to eliminate cells bearing mutations in non-signaling molecules such as TET2.

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G6 ALLEVIATES JAK2-V617F MEDIATED MYELOPROLIFERATIVE NEOPLASIA BY PROVIDING SIGNIFICANT THERAPEUTIC EFFICACY TO THE BONE MARROW

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The discovery of the Jak2-V617F mutation in a large percentage of myeloproliferative neoplasm (MPN) patients has been a driving force behind the development of small molecule Jak2 inhibitors. Unfortunately, most drugs have been found to be merely palliative as they have little to no efficacy in the bone marrow and, therefore, cannot alter the natural history of the disease. We recently developed a small molecule Jak2 inhibitor called G6 and found that, in a xenograft model of Jak2-V617F mediated hyperplasia, it had noticeable efficacy in the peripheral blood, spleen, and bone marrow. Here, we hypothesized that it would have similar efficacy in a transgenic mouse model of Jak2-V617F mediated MPN. We implemented such a study and found that G6 provided therapeutic benefit to the peripheral blood in this model as determined by elimination of leukocytosis, thrombocytosis, and erythrocytosis. With respect to the spleen, G6 provided marked therapeutic efficacy as measured by normalization of spleen size and elimination of megakaryocytic hyperplasia. In the critically important bone marrow, G6 normalized the pathologically high levels of pJak2 and pSTAT5. It significantly reduced the megakaryocytic hyperplasia in the marrow and completely normalized the M:E ratio. Most importantly, G6 selectively reduced the mutant Jak2 burden by 67% on average with virtual elimination in one-third of all treated mice. This significant reduction in the Jak2 mutant burden correlated with the presence of G6 in the plasma. Lastly, clonogenic assays using marrow stem cells from the MPN mice revealed a time-dependent elimination of the clonogenic growth potential of these cells by G6. Collectively, these data indicate that G6 exhibits exceptional efficacy in the peripheral blood, spleen, and most importantly, in the bone marrow. As such, G6 appears to alter the natural history of Jak2-V617F mediated MPN.

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NF-E2 MEDIATES EXPRESSION OF THE CYTOKINE IL-8, AN INDEPENDENT PREDICTOR OF INFERIOR OUTCOME AND PRESENCE OF CIRCULATING BLASTS IN PMF PATIENTS

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Background. The transcription factor nuclear factor erythroid-2 (NF-E2) is overexpressed in patients with myeloproliferative neoplasms (MPN) irrespective of the presence of the $JAK2^{V617F}$ mutation.[1,2] We have recently engineered a transgenic mouse model which recapitulates many features of MPN including thrombocytosis, leukocytosis, typical bone marrow features and transformation to acute leukemia[3,4] demonstrating a role for NF-E2 overexpression in the pathophysiology of MPN. Because the targets mediating NF-E2 effects are not well characterized, we conducted microarray analysis of $CD34^+$ cells lentivirally transduced to overexpress NF-E2 or to silence NF-E2 via shRNA, in order to identify novel NF-E2 target genes. *Aims.* To identify novel target genes of the transcription factor NF-E2. *Methods.* Peripheral blood $CD34^+$ cells from healthy donors were lentivirally transduced with a NF-E2 cDNA or with a shRNA directed against NF-E2. Cells were FACS sorted to obtain pure populations of transduced cells and assayed for gene expression using an Affymetrix Exon-Array (ST 1.0). IL-8 mRNA and protein expression were investigated by qRT-PCR of independently transduced $CD34^+$ cells, by intracellular FACS staining and by ELISA. The proximal 262 bp of the IL-8 promoter were used in a luciferase reporter gene assay. *Results.* Transduction of $CD34^+$ cells with the NF-E2 cDNA induced IL-8 mRNA expression 3-4 fold, while transduction of an shRNA against NF-E2 lowered IL-8 mRNA expression by 50% (n=4; p=0.008 by repeated measures ANOVA). Concurrently, IL-8 protein expression in the supernatant increased and decreased 1.7- and 2-fold, respectively. This effect was reproducible in the U937 cell line, where NF-E2 transduction also significantly increased IL-8 production, as measured both by ELISA and intracellular FACS staining (n=4 each; p=0.005 and p=0.02, respectively). Co-transfection of the proximal 262 bp of the IL-8 promoter with both NF-E2 and its obligate heterodimeric partner MafG, but not with either subunit alone, increased reporter gene activity 3-fold (n=3; p<0.001). NF-E2 binding to the IL-8 promoter *in vivo* was previously demonstrated by ChIP assay in K562 erythroleukemia cells[7]. *Summary/Conclusions.* We have identified IL-8 as a novel NF-E2 target gene. Serum levels of IL-8 are elevated in both PV and PMF patients.[5,6] Recently, increased IL-8 levels have been shown to be predictive of inferior survival in PMF patients in multivariate analysis.[6] Likewise, elevated IL-8 levels were associated with the presence of > 1% circulating blasts.[6] We therefore propose that one mechanism through which aberrant NF-E2 expression in MPN patients exerts its pathophysiological effect is by increasing IL-8 production.

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Acute leukemia - Cytogenetics & genomics

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INTEGRATED TRANSCRIPT AND GENOME ANALYSES REVEAL NKX2-1 AND MEF2C AS NOVEL ONCOGENES IN T -CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. T-lineage acute lymphoblastic leukemia (T-ALL) is a malignancy of thymocytes. T-ALL represents about 15% of pediatric ALL cases but has an inferior outcome compared to B-ALL as approximately 30% of T-ALL cases relapse during therapy or within the first 2 years following treatment and eventually die. T-ALL is mostly characterized by genetic abnormalities that are crucial for T-cell pathogenesis. Various genetic rearrangements in T-ALL occur in a mutually exclusive pattern (such as *TLX1, TLX3, TAL1* and *LMO2* rearrangements) and these are identified in ~60% of pediatric T-ALL cases. For the remaining 40% the underlying oncogenic rearrangements remain unresolved. *Aims.* To identify novel oncogenic pathways in T-cell acute lymphoblastic leukemia (T-ALL) cases that lack a currently known (mutually exclusive) oncogenic rearrangement. *Methods.* We combined expression profiling of 117 pediatric patient samples and detailed molecular cytogenetic analyses including the Chromosome Conformation Capture on Chip (4C), arrayCGH, FISH and LM-PCR. *Results.* In a supervised cluster analysis based on the gene expression data, two T-ALL subtypes were identified that lacked rearrangements of known oncogenes, both comprising approximately 10% of the 117 pediatric T-ALL cases studied (n=12 in both groups). One subtype associated with cortical arrest, expression of cell cycle genes and ectopic *NKX2-1* or *NKX2-2* expression. In this subgroup 7 out of 12 cases carried *NKX2-1* or *NKX2-2* rearrangements involving T-cell receptor or *IgH@* loci, which have not been described in human cancer before. The second subtype associated with immature T-cell development and could also almost completely be predicted by an early thymic precursor signature (ETP-ALL) as previously published by Coustan-Smith *et al.* (2009). This entity highly expressed *LMO2, LYL1, HHEX* and the *MEF2C* transcription factor. In this subgroup we found several genetic rearrangements that all converge on the upregulation of *MEF2C*, a gene involved in muscle development. We also demonstrated by ChIP and knock-in and knock-out models, that MEF2C is a transcription factor that binds the promoters of LMO2 and *HHEX* and is responsible for part of the expression signature of this immature cluster. Knockdown of MEF2C also induced cellular differentiation in a cell line model. Furthermore, in cellular transformations assays using NIH-3T3 and BJ-EHT cells we could demonstrate transforming potential for MEF2C as well as NKX2-1 in combination with *RAS* or *MYC* oncogenes. *Summary/Conclusions.* We propose *NKX2-1, NKX2-2* and *MEF2C* as novel T-ALL oncogenes that are activated by various rearrangements.

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IGH@ TRANSLOCATIONS, CRLF2 DEREGULATION AND DELETIONS OF B-CELL DIFFERENTIATION GENES ARE PREVALENT IN PH-NEGATIVE B-CELL PRECURSOR ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA AND ARE LINKED TO OUTCOME

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Adult ALL is a heterogeneous disease at the genetic level. We have previously demonstrated a strong association between cytogenetics and outcome which is independent of other risk factors. The widespread use of fluorescence *in situ* hybridisation (FISH) and genomic technologies has revealed a plethora of genomic abnormalities in this disease: e.g. translocations involving the *IGH@* locus (*IGH@*-t); deregulation of the *CRLF2* gene (*CRLF2*-d) via *IGH@-CRLF2* or *P2RY8-CRLF2* (aka PAR1 deletion); and micro-deletions of *CDKN2A/B, IKZF1, PAX5, ETV6, RB1, BTG1* and *EBF1*. The aim of the study was to estimate the frequency of these abnormalities and assess their prognostic relevance among adults with Philadelphia-negative B-cell precursor ALL. The cohort comprised 454 adults (15-60 years old) treated on MRC UKALLXII and had a median follow-up time of 5.6 years. FISH, using a commercial IGH break-apart probe and validated home-grown probes, was used to detect *IGH@*-t (and partner genes), *IGH@-CRLF2* and *P2RY8-CRLF2*. Multiplex Ligation Probe Amplification (MLPA) using the SALSA P335-A1 kit was used to detect micro-deletions and *P2RY8-CRLF2*. A total of 49/439 (11%) patients had an *IGH@*-t: *IGH@-CRLF2* (n=12); *IGH@-ID4* (n=3); *IGH@-CEPBA/B/E* (n=6); unknown (n=28). Another eight patients harboured *P2RY8-CRLF2*, thus 20 (5%) patients had *CRLF2*-d. There was little overlap between *IGH@*-t, *CRLF2*-d and established chromosomal abnormalities (*MLL* translocations, t(1;19)(q23;p13), *ETV6-RUNX1*, high hyperdiploidy (HeH), low hypodiploidy/near-triploidy (HoTr), complex karyotype (CK) and iAMP21): *CRLF2*-d with HeH/iAMP21 (one each) and *IGH@*-t with *ETV6-RUNX1*/CK/iAMP21 (one each), HeH (n=2). Micro-deletions were highly prevalent with 203/304 (67%) cases harbouring at least one deletion. However, the frequency of each deletion varied by cytogenetic subgroup (see table). *IKZF1* deletions were present in 66% *CRLF2*-d cases but were rarer among t(4;11), t(1;19) and HeH cases. There was no correlation between age or sex and the presence of any of these new abnormalities. Patients with *IGH@-CRLF2* or *IKZF1* deletions had higher white cell count (WCC) (median 102x10e9/L v 10, 16 v 11) whereas patients with *ETV6* deletion had lower WCC (5 v 15). The 4-year EFS and OS for the 441 patients with follow-up data were 41% (95%CI 37-46) and 46% (41-50). Univariate Cox analysis showed that four abnormalities were associated with poor OS: *CRLF2*-d - hazard ratio 2.03 (95%CI 1.20-3.44), p=0.008; *IGH@*-t 1.65 (1.11-2.45), p=0.013; *EBF1* deletion 2.52 (1.11-5.71), p=0.027 and *IKZF1* deletion 1.54 (1.10-2.14), p=0.011. In contrast, the EFS and OS hazard ratios for patients with a *PAX5* deletion were 0.65 (0.42, 1.01), p=0.056 and 0.68 (0.43-1.06), p=0.09. Multivariate analysis adjusting for sex, age, WCC and cytogenetic risk group revealed that *CRLF2*-d, *IGH@*-t and *PAX5*

Table 1. Distribution of deletions by cytogenetic subgroup.

Deletion	Cases	Frequency							
		Overall	t(4;11)	HoTr	CK	*CRLF2*-d	*IGH@*-t	HeH	t(1;19)
Tested	304	304	34	16	12	12	20	32	10
CDKN2A/B	114	38%	18%	44%	50%	58%	45%	28%	40%
IKZF1	87	29%	15%	25%	33%	66%	30%	13%	10%
PAX5	56	18%	3%	6%	33%	42%	10%	9%	30%
ETV6 *	32	11%	3%	0%	8%	8%	15%	22%	0%
RB1	30	10%	0%	19%	17%	33%	10%	9%	0%
BTG1	26	9%	0%	0%	8%	50%	10%	3%	0%
EBF1	7	2%	0%	6%	17%	0	10%	0%	0%

* *ETV6-RUNX1*-positive (n=2), *ETV6-RUNX1*-negative (n=26), Unknown (n=4)

deletions were independent risk factors. The 4-year OS rates for these aberrations were: *CRLF2*-d 15% (3-37%); *IGH@*-t 29% (16-44%); *PAX5* deletions 58% (44-71%). In conclusion, micro-deletions of key B-cell differentiation and cell cycle control genes are highly prevalent in adult ALL but vary in frequency by genetic subgroup. There was evidence to suggest that deletions of B-cell differentiation genes were linked to outcome. *CRLF2*-d and *IGH@*-t represent distinct and prevalent subgroups of adult ALL which are associated with a poor outcome.

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GENE EXPRESSION BASED OUTCOME PREDICTION IN CYTOGENETICALLY NORMAL AML: A MULTICENTER APPROACH FOLLOWED BY INDEPENDENT VALIDATION PROVES CLINICAL POTENTIAL

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Background. Cytogenetically normal acute myeloid leukemia (CN-AML) is biologically and clinically heterogeneous. During recent years genomic aberrations and deregulated gene expression signatures have been identified to provide important prognostic information. *Aims.* Our intention was to assess whether gene expression-based outcome prediction using novel biostatistical approaches applied to large data sets generated across four different laboratories could refine previous prognostic signatures in CN-AML. *Methods.* We generated gene expression profiles of 220 clinically well annotated CN-AML cases in a multicenter setting, comprising three different expert laboratories. For the analysis Affymetrix Human Genome U133plus2.0 Arrays and a standardized labeling protocol were used. Following data normalization and correction of batch artifacts, we applied L1-penalized Cox proportional hazards regression to develop a sparse prognostic model for overall survival. Our model was then validated by (i) leave-one-out cross-validation and (ii) by evaluating the prognostic accuracy in two independent CN-AML data sets (n=163 and n=79 cases) generated in a fourth laboratory. *Results.* We identified a 13-gene signature for overall survival that was successfully validated by means of cross-validation [P<0.001, Hazard Ratio (HR) 1.56, 95% Confidence Interval (CI) 1.21-2.00] as well as in both independent data sets (P<0.001, HR 1.85, 95%CI 1.40-2.46; and P=0.004, HR 1.85, 95%CI 1.22-2.81, respectively) using a Cox Proportional Hazards model. The gene signature retained its prognostic relevance in a multivariable model adjusting for age, FLT3-ITD and NPM1 alterations (P=0.007, P=0.004 and P=0.013). Genes contained in our outcome signature comprised candidates previously reported to be associated with molecular markers such as APP (amyloid beta precursor protein). Its expression pattern has been associated with NPM1 mutations and it was also found amplified in AML with complex karyotype. With regard to its potential biological impact APP can bind to acetyltransferase complexes to promote transcriptional activation and has been linked to gamma-secretase function and NOTCH signaling. Furthermore, we also confirmed candidates known to confer prognostic information such as LEF1 that might play a significant role in self-renewal. *Summary/Conclusions.* This is the first multicenter study to identify a refined prognostic gene signature followed in CN-AML. Our independent validation further proves the clinical potential of gene expression profiling in this AML subgroup.

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RECURRENT NADH DEHYDROGENASE SUBUNIT 4 (ND4) MUTATIONS IN ACUTE MYELOID LEUKEMIA

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Background. Recently, mutations in *NADH dehydrogenase subunit 4* (*ND4*), a mitochondrial encoded transmembrane component of the electron transport chain respiratory Complex I, have been described in acute myeloid leukemia (AML). *Aims.* In the present study, we investigated the prevalence and prognostic importance of *ND4* mutations in 452 AML patients. *Methods.* After obtaining informed consent according to the Declaration of Helsinki, DNA from diagnostic bone marrow or peripheral blood samples were analyzed from 452 adult patients (aged 17-60 years) with de novo (n=402) or secondary AML (n=50). All patients received intensive induction and consolidation therapy. *ND4* mutations were detected by direct sequencing and, when possible, the somatic or germline status was established by evaluating matched buccal swabs or healthy $CD3^{+}CD45^{bright}CD34^{-}$ T-cells sorted from diagnostic leukemia samples. *Results.* Homoplasmic and heteroplasmic *ND4* mutations (e.g. mutations affecting all or only a fraction of mitochondrial DNA copies) predicted to affect translation were detected in 34 of 452 patients (7.5 %). Thirty-one cases had single point mutations resulting in amino acid substitutions, two cases had two separate missense *ND4* point mutations, and another case had a one base pair deletion predicted to result in a truncated protein. *ND4* mutations were associated with younger age (P=.042) and *NRAS* mutations (P=.019), but were inversely associated with *NPM1* mutations (P=.039). Analysis of buccal swab samples available for three *ND4mutated* patients demonstrated that two heteroplasmic mutations were somatic and one homoplasmic mutation was germline. In six additional patients (five with heteroplasmic and one with homoplasmic *ND4* mutations), we sorted T-cells ($CD3^{+}CD45^{bright}CD34^{-}$) and leukemic blasts ($CD3^{-}CD45^{dim}$) from diagnostic leukemia samples. In the five heteroplasmic *ND4mutated* cases, we detected only wildtype *ND4* in the healthy T-cell population, while the *ND4* mutations were restricted to the leukemia blast population in all five cases, thus confirming the somatic nature of these mutations. In contrast, the homoplasmic *ND4* mutation was detected in both the healthy T-cell and the leukemia blast populations, demonstrating that this is a germline mutation. Additionally, analysis of *ND4* mutation status in complete remission samples revealed loss of the *ND4* mutation in 4/5 AML cases who had heteroplasmic *ND4* mutations at diagnosis, while the same was true for only 1/7 cases who presented with homoplasmic *ND4* mutations. Although univariate analysis demonstrated similar relapse-free (RFS; P=.676) and overall survival (OS; P=.948) for *ND4mutated* patients (n=34) compared to *ND4wildtype* patients (n=418), comparison of patients according to the heteroplasmy status of *ND4* mutations revealed longer RFS (P=.025) and OS (P=.012) for patients with heteroplasmic *ND4* mutations (n=12). *Conclusions.* These observations extend our knowledge of *ND4* mutations and further establish the link of mitochondrial mutations with leukemia. Our data suggests that hetero- and homoplasmic *ND4* mutations may elicit different effects in leukemia biology, such as contribution to leukemia maintenance or sensitization of leukemia cells to therapy.

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RUNX1-MUTATIONS ARE COMMON IN ADULT PRECURSOR T- CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL)

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Precursor T cell acute lymphoblastic leukemia (T-ALL) accounts for 20% of all lymphoblastic leukemia cases. The runt-related transcription factor 1, RUNX1, is crucial in the development of myeloid and lymphoid cell lineages. Mutations have been reported in approximately 30% of cases with MDS, CMML and AML and are associated unfavorable impact on outcome, at least in MDS and AML. We recently investigated 39 patients with chronic myeloid leukemia in blast crisis (BC-CML) with either myeloid or lymphoid features. In that study, 3/10 patients with a lymphoid BC-CML harbored RUNX1 mutations, indicating a potential role of these alterations in lymphatic malignancies. Recently, RUNX1 has also been demonstrated to be involved in lymphoid differentiation in particular in T cell differentiation. Expression of RUNX1 is found in naïve CD4-positive T cells and represses the expression of GATA3 and IL4, thereby influencing their further development into Th2 T helper cells. Here, we for the first time analyzed the RUNX1 mutation status in a cohort of 101 adult ALL patients (T-ALL, n=49; B-ALL, n=52). In addition, 5 cases with natural killer cell leukemia were analyzed. For molecular analyses, using the purified fraction of mononuclear cells after ficoll density centrifugation, a sensitive next-generation amplicon deep-sequencing assay was applied (454 Life Sciences, Branford, CT). A sequencing library was prepared interrogating the complete coding region of RUNX1, split

into 7 distinct amplicons (median length: 342 bp). NGS data were analyzed using GS Variant Analyzer Software 2.3 (454 Life Sciences) and Sequence Pilot version 3.4 (JSI Medical Systems, Kippenheim, Germany). In median, 821 reads per amplicon and patient (range 217-1687) were obtained, thus yielding a sufficient coverage for mutation detection with high sensitivity (<5%). Overall, 15 mutations were detected in 13 patients: 7 missense alterations, 1 nonsense mutation, 6 frameshift alterations, and 1 in-frame insertion. RUNX1 mutations were distributed across several exons, but like in myeloid malignancies predominantly clustered in the RUNT domain (aa 50-177; 11/15 mutations) and TAD domain (aa 291-371; 4/15 mutations). In the cohort of B-ALL (n=52 including 22 cases with BCR-ABL1 rearrangement), only 2 cases (3.8%) were RUNX1-mutated. Both of them harbored a BCR-ABL1 rearrangement. In contrast, in T-ALL 13 distinct mutations were observed in 11/49 cases (22.4%; p=0.007 vs. B-lineage). With respect to clinical associations, RUNX1 mutations in T-ALL were associated with a higher age (mean±SD 58±17 vs. 39±17 years; p=0.003; median age overall: 42 years) and with a lower white blood cell count (mean±SD 27±22 vs. 81±95 G/L; p=0.006), but not with platelet count, hemoglobin level, gender or karyotype. These findings may be a rationale for a pathogenetic role of RUNX1 mutations in T-cell precursor ALL as observed in the present series. In summary, our study revealed a high incidence of RUNX1 mutations in 22.4% of T-ALL patients. This data further support that RUNX1 plays an integral part in hematopoiesis in general and is not limited to myeloid differentiation. Considering the present results, RUNX1 mutation screening is warranted in future clinical studies and may enable an even more specific diagnosis of this disease.

Acute lymphoblastic leukemia - Clinical

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PROGNOSTIC SIGNIFICANCE OF LEF1 EXPRESSION IN ADULT B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. The Wnt signaling pathway is linked to proliferation and survival of leukemia cells and has emerged as a potential target for antileukemic therapy. Lymphoid Enhancer Factor (LEF) 1, a key mediator of the Wnt pathway, was shown to directly promote leukemic transformation. Recently, microdeletions and mutations of the *LEF1* gene were identified in pediatric T-cell acute lymphoblastic leukemia (ALL). *Aims.* To further investigate the yet undefined role of *LEF1* in B-lineage ALL, we determined the prognostic impact of *LEF1* expression in adult B-precursor ALL patients. *Methods. LEF1* mRNA expression was determined by quantitative real time PCR in pretreatment bone marrow samples of 282 adults with newly diagnosed B-precursor ALL enrolled on the 06/99 (n=138) and 07/03 (n=144) GMALL multicenter trials. For statistical analyses, patients were grouped into quartiles according to *LEF1* expression levels [*LEF1* high (Q4; n=71); *LEF1* low (Q1-3; n=211)]. The median follow-up for living patients was 42.6 months. Patients who received allogeneic stem cell transplantation in first remission were censored at the time of transplantation for survival analyses. Multivariate analyses were performed according to the Cox proportional hazards model, including the following variables in the full model: white blood cell (WBC) count (10/nl increase), age (10-year increase), CD20 positivity, *BCR-ABL*, *MLL-AF4*, and immunophenotype. We focussed our analysis on standard risk (SR) patients as defined by GMALL (*BCR-ABL*- and *MLL-AF4*-negative patients with CR after first induction therapy and WBC ≤30/nl; n=91), as this patient group lacks molecular markers for further risk stratification. To evaluate the presence of *LEF1* mutations, we performed DNA sequencing of *LEF1* exons 2 and 3, as the observed hot spot regions in T-ALL, in 41 B-precursor ALL patients. *Results.* No significant differences regarding age, WBC, the immunophenotype, or GMALL risk groups were found between the two *LEF1* groups. High *LEF1* expression was associated with CD20 positivity (P=0.01) and inversely associated with the expression of myeloid markers (P=0.001). Patients with high *LEF1* expression had a significantly shorter relapse-free survival (RFS) compared to low *LEF1* expressers (5-year RFS: *LEF1* high: 29%, *LEF1* low: 51%; P=0.008). In mulitvariate analyses, *LEF1* was independently predictive for RFS [hazard ratio 1.9 (95% CI 1.1-3.3); P=0.02]; the other factors in the final model were WBC, age, and immunophenotype. Similarly in SR patients, high *LEF1* expression was associated with a significantly inferior RFS (5-year RFS: *LEF1* high: 39%, *LEF1* low: 61%; P=0.01). Upon Cox analysis, *LEF1* expression was the only factor with prognostic significance for RFS [hazard ratio 2.8 (95% CI 1.2-6.3); P=0.01] in SR patients. The mutational analyses revealed no *LEF1* mutations in exons 2 and 3, suggesting that in B-precursor ALL transcriptional activation of *LEF1* rather than inactivating mutations as in T-ALL might play a role. *Conclusions.* High *LEF1* expression identifies adult B-precursor ALL patients with significantly inferior RFS, supporting a pathogenetic role of Wnt signaling in ALL. Thus, determination of *LEF1* might improve pretreatment risk assessment in SR ALL patients. Moreover, patients with high *LEF1* expression may be considered for new molecular therapies, including agents targeting the Wnt pathway.

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DEXAMETHASONE VERSUS PREDNISOLONE FOR THE TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Corticosteroids are essential and one of the mainstays in the treatment of acute lymphoblastic leukemia (ALL). The current randomized comparisons between dexamethasone (DXM) and prednisolone (PDN) in childhood ALL indicated a statistically significant

and clinically important decrease in rate of isolated central nervous system (CNS) relapses and an increase in event-free survival (EFS) with DXM. But the data were limited in adult ALL. *Aims.* We evaluate the role of DXM compared to PDN during induction or subsequent phases of therapy in adult ALL patients. *Methods.* From January 2000 to January 2010, ninety five standard risk (SR) and 132 high risk (HR) or very high risk (VHR) newly diagnosed adult ALL patients entered the randomized comparison of DXM at 6 mg/m2/d vs PDN at 60 mg/m2/d with ALL treatment protocol. HR ALL was defined as patients fulfilling at least one of the following criteria: age 35 years and older, WBC count greater than 30x109/L for B-lineage ALL or more than 100x109/L for T-lineage ALL at diagnosis, time to complete remission (CR) more than 4 weeks. Patients who had none of these risk factors were considered as SR, and patients with cytogenetics of t (9; 22) or BCR-ABL fusion gene positive were considered as VHR. *Results.* The median follow-up time was 3.5 years. There were no significant differences in terms of CR between the PDN and DXM arms, no matter in SR group (84% vs 87%, p =0.53) or in HR+VHR group (72% vs 75%, p =0.42). In patients with SR, the cumulative incidence of isolated CNS relapse was lower for DXM patients than for PDN patients, with 3-year cumulative estimates of 7.5% and 12.1%, respectively (p =0.015). The 3-year disease-free survival (DFS) and 3-year overall survival (OS) rates were 49% and 52% in SR patients who received DXM, which were significantly higher than 40% and 46% in SR patients who received PDN (p =0.005, 0.01). For patients with HR+VHR, the isolated CNS relapse rate was similar for patients assigned to DXM compared with that of patients assigned to PDN (3-year cumulative estimates: 11.6% vs 14.7%; p =0.15), and there were no differences in the 3-year DFS rates (32% vs 30%, p =0.59) and 3-year OS rates (35% vs 33%, p =0.36) based on steroids type (DXM vs PDN). During induction the incidence of grade 3- 4 infection was similar in the two arms (DXM 18.5% vs. PDN 15.3%, p =0.20), and during post-induction consolidation, it was higher in the DXM arm (15.5% vs 8.9%, p =0.02). Reversible hyperglycemia and myopathy were higher in the DXM arm than in the PDN arm (5.8% vs 4.8%, 4.2% vs 3.1%), but the differences were not statistically significant (p =0.5, 0.35). *Conclusions*. Our results indicates that DXM might have benefit in adult SR ALL patients, as it was proved in children, especially concerning the long-term survival and the CNS relapse. But for patients at HR of relapse, it is unlikely that only modification of steroids replacement would alter their dismal prognosis.

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LONG-TERM FOLLOW UP OF ADULT PATIENTS WITH NEWLY DIAGNOSED PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH+ALL) RECEIVING IMATINIB AND CHEMOTHERAPY AS FRONT-LINE TREATMENT WITHIN GMALL STUDIES

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Background. Imatinib (IM) in combination with chemotherapy regimens followed by allogeneic SCT has become the standard treatment for younger patients with Ph+ ALL. Complete remission (CR) rates generally exceed 90%. The efficacy on the long-term outcome data obtained from a large group of prospectively evaluated patients is still unclear. *Aim.* The aim of this analysis was to analyze the efficacy of an early imatinib administration started simultaneously with induction chemotherapy according to the GMALL protocol 07/03 (A3). These results were compared with two consecutive patient cohorts of the GMALL study group, where IM was given alternating and/or concurrent, which has been previously reported by Wassmann *et al.* (Blood 2006;108:1469). *Methods.* A total of 335 patients with newly diagnosed Ph+ ALL who received IM given at a single daily oral dose of 600 mg within 3 successive treatment cohorts were compared. A1: IM was administered between induction (IND) and first consolidation (CONS1) and again after CONS1 (n=51); A2: IM was given during the second half of IND and then continued throughout CONS1 until SCT (n=105); A3: IM has been initiated together with start of induction chemotherapy and continued throughout CONS1 until SCT (n=179). Minimal residual disease (MRD) was serially assessed by quantitative RT-PCR, mutational analyses was performed by D-HPLC and direct sequencing. *Results.* The median age of all patients was 43 years (17-65 y), 57 (17%) patients were 55 years of age or older. CR rates in cohorts A2 and A3 were 89.4% and 85.7%, induction deaths occurred in 5.8% and 11.3% of patients, treatment failure was observed in 4.8% and 3% of pts., respectively. The molecular response rate based on PCR negativity for bcr-abl transcripts after CONS1 was superior in cohort A3 with 33% (26/79) as compared to 12.5% (5/40) and 4.2% (2/47) in cohorts A2 and A1, respectively (p=0.01). Overall treatment outcome improved with earlier initiation and more prolonged administration of IM in the three successive patient cohorts: Overall survival (OS) at 4 years was 31%, 40% and 50% in cohorts A1, A2 and A3, respectively. To date, 219 patients (66.4%) underwent SCT in CR1 (A1: n=39; A2: n=74; A3: n=106), with a median age of 39.5 years. The 3 year probability of DFS of pts. in cohort A3 who received myeloablative conditioning regimens combining TBI with cyclophosphamide or etoposide was 72. The incidence of relapse after SCT was lower among patients in cohort A3 (11.3%) than those in A2 (24.3%) or A1 (30.8%). For all pts. transplanted in CR1 irrespective of treatment cohort, median OS was 57% after 3 yrs. and 52% after 7 yrs. Patients who did not undergo SCT in CR1 had a dismal outcome, with a median OS of 9.4 months and 14% alive after 3 years. *Conclusion.* In conclusion, intensive chemotherapy and early administration of IM is feasible in patients with newly diagnosed Ph+ALL and is associated with superior treatment outcomes after SCT. SCT in CR1 remains the treatment of choice even in patients who achieve a good molecular response to initial therapy.

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PROMISING RESULTS OF SPECIFIC IMMUNOCHEMOTHERAPY IN BURKITT'S LEUKEMIA OR LYMPHOMA REGARDLESS OF THE HIV INFECTION STATUS: RESULTS OF A PHASE II STUDY

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Background and aims. The addition of rituximab to specific chemotherapy for Burkitt's leukemia or lymphoma (BLL) has yielded promising results in phase II studies. However, most of these studies excluded patients infected by the human immunodeficiency virus (HIV). The results and toxicity of a phase II study including patients with BLL regardless of their HIV status are presented. *Methods.* This trial (Burkimab) derived from the B-ALL/NHL2002 (German Multicenter Adult Acute Lymphoblastic Leukemia Group, GMALL) consisted of a prephase with cyclophosphamide and prednisone, followed by cycles (A, B, C) including rituximab, iphosphamide, high-dose methotrexate and cytarabine. Patients in localized stages (I, II non-bulky) received 4 cycles (A1,B1,C1 and A2) and those with bulky stage II or in advanced stages (III, IV) or Burkitt's leukemia received 6 cycles (A1, B1, C1, A2, B2, C2), plus two additional doses of rituximab. Patients ≥55 yr. received only A and B cycles, with 50% reduction in doses of methotrexate and cytarabine. Informed consent was obtained from all patients. *Results.* 121 patients were included (2004-2010). Median age was 45 yr (range 15-83), 97 (80%) were in advanced stages (III-IV), with extranodal involvement ≥2 sites in 56 (46%) and Burkitt's leukemia in 24 (20%). LDH was elevated in 108 (91%), ECOG score was >2 in 54 (45%), and age-adjusted IPI was:

low 6 (5%), low-intermediate 17 (14%), intermediate-high 48 (41%) and high 47 (40%). The only difference between HIV- (n=80) and HIV+ (n=41) cohorts was age (48 vs. 42 yr, respectively, p=0.03). Seventy-three (HIV-) and 40 (HIV+) patients were evaluable for treatment results. Complete response (CR) was achieved in 63 (86%) vs. 33 (83%), 4 (5%) vs. 5 (13%) died during induction and 4 (5%) vs. 2 (5%) did not respond. The remaining 2 patients (HIV-) were removed early from the trial. After a median follow-up of 2 yr. (range 0.4-7), 4 relapses (5%) vs. 2 (5%) were observed (isolated BM: 2, isolated CNS: 2, combined BM+CNS: 1, extranodal: 1) and 3 (4%) vs. 5 (13%) patients died in remission during chemotherapy (p=0.12). The 4-yr. OS probabilities were 79% (95%CI, 69%-89%) vs. 67% (95%CI, 52%-82%)(p=0.11), the 4-yr. DFS probabilities were 90% (95%CI, 82%-98%) vs. 79% (95%CI, 65%-93%)(p=0.12), and the 4-yr. EFS probabilities were 76% (95%CI, 66%-86%) vs. 62% (95%CI, 47%-77%)(p=0.17). For patients aged <55yr. grade 3-4 hematological toxicity, mucositis and infections were significantly more frequent in HIV+ patients either as a whole or considering cycles A, B and C separately. For patients aged ≥55yr. only grade 3-4 neutropenia in A cycles was significantly more frequent in HIV+ patients. *Conclusions.* This phase II trial showed promising results of specific immunochemotherapy in adult patients with BLL. Although hematological, mucosal and infectious toxicity was higher in HIV+ cohort, the CR, DFS, OS and EFS were not significantly different according to HIV infection status.

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GENOMIC VS. GENE EXPRESSION-BASED METHODS IN THE DETECTION OF IKAROS (IKZF1) ALTERATIONS AND EVALUATION OF THEIR PROGNOSTIC IMPACT IN CHILDHOOD ALL

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Background. Recently, Ikaros (IKZF1) gene deletions have been described as adverse prognostic factors in childhood acute lymphoblastic leukemia (ALL). Nevertheless, there is still lack of data on IKZF1 impact concerning different treatment protocols, minimal residual disease (MRD) and the most suitable diagnostic method in BCR/ABL-negative ALL. *Aim.* To compare the DNA- vs. RNA-based approach assessing Ikaros status in a cohort of children with BCR/ABL-negative ALL treated by non-MRD based protocol ALL IC-BFM 2002. *Methods.* A) Gene expression of functional (Ik1, Ik2) vs. short (Ik4, Ik4A, Ik4del, Ik6, Ik6del, Ik8) IKZF1 isoforms was evaluated using Lab-on-a-chip (Agilent) electrophoresis and reported either as an absolute level or relative to the total signal. Thresholds for abnormal expression were set based on the analysis of peripheral blood of healthy donors, remission bone marrow (BM), and sorted B- and T-cell precursor subpopulations. B) MLPA (multiplex ligation-dependent probe amplification) was performed on BM DNA with probes for Ikaros exons 1 to 8. *Results.* Results of both gene expression and MLPA analysis were available for 182/244 children diagnosed between 2002 and 2007. MLPA revealed a deletion of at least one exon of IKZF1 in 12 (7%) patients. The proportion of non-DNA binding isoforms to the total IKZF1 expression was significantly increased (>80%) in 13 (7%) patients. The expression of a dominant-negative Ik6 isoform was significantly elevated (>50% of total) in 10 (6%) patients. Surprisingly, changes on DNA level were not always reflected in gene expression. Of 12 patients with gene deletion, only five had an increased short/long isoform ratio (4 pts due to Ik6 overexpression). The deletion on one allele did not cause a decrease in total IKZF1 gene expression level. Conversely, of 10 patients with Ik6 overexpression, six patients had no DNA alteration, suggesting a different mechanism of altered gene expression. Patients with IKZF1 gene deletion had significantly worse relapse-free survival (RFS) than other patients (5-year RFS 46±15% vs. 91±2%, p<0.0001). Patients with IKZF1 deletion had higher MRD in BM at day 33 (p=0.008). In gene expression analysis, Ik6 overexpression was the most important negative prognostic factor (5-year RFS 50±16% vs. 91±2%, p<0.0001), whereas elevated short/long isoform ratio (>80%) had only weaker impact (5-year RFS 69±13% vs. 90±2%, p=0.02). Gene expression of no other single IKZF1 isoform had impact on prognosis. Patients with Ik6 overexpression had higher MRD in BM at day 15 (p=0.009), day 33 (p=0.02) and at week 12 (p=0.01) and higher peripheral blood MRD at day 15 (p=0.05). *Conclusion.* Contrary to previous studies, we showed that deletions within IKZF1 locus do not necessarily correlate with altered Ikaros gene expression. Conversely, Ik6 overexpression was not accompanied by a deletion within IKZF1 locus in 60% of patients. Both DNA- and RNA-IKZF1 alterations had a strong negative prognostic impact in a cohort of children with BCR/ABL-negative ALL treated by a BFM-based protocol that did not use MRD in the risk group stratification. Ideally, both genomic and gene expression-based approach should be applied together for the evaluation of prognosis.

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Congenital neutropenia and host defence

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PLERIXAFOR IS A POTENTIAL THERAPY FOR MYELOKATHEXIS, WHIM SYNDROME

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Background. Myelokathexis or WHIM syndrome (warts, hypogammaglobulinemia, immunodeficiency and myelokathexis) is a rare autosomal dominant disorder attributable to mutations in the CXCR4 gene. The WBC is usually <1.0 x 109/L. with severe neutropenia and lymphocytopenia. Marrow examination shows abundant neutrophils with hypersegmented nuclei and remnants of neutrophils in marrow macrophages. The mutations in CXCR4 are activating and prevent the normal release of neutrophils from the marrow into the blood. Plerixafor is a small molecule inhibitor of the binding of CXCR4 to its ligand CXCL12. Subcutaneous administration of plerixafor causes a dose-dependent increase in circulating leukocytes. It increases circulating CD34+ cells and is currently used as an adjunct to granulocyte colony-stimulating factor (G-CSF) for mobilization of hematopoietic stem cells. *Aim.* Investigated the potential of plerixafor as therapy for myelokathexis/WHIM syndrome. *Methods.* We enrolled 6 patients (4 female, 2 male, ages 28 to 73 years) in this study, with informed consent and investigational review board approval of the University of Washington and Federal approval for investigation use of plerixafor. Five patients from three different families had the same mutation (R334ter); the other patient had a novel mutation (S324fs365ter). Single subcutaneous doses of plerixafor, increasing from 0.04 to 0.24 mg/kg, were administered at 2 to 4 day intervals. Complete blood counts were determined with an automated counter and leukocyte differential counts confirmed manually. CD34+ cells and lymphocyte subtypes were measured by FACS before and 6 hours after the 0.08 mg/kg dose. Plerixafor was discontinued if neutrophils were >2.0 x 109/L at 24 hours, after all doses were tested or if serious adverse events or illness occurred. Results were compared with five similarly studied normal subjects. *Results.* All 6 patients showed a prompt leukocytosis with maximum blood neutrophils and lymphocytes at 6 to 12 hours, declining toward baseline by 24 hours. Blood neutrophils peaked at 3.9 +/- 0.55 x 109/L (range 1.8 to 5.1 x 109/L) at 6 to 12 hours. Two of the 6 patients achieved > 2.0 x 109/L neutrophils at 24 hours. The lymphocyte responses were proportionally greater than the neutrophil responses. The greatest increase was in B cells (CD20+cells), a 60 fold increase at 0.08 mg/kg. CD34+ cells increased 6.8 fold at 0.08 mg/kg. Comparisons of patients and normal subjects showed larger proportional B and T cell responses in the patients. Hematocrit, hemoglobin and platelet counts were stable through the 10 day testing period, except for one patient with severe iron deficiency anemia who responded well to oral iron initiated during the study. None of the patients experienced any significant adverse effects. One patient discontinued the trial when she had a recurrence of pneumonia. All of the patients who had previously received G-CSF had neutrophil responses but no changes in lymphocyte counts. *Conclusions.* This trial shows that plerixafor can correct neutropenia and lymphocytopenia in patients with myelokathexis/WHIM syndrome and suggests that this agent may correct the underlying immunodeficiency. Plerixafor is a promising molecularly targeted therapy for this condition. Plans for a therapeutic trial are now underway.

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ACHIEVEMENT OF EFFICIENCY AND SAFETY BY THE USAGE OF A NOVEL SINGLE CHAIN TCR DESIGN IN HUMAN CYTOMEGALOVIRUS PP65 / P53 TUMOR ANTIGEN-BISPECIFIC T CELLS

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Allogeneic stem cell transplantation leads in immunosuppressed seropositive leukemia patients to reactivation of human cytomegalovirus (HCMV) and in this case to increased virus titer and mortality. Therefore, our aim is the generation of p53 tumor antigen- and HCMV-bispecific T cells for donor lymphocyte infusions (DLI). First, we isolated HCMV+ T cells from seropositive blood donors using pp65(NLVPMVATV(495-503)) peptide-specific stimulation. After one or two pp65 peptide-specific restimulations followed by CMV pp65-specific multimer cell-sorting in flow cytometry, we were able to enrich HCMV-specific T cells and assess their cytotoxic effector function using either pp65-loaded K562-A2 or recombinant HCMV-infected HLA-A*0201+ human fibroblasts as target cells. Different HCMV+ donors indicated high TCR affinities for the pp65 antigen by EC50-values ranging from 0.1 nM to 1.8 nM in peptide titration. Moreover, the HCMV+ T cells recognized HCMV-infected fibroblasts with superior efficiency. Next, we expanded the cells with irradiated and peptide loaded PBMCs and performed their retroviral transduction with a single chain (sc) as well as double chain (dc) p53(264-272) tumor antigen-specific TCR. In both cases the T cells acquired specific bifunctionality after stimulation with pp65- and p53- peptide loaded K562-A2 target cells in terms of cytotoxicity in chromium release assays as well as in terms of interferon-γ secretion in intracellular cytokine staining analysis. An alternative approach aims at the simultaneous retroviral transduction of HCMV- and p53 tumor antigen-specific TCRs in bulk human T cells which may be used in particular in case of HCMV-negative donor grafts. Analysis of tetramer staining elicited the expression of a substantial fraction of HCMV/p53 double TCR+ T cells which also proved bifunctionality towards their cognate antigen in CTL- and in intracellular IFNγ secretion-assays. However, the presence of endogenous TCRs which may mispair with the exogenous ones potentially leading to neoreactivity prompted us to assess whether residual mispairing takes place in our chosen scTCR approach and if yes to entirely prevent it by either protein design or shRNA technology. We performed mispairing analysis of the murine p53-specific scTCR with various human TCR alpha chains (TCRα) of different Vα subfamilies mimicking any endogenous TCRα chain. For this, we used the Jurkat-76 T cell line which lack endogenous TCRs and bulk human T cells. Flow cytometry analysis revealed residual mispairing of the murine p53-specific scTCR with any TCRα chain presumably by displacing the Variable alpha (Vα) domain of scTCR. Mispairing was less pronounced in human T cells most likely due to a more competitive situation between exogenous and human TCRα/β- chains. Preliminary results indicated less mispairing of the p53-specific scTCR encoded along with Cα as a transient bicistronic mRNA construct on a self-cleaving 2A peptide-based retroviral vector. In conclusion, we describe two approaches for the generation of HCMV- and p53 tumor antigen-bispecific human T cells. Importantly, our mispairing analyses emphasize the need to further optimize both TCR structures and the design of TCR transgene cassettes in retroviral vectors to safely apply them in adoptive immunotherapy.

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IMMUNE FUNCTION AGAINST MURINE CYTOMEGALOVIRUS IS SUPERIOR IN MICE TRANSPLANTED WITH PURE ALLOGENEIC HEMATOPOIETIC STEM CELLS AS COMPARED TO RECIPIENTS OF T-CELL REPLETE GRAFTS

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Background. Infections due to impaired immune function are major causes of morbidity and mortality after allogeneic hematopoietic cell transplantation (HCT). Donor (do) T cells (TC) in the graft are thought to provide protective immunity, however, cause graft-versus-host disease (GVHD) and therefore require pharmacologic immunosuppression. *Aim.* To study how cell subsets contained in hematopoietic grafts influence functional immunity against murine cytomegalovirus (MCMV) post-transplant, using a minor-mismatched mouse model. *Methods.* Lethally irradiated BALB.B mice received grafts composed of purified hematopoietic stem cells (HSC; $cKit^+Sca1^+Thy1.1^{lo}Lin^-$) and TC populations from congenic C57BL/6 strains which were distinguished based upon CD45 alleles. At 2 or 8 weeks (w) post-transplant hosts were infected with MCMV. 2w post-infection immune function of: 1) transferred doTC from uninfected and pre-immunized donors, 2) HSC-derived doTC, and 3) residual host TC were assessed using MCMV-specific M45 tetramer staining for FACS analysis and ELISPOT. *Results.* Our key finding is that immune function against MCMV was superior in recipients of HSC alone compared with mice given allografts containing total TC (ToTC). In recipients of HSC alone residual host TCs provided the strongest responses early post-transplant, while at later times HSC-derived doTC also contributed to anti-viral reactivity. Adoptively transferred doTC from unimmunized or immunized donors did not yield higher anti-viral responses in allogeneic recipients,

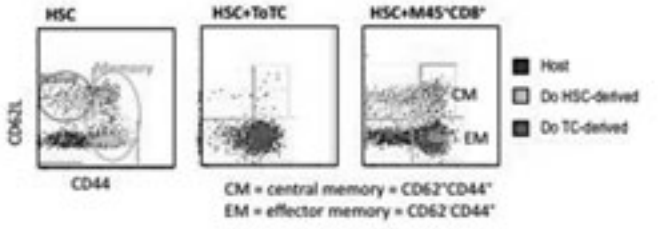

Figure 1. Naive/memory T cell reconstitution post-transplant.

although they reacted vigorously when infused into congenic mice. Likewise, immune function of allogeneic doTC was not restored when pharmacologic GVHD prophylaxis with cyclosporine A (CSA) was administered post-transplant. Separation of GVH-reactions and transfer of immunity was possible by strict selection of TC subpopulations that are enriched for or contain exclusively MCMV-specific TC, such as memory ($CD62L^{+/-}CD44^{+}$) or M45-tetramer^{+} CD8TC ($CD8_{M45}$). HSC+$CD8_{mem}$ or +CD_{8M45} resulted in better survival and significantly stronger anti-viral responses in MCMV target organs ($CD8_{mem}$: median 0.65%; $CD8_{M45}$: 8.7% M45^{+}/hepatic CD8TC) than HSC+ToTC (<0.05% M45^{+}/CD8TC). Furthermore, transplantation of HSC alone or grafts composed of HSC+CD8TC subsets allowed the regeneration of a nascent doHSC-derived TC pool with naïve and central memory TC, that could theoretically provide better protection against a broad range of future infections. In mice given HSC+ToTC >90% of TC were expanded doTC with an effector memory phenotype (Figure). *Conclusions.* 1) Recipients of HSC alone have stronger immune responses against MCMV compared to hosts given TC-replete grafts; 2) Residual host cells contribute substantially to protective immunity in recipients of HSC alone early pTX, but are eradicated by conventional TC-replete grafts; 3.) GVHD-prophylaxis with CSA did not improve immune function; 4.) HSC-derived TC arising in a healthy host are superior to those that develop and undergo selection in a GVHD-affected lymphoid system; and 5) Transfer of small numbers of highly selected cell subsets, such as tetramer-sorted MCMV-specific CD8 TC, is feasible, as these cells expand dramatically in a lymphopenic environment and provide functional protection against infections. Our results challenge the conventional assumption that doTC in a hematopoietic allograft are required for optimal regeneration of the immune system. Rather, our studies suggest that long-term lymphoid function will greatly benefit from rigorous TC-depletion of the graft, and avoidance of GVHD.

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DIMINISHED LEVELS OF HAX1 OR ELA2 PROTEIN, BUT NOT MUTATED ELA2 PROTEIN LED TO DEFECTIVE MYELOID DIFFERENTIATION: IN VITRO MODEL OF CONGENITAL NEUTROPENIA

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Severe congenital neutropenia (CN) is a heterogenous disorder of hematopoiesis characterized by a maturation arrest of granulopoiesis in bone marrow at the promyelocyte stage. CN is a multigene syndrome caused by e. g. inherited mutations in the elastase 2 (ELA2) gene or HAX1 gene. The pathomechanism of defective granulopoiesis in CN patients downstream of ELA2 or HAX1 mutations is not completely understood. It is also not clear, why the same ELA2 mutations lead to cyclic neutropenia (CyN) or CN. Mutations in the HAX1 gene led to absent HAX1 protein. Although it has been demonstrated that mutations in HAX1 could contribute to the apoptosis of myeloid cells due to defective mitochondrial membrane potential, definitive effects of HAX1 mutations leading to isolated ineffective granulopoiesis in CN are still not well understood.We aimed to analyse the effects of mutations in HAX1 or ELA2 genes on granulocytic differentiation *in vitro*. Intriguingly, in CD33^{+} granulocytic progenitor cells of CN patients harboring HAX1 mutations, G-CSF failed to upregulate mRNA levels of the HAX1 interaction partner, HCLS1, as compared to healthy individuals. We found that HCLS1 in a complex with HAX1 transduced the G-CSFR signal via LEF-1 to the nucleus.We transduced the promyelocyte leukemia cell line NB4 with shRNA constructs specific for HAX1 or HCLS1. We also transduced NB4 cells with WT ELA2 cDNA or cDNA encoding mutated ELA2 (C42S and ΔV145-152 mutations are CN-specific; S97L mutation is typical for both CN and CyN). We assessed myeloid differentiation of transduced cells by FACS analysis, RT-PCR and cell morphology.We found that inhibition of HAX1 or its interaction partner HCLS1 led to a significantly reduced differentiation of the NB4 promyelocytic cell line in response to ATRA (16,9 % of CD11b^{+} cells in HAX1 shRNA group, 20,1 % of CD11b^{+} cells in HAX1 shRNA group vs. 63,2 % of CD11b^{+} cells in ctrl shRNA group), which mirrors the situation in CN. However, mutated ELA2 did not affect myeloid differentiation and even lead to increased proliferative capacity of cells. Previously, we demonstrated that levels of ELA2 mRNA expression in myeloid progenitors as well as of plasma NE protein were markedly reduced in CN patients harboring mutations in either ELA2 or HAX1 genes, as compared to CyN patients and to healthy individuals due to a lack of LEF-1 expression (Skokowa *et al.* Blood 2009). Therefore, we analysed if inhibition of ELA2 by ELA2-specific shRNA had any effects on myeloid differentiation. As a result, we found significant inhibition of ATRA-induced differentiation of the promyelocyte leukemia cell line NB4 after inhibition of ELA2, as compared to ctrl shRNA transduced cells (25,3 % of CD11b^{+} cells in ELA2 shRNA group vs. 41,2 % of CD11b^{+} cells in ctrl shRNA group). In line with diminished myeloid differentiation, mRNA expression of LEF-1 transcription factor was downregulated after inhibition of HAX1, HCLS1 or LEF-1.Taken together, diminished levels of HAX1, HCLS1 or ELA2 proteins leads to disturbed granulocyte differentiation. However, introduction of mutated ELA2 protein into cells has no effects on myeloid differentiation.

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UPDATE ON THE RISK OF SECONDARY LEUKEMIA IN GENETIC SUBGROUPS (ELA2, HAX1, WASP, G6PC3, P14) OF CONGENITAL NEUTROPENIA IN EUROPE

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Congenital neutropenia (CN) is well known as one of the premalignant bone marrow failure syndromes with an overall incidence of secondary leukemia of more than 10 percent. With the identification of new causative gene mutations the number of genetic subgroups is still increasing. In this study we assessed the incidence and potential risk factors of leukemic transformation in CN patients with known gene mutations in ELANE, HAX1, G6PT, G6PC3, WAS, SBDS, TAZ1 and p14 or no identified mutation, respectively, by analyzing all available data from the European Branch of the Severe Chronic Neutropenia Registry (SCNIR). For comparison we also analyzed patients with cyclic neutropenia (CyN) with or without ELANE mutations. Results from genetic testing were available for 195 of 311 CN patients. Of the 195 CN patients 68 patients revealed ELANE mutations, 25 HAX1 mutations, 46 SBDS, 18 WAS, 21 G6PT, 8 G6PC3, 4 p14 and 5 TAZ1 mutations. In addition, in 35 patients neither ELANE nor HAX1 mutation was detectable. 81 patients were not tested to date, but further genetic evaluation is not yet completed. Results from genetic testing were also available in 29 of 67 patients with CyN of whom 23 revealed ELANE mutations and 6 were negative for ELANE mutations. Secondary malignancies occurred in 37 of the 311 CN patients and 1 of the 67 patients with CyN. The distribution by genetic subtypes is shown in the table below:

Table 1.

Gene Mutation	Patients (N)	MDS/Leukemia n / (%)
Total CN	311	37 (11.9)
- ELANE-CN	68	11 (16,2)
- HAX1-CN	25	5 (20,0)
- ELANE neg/HAX1neg	35	6 (17,1)
- SBDS	46	4 (8,7)
- WAS	18	2 (11,1)
- G6PT	21	0
- G6PC3	8	0
- TAZ1	5	0
- p14	4	0
- not testet	81	9 (11,1)
Total CyN	67	1 (0.2)
- ELANE-CyN	23	0
- ELANE neg-CyN	6	0
- not testet	38	1 (0,3)

All subgroups benefit from G-CSF treatment. However, patients requiring higher maintenance doses of G-CSF are at greater risk of leukemic transformation (Rosenberg, Zeidler *et al.*, 2010). *Conclusion.* The incidence of secondary leukemia reflects the heterogeneity of congenital neutropenia ranging between no leukemia and 20 percent in patients with an underlying HAX1 mutation. However, patient numbers within each genetic subgroup are still limited. Patients with severe congenital neutropenia who have mutations in ELANE, HAX1, SBDS or WAS as well as those with no recognized mutation are at risk of secondary leukemia. Progression to MDS or leukemia has so far not been reported in G6PT, G6PC3, TAZ1 or p14 CN cases in our Registry. Despite mutations in the ELANE gene patients with cyclic neutropenia exhibit no increased risk for malignant transformation. Mutational analysis is helpful to identify the genetic cause of severe congenital or cyclic neutropenia but with limited numbers in genetic subgroups still does not serve to identify patients at risk of leukemic transformation.

Stem cell transplantation - Clinical 3

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CD4+CD25+ REGULATORY T-CELL DEPLETION TO IMPROVE GRAFT-VERSUS-TUMOR EFFECT AFTER DONOR LYMPHOCYTES INFUSION: BIOLOGICAL PREDICTORS OF CLINICAL RESPONSE

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Background. We very recently published the first clinical trial showing that regulatory T-cells (Treg) depletion can improve alloreactivity and likewise the GVT effect in humans in the setting of donor lymphocyte infusion (DLI) after HSCT (Maury *et al.* Sci Transl Med 2010). Here, we aim to analyze T cell contents of donors and recipients before and sequentially after DLI to identify relevant biomarkers of clinical response. *Methods.* CD25+ Treg-depleted DLI (d-DLI) were given in 17 adult patients with malignancy relapse after HSCT. All but one had previously failed to respond to at least one standard DLI, and none had experienced GVHD. Overall, GVHD induction through Treg depletion was obtained in 6 out of the 17 patients and associated with partial or complete remissions of hematological malignancies. With a median follow-up of 24 months after d-DLI, this group of patients (GVH+) had an improved survival (p=0.035) as compared to the 11 others without GVHD induced (GVH-). CD4+, CD8+, B and NK cell subsets were monitored using flow cytometry and FoxP3 expression assessed by RTq-PCR on (i) d-DLI and (ii) PBMC collected from recipients before and at 7 time-points during the first year after d-DLI. *Results.* The CD25 magnetic depletion led to a very high Treg-depletion rate in all d-DLI products (mean CD4+FoxP3+ cell-depletion rate of 98% in accordance with a FoxP3 expression decrease by 92% by RTq-PCR) with no significant difference between GVH+ and GVH- patients. We could also not evidence any correlation between Treg monitoring in recipients after d-DLI and GVHD onset. However, a longitudinal monitoring of lymphoid subpopulations using a hierarchic clusterization (Genesis software, Graz, Austria) revealed that a lowering relative number of naïve CD4+CD45RA+ cells among CD4+ T-cells over time correlated with GVHD induction. At day 15 after d-DLI, i.e. before the occurrence of any GVHD, a low level of naïve CD4+CD45RA+ +/- CD62L+ cells correlated with GVHD onset (p=0.04, Fig 1). This remained significant at day 30 for both phenotypes of naïve cells. *Conclusion.* The ability of Treg depletion to break immune tolerance in HSCT patients refractory to alloreactivity seems to be associated with the decrease of the naive CD4+ T-cell population in recipients. Such a polarization to a memory phenotype that we found associated with GVH/GVT effects might represent a new relevant parameter to improve and predict responses to Treg-based antitumor immunotherapies.

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RELAPSE AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOLLOWING REDUCED INTENSITY CONDITIONING FOR AML AND MDS: DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS FOR GRAFT CELLS

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Relapse after allogeneic hematopoietic cell transplantation (HCT) following reduced-intensity-conditioning (RIC) in patients with AML and MDS remains a major hazard. Therefore, identifying predictive factors which allow successful prevention and management of relapse is still a major challenge. For this purpose, we analysed 256 consecutive patients (138 male/118 female); median age 62 years with AML, n=205 (80%) and MDS, n=51 (20%) who received HCT after 200 cGy TBI +

fludarabine 30 mg/m^2 for 3 days followed by mycophenolate mofetil and cyclosporine. Donors were matched unrelated (MUD) in 201 (78.5%) and matched related (MRD) in 55 (21.5%) patients. Disease-stage at HCT was complete remission (CR)1,n=156 (61%), CR2,n=42 (16.4%), beyond CR2,n=37 (14.4%) and untreated-MDS,n=21 (8.2%). Cytogenetics were intermediate and high risk in 169 (66%) and 74 (29%) patients respectively. An initial positive leukemic CD34-phenotype was present in 54.7% of patients. Donor-cell-chimerism (DCC) in flow-sorted CD34+-marrow cells at days 28, 56, 84, and at 3 months interval thereafter was monitored by PCR of polymorphic micro satellite regions. After a median follow-up of 42 months, engraftment was 94%. Survival, disease-free-survival, and non-relapse-mortality at 5-years were 40%, 35%, and 27% respectively. Relapse-incidence was 48%. Interestingly, disease-specific parameters such as diagnosis, disease- stage, high-risk cytogenetics, and initial leukemic CD34-phenotype had no impact on relapse. While the donor-associated factors graft-versus-host-disease (GvHD) and CD34-DCC day28 strongly correlated with later relapse (p<0.0005). Irrespective of the severity, patients with acute and/or chronic GvHD relapsed less frequently (13.6%) compared to 48% of patients without GvHD (p<0.0005). Irrespective of initial leukemic CD34-phenotype, CD34-DCC day28 <90% was highly predictive of hematological relapse (HR) with 82% versus 38% relapse-risk if >90% (p<0.0005). Only 4.5% of patients who never relapsed had a CD34-DCC day28 <90%. Management of patients with (HR) and those with decreasing CD34-DCC below 90% without HR consisted of immunomodulation [tapering of immunosuppression and/or donor lymphocyte infusion] in 82.5%. Initial leukemic CD34-phenotype changed in 37% of patients at relapse with only 31% of patients expressing a positive CD34-phenotype. Overall, 34% of patients with overt HR achieved CR and 47.5% of patients with declining CD34-DCC below 90% a sustained complete CD34-DCC. A negative leukemic CD34-phenotype at relapse (p=0.02), relapse beyond day 100 after HCT, therapy for a decreasing CD34-DCC rather than a HR (p=0.05), and inducing GvHD with immunomodulation particularly in relapses within 100 days post-HCT (p=0.005) correlated with a superior response and survival after relapse. CR in the 51% of patients where GvHD was induced was 63% compared to 30% in patients without GvHD (p=0.004) highlighting the graft-versus-leukemia activity. After RIC-HCT, donor- rather than disease-related factors predict relapse and its treatment outcome in AML and MDS. Irrespective of the initial leukemic CD34-phenotype, monitoring of CD34+-DCC is an excellent marker to identify patients at risk of relapse and guide early immunomodulation which effectively enhances the graft-versus-leukemia effect thereby preventing hematological relapses or successfully treating them. Nevertheless, further research is needed to optimize immunosuppressive regimens to maximize the graft-versus-leukemia effect without the injurious effects of graft-versus-host-disease particularly in the early post-HCT phase.

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IMPACT OF PROPHYLACTIC CD8-DEPLETED DONOR-LYMPHOCYTE INFUSIONS AFTER T-CELL DEPLETED ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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The combination of reduced-intensity conditioning and *in vivo* T-cell depletion by alemtuzumab prior to hematopoietic stem cell transplantation (HSCT) has demonstrated efficient engraftment and reduced graft-versus-host disease (GVHD). However, this regimen is associated with slow lymphocyte recovery leading to a delayed anti-infectious and anti-malignant immunity. In a phase I study, we investigated the prophylactic use of CD8-depleted donor lymphocyte infusions (DLIs) to improve immune reconstitution. We have previously shown the feasibility of this approach and demonstrated that the CD8-depleted DLI reliably converted a decreasing T-cell chimerism (Meyer *et al.* Blood 2007 & BMT 2010). Here we provide clinical follow up data of 101 patients with different hematological malignancies with a median observation time of 1 year post HSCT (range, 1-80 months). The majority of patients either suffered from an acute leukemia / MDS (n=42), lymphoma (n=28), myeloma (n=17), or myeloproliferative neoplasms (n=12). The median age of the patients was 56 years (range, 20-71) and none of them qualified for a conventional conditioning regimen. 45 patients had undergone previous transplantations (autologous: n=43, allogeneic: n=2). The donors were matched siblings (n=15), matched unrelated (n=48), or unrelated donors with single HLA mismatches (n=38). The calcineurin-inhibitor used for GVHD-prophylaxis was intended to be tapered until day 50 post HSCT. In the absence of GVHD, CD8-depleted DLI were subsequently administered prophylactically in escalating doses starting with 1x106 CD4 T cells / kg bodyweight. Following this procedure, 39 patients received at least one dose of DLI. Among those patients who did not qualify for DLI, 46 patients had primary GVHD. In 16 patients DLI were not administered for other reasons (donor unavailable, infections, relapse). In 64% DLI induced acute GVHD, which was the major reason for withholding the next DLI-dose step. The rate of acute GVHD > grade 2 was 30%. 10% suffered from extensive chronic GVHD. The 1 and 3 year overall survival was 63% and 43%, respectively. Survival significantly differed between the DLI and the non DLI group after 3 years (63% vs. 27%, p=0.002). Since this trial was not randomized, we also compared the DLI group to only those patients who did not receive DLI for other reasons than primary GVHD and found similar results (62% vs. 28%, p=0.01). Although DLI was associated with a survival benefit, the relapse rate did not differ from that of the no-DLI cohort. When we analyzed the diseases separately, there was no significant effect of DLI on the relapse rate, but we found a trend towards a lower relapse rate among AML/MDS- and delayed relapses in myeloma-patients. As expected, the presence of GVHD at any time was associated with a reduced relapse rate (55.8% vs 30.8%, p=0.013). In summary, the prophylactic application of CD8-depleted DLI in the absence of GVHD was associated with a survival benefit. However, we were not able to relate this benefit to a decreased relapse rate. Our data strongly support a randomized trial, comparing prophylactic vs. preemptive / therapeutic DLI application in the context of T-cell depleted HSCT.

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SECONDARY MALIGNANCIES AFTER ALLOGENEIC STEM-CELL TRANSPLANTATION IN THE ERA OF REDUCED-INTENSITY CONDITIONING; THE INCIDENCE IS NOT REDUCED

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Background. Allogeneic stem-cell transplantation (SCT) is a potentially curative therapy for a variety of hematological malignancies and non-malignant diseases. Secondary malignancies are a known complication in long-term survivors. The incidence and kinetics of secondary malignancies have been reported mostly after myeloablative conditioning (MAC). Reduced-intensity conditioning (RIC) has been introduced over the last decade to allow SCT in patients not eligible for standard SCT. RIC has been shown to reduce the incidence of transplant-related complications, however due to the relative limited long-term follow-up of RIC recipients, the incidence and risk-factors for secondary malignancies following RIC have not been well defined. *Aims.* To determine the relative risks and risk-factors for secondary malignancies following RIC compared with MAC. *Methods.* A single institution database of 902 allogeneic SCTs given over the last 12 years was retrospectively reviewed to identify patients with secondary malignancies. Conditioning regimens included standard MAC (n=252), fludarabine-based RIC (n=452) or fludarabine-based reduced-toxicity myeloablative conditioning (RTC, n=198). The incidence of secondary malignancies was calculated by cumulative incidence analysis with death due to other causes considered competing risk. The incidence was correlated with patient and transplant characteristics. Three patients with PTLD and 2 with secondary leukemia and relapse of the prior malignancy after SCT were not considered a having secondary malignancies in this analysis. *Results.* Twenty-two patients had secondary malignancies including squamous cell carcinoma of the skin (n=5), penis (n=1) vagina (n=1), tongue (n=1) and esophagus (n=2), colon cancer (n=3), breast cancer (n=2), metastatic cancer of unknown primary (n=1), pancreatic cancer (n=1), metastatic sarcoma (n=1), Kaposi sarcoma (n=1) and donor-derived MDS/AML (n=3). The median age at SCT was 55 years (29-70). Nineteen patients were given fludarabine-based RIC/RTC and none had total-body irradiation. The median time from SCT to diagnosis of secondary malignancy was 38 months (7 months-11 years). Eighteen patients had prior chronic GVHD and 10 were still on immunosuppressive therapy at the time of diagnosis of secondary malignancy. The cumulative incidence of secondary malignancy 10 years after SCT was 5.5% (95%CI, 3.3-9.1%). It was higher in older patients (>50 years) than in younger patients; 7.3% Vs 4.4% (p=0.004). It was also higher in patients with a history of chronic GVHD; 11.2% Vs 2.1% (p=0.05). Secondary malignancies were less common in patients with CML and nonmalignant diseases compared with patients with prior chemotherapy; 1.1% and 6.5% (p=0.07). Patients given MAC had

a cumulative incidence of 1.9%, compared with 7.8% for patients given fludarbine-based RIC or RTC (p=0.02). Multivariable analysis identified chronic GVHD and advanced age as adverse prognostic factors with hazard-ratios of 3.5 (p=0.03) and 2.9 (p=0.05), respectively. *Conclusions.* Secondary malignancies are rare but significant complication after allogeneic SCT. Chronic GVHD and advanced age at SCT predict for higher incidence. The incidence is not reduced in the RIC era, possibly due to the inclusion of older and more heavily pretreated patients to these protocols. The possible adverse effect of fludarabine in the conditioning regimen can not be ruled out. Larger studies with a larger number of events are needed to confirm these observations.

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EBV-ASSOCIATED POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE FOLLOWING ALEMTUZUMAB-BASED ALLOGENEIC STEM CELL TRANSPLANT: CLINICOPATHOLOGICAL FEATURES AND PREDICTORS OF OUTCOME

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Background. Epstein-Barr virus-associated post-transplant lymphoproliferative disease (EBV+PTLD) remains an important clinical problem following allogeneic stem cell transplantation (alloSCT). *In vivo* T cell depletion with Alemtuzumab has been previously thought to confer only a small increase in PTLD risk and the characteristics and outcome of such patients have not received detailed study. Furthermore, although quantitative-PCR monitoring for EBV reactivation post-HSCT is now commonplace, it's diagnostic and predictive value remains unclear. *Aims.* To characterise the clinicopathological features of PTLD occurring after an Alemtuzumab-based alloSCT, correlate with EBV qPCR data and analyse factors predicting response and outcome. *Methods.* This retrospective multicentre study analysed 75 cases diagnosed with PTLD following Alemtuzumab-based alloSCT during 2001-2010. Six cases were excluded because of insufficient evidence of PTLD and 7 cases comprised EBV-associated encephalitis or haemophagocytic syndrome. 62 patients with EBV+PTLD were included in the analysis, equating to an incidence of 4% following Alemtuzumab-based alloSCT. Forty-six were biopsy-proven whilst 16 had probable PTLD based on viral load and robust clinical/radiological evidence of lymphadenopathy or visceral lesions. *Results.* The median age at onset was 50 years (16-62). 17 patients received myeloablative (MA) whilst 45 received reduced-intensity (RI) conditioning and 44 occurred following an unrelated donor alloSCT. The median time from HSCT to PTLD onset was 120 days. Substantial clinicopathological heterogeneity was observed but B symptoms were manifest in 80% and extra-nodal disease was frequent (60%). The median viral load at the onset of PTL D was 49,300 copies/ml (50-65,200,000 copies/ml). Notably, the viral load was ≤10,000 and ≤40,000 copies/ml in 23% and 45% of cases respectively. With a median follow-up of 20.4 months, mortality attributable to PTLD was 31% (19/62). Eleven cases were post-mortem diagnoses or died rapidly after initiation of therapy. Fifty-one patients were therefore evaluable for treatment-response: 3 underwent a reduction in immunosuppression only; 36 received rituximab monotherapy (median total dose 1500mg/m2); 6 received rituximab-chemotherapy and 6 received donor T lymphocytes. The overall response rate was 78% and although there were no documented relapses, all those with progressive disease died of PTLD at a median of 33 days (13-257 days) from diagnosis. A four-point prognostic score (age ≥60; stage III/IV disease; ≥2 extranodal sites and poor performance status [ECOG 2-4]) was tested in multivariate analyses. A score of 3-4 correlated both with inferior response to therapy (odds ratio 0.10 [CI 0.02-0.55], p=0.008) and overall survival (hazard ratio 2.89 [CI 1.39-6.02], p=0.005). Donor-type, conditioning intensity, Alemtuzumab dose, lymphocyte count and, importantly, viral load at PTLD onset, were not associated with response or survival. *Summary/Conclusions.* This is the largest reported series of EBV+PTLD after Alemtuzumab-based alloSCT and describes a clinically diverse disease that is often rapidly progressive. At onset of PTLD, EBV loads are frequently below accepted thresholds for pre-emptive therapy, challenging current paradigms for monitoring and intervention. Although a majority of patients responded to Rituximab, 22% experienced progressive disease following immunochemotherapy. A 4-point score identifies those predicted to have a poor outcome and for whom novel antibody therapies or adoptive cellular therapy could be targeted.

Publication only

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A NOVEL SPLICED FUSION OF MLL WITH CT45A2 IN A PEDIATRIC BIPHENOTYPIC ACUTE LEUKEMIA

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Background. Abnormalities of 11q23 involving the MLL gene are found in approximately 10% of human leukemias. To date, nearly 100 different chromosome bands have been described in rearrangements involving 11q23 and 64 fusion genes have been cloned and characterized at the molecular level. In this work we present the identification of a novel MLL fusion partner in a pediatric patient with de novo biphenotypic acute leukemia. *Aims and Methods*. Cytogenetics, fluorescence in situ hybridization (FISH), molecular studies (RT-PCR and LDI-PCR), and bioinformatic sequence analysis were used to characterize the CT45A2 gene as novel MLL fusion partner in pediatric acute leukemia. *Results*. Fluorescence in situ hybridization of the patient G-banded metaphases demonstrated a cryptic insertion of 11q23 in Xq26.3 involving the MLL gene. Breakpoint fusion analysis revealed that a DNA fragment of 653 kb from 11q23, containing MLL exons 1-9 in addition to 16 other 11q23 genes, was inserted into the upstream region of the CT45A2 gene located at Xq26.3. In addition, a deletion at Xq26.3 encompassing the 3' region of the DDX26B gene (exons 9-16) and the entire CT45A1 gene was identified. RNA analysis revealed the presence of a novel MLL-CT45A2 fusion transcript in which the first 9 exons of the MLL gene were fused in-frame to exon 2 of the CT45A2 gene, resulting in a spliced MLL fusion transcript with an intact open reading frame. The resulting chimeric transcript predicts a fusion protein where the N-terminus of MLL is fused to the entire open reading frame of CT45A2. Finally, we demonstrate that all breakpoint regions are rich in long repetitive motifs, namely LINE/L1 and SINE/Alu sequences, but all breakpoints were exclusively identified outside these repetitive DNA sequences. *Summary /Conclusions*. We have identified CT45A2 as a novel spliced MLL fusion partner in a pediatric patient with de novo biphenotypic acute leukemia, as a result of a cryptic insertion of 11q23 in Xq26.3. Since CT45A2 is the first Cancer/Testis antigen family gene found fused with MLL in acute leukemia, future studies addressing its biologic relevance for leukemogenesis are warranted.

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THE SINGLE NUCLEOTIDE POLYMORPHISM OF GENE XPA?XPC?XPD?XRCC1 AND IN ASSOCIATION WITH THE RISK OF ACUTE LYMPHOBLASTIC LEUKEMIA

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Backgroud. Polymorphisms of DNA repair genes can alter protein structure and may impair DNA repair capacity. It is becoming clear that defects in repair pathways are connected to many different types of diseases, including leukemia and cancer.This study was performed to evaluate the effect of the polymorphisms of DNA repair genes on risk of adult acute lymphoblastic leukemia(ALL) in a Chinese population. Aims: To study the relationship between polymorphism of gene XPA、XPC、XPD XRCC1 and the susceptibility to ALL in a Chinese population. Methods: Genotypes were determined by the MALDI-TOF mass spectrometry method in 114 confirmed ALLcases and 169 controls whose age and sex matched to above cases. Results: Multivariate logistic regression analysis revealed that individuals carrying at least one 23G variant allele(AG+GG genotypes)had a significantly increased risk for ALL (adjusted OR=2.00; 95%CI=1.07～3.76)compared with the wild-type genotype(23AA), and evidence that positive interactions between the polymorphisms in XPC Ala499Val/XPA A23G and XPD 751/XPA A23G may occur. Furthermore, individuals with both putative risk genotypes had a significantly higher risk (adjusted OR=5.6; 95%CI=1.57～19.9), compared with those with both wild-genotypes. By contrast, no significant association was observed between the XPD Lys751Gln、XRCC1 Arg399Gln、Arg194Trp polymorphism and ALL risk.Conclusions: These results suggest that the XPA A23G and XPC Ala499Val polymorphisms may contribute to the risk of developing ALL.There are signigicant combinations between XPC Ala499Val and XPA A23G. Further studies are needed to elucidate potential functional relevance of the those variant allele.

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ACUTE LEUKEMIA IN CHILDREN WITH DOWN SYNDROME

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Background. Down syndrome (DS) is the most common and the best known chromosomal disorder in human.The frequency is about 1 case in 800 live births and it is proportional with maternal age.The extra copy of the proximal part of 21q22.3 appears to result in the typical physical phenotype, mental retardation, hand anomalies, and congenital heart defects. Children with DS are predisposed to developing leukaemia (1 case to 300 or 15-20 times more than other children) with a ratio of acute lymphoblastic leukaemia (ALL) to acute myeloid leukaemia (AML) typical for childhood acute leukaemia. During the first 3 years of life AML is dominant , particularly transient myeloproliferative disorder (TMD) and acute megakaryocytic leukemia. Nearly all children with DS and leukaemia have mutations in the hematopoietic transcription factor gene, GATA1. Children with DS -ALL are mostly of B-cell precursors origin and aberrant expression of cytokine receptor CRLF2, associated with mutated JAK2. *Aim*. To present our experience regarding to frequency, clinical features, treatment results and follow up of children with DS and leukaemia. *Methods*. During the period from January 2006 to January 2011, 112 children with DS was diagnosed in Macedonia. The frequency of DS is 1 to 1000 live births. Three children developed acute leukaemia. Two boys, 1 and 2 years old, have AML (M0 and M7),one 3 years old boy has cALL and TMD was diagnosed in two newborns. First symptoms in all patients with acute leukaemia were fatigue, petechial haemorrhagy, recurrent respiratory infection. Two newborns had connatal infection. Child with AML M7 is without cardiopathy. Child with AML M0 has VSD. The thirth with ALL has hypothyreosis, atrioventriculat septal defect and Morgagni hernia which bouth were corrected in the age of 3 months. One of newborns with TMD had Tetralogy of Fallot and the other VSD. Moderate splenomegaly was detected in all of the patients. Results. Laboratory data confirmed anemia (Hb range: 49-60g/l), WBC number from 4,7 to 74x10^9/l and severe thrombocytopenia (11 to 15x10^9/l). Bone marrow analyses confirmed M0, M7 and common ALL in three patients. Imunophenotype profiles are: patient with AML M0 had CD68 pozitive in 60% blast cells,CD79,CD20 and CD34 in 30%. Patient with AML M7 had expression of CD33, CD13, CD117, CD56, CD36 and parcial CD42b. Patient with cALL had BCR-ABL1 negative, MLL and TCF3 rearrangement negative, ETV6-RUNX1 positive. CD19,CD34, CD22, CD79A positive. Children with AML were treated according ML DS-BFM 2006 protocol and they are in complete remission 2 and 2,5 years after completed therapy. Child with ALL is in remission but still on chemotherapy. Newborn with complex cardiopathy died six month later during serious pulmonary infection but with normal blood and bone marrow features on 1 month age. The other is still alive without any sign of TMD. *Conclusions*. Children who develop AML have generally favourable prognosis. Outcome of DS-ALL has been considered worse than the outcome of ALL without DS. Majority DS-ALL may benefit from therapy blocking the CRLF2/JAK2 pathway and for DS-AML, mutations of GATA1 gene.

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IMMUNOGLOBULIN AND T-CELL RECEPTOR GENE REARRANGEMENT PATTERN IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA WITH MLL GENE REARRANGEMENTS

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Background. Rearrangements of the mixed lineage leukemia (MLL) gene are the most common genetic aberrations found in infant acute lymphoblastic leukemia, occurring in approximately 75% of patients younger than 1 year. Although less frequently, the MLL gene is also rearranged in older children (≥1 year), particularly in CD10-negative ALL

cases. These three factors: MLL rearrangements, younger age and pro-B immunophenotype are inter-correlated and closely associated with poor prognosis. They have also been postulated to influence the immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangement pattern. *Aims*. We aimed at characterization of Ig/TCR gene rearrangement pattern in a group of 19 MLL-positive ALL patients: 12 infants (10 pro-B-ALL, 2 B-cell precursor ALL with myeloid co-expression) and 7 patients older than 1 year, aged 1.6-17.5; mean age: 7.8, median age 6.5 (6 pro-B-ALL and 1 common/pro-B-ALL). We also aimed at comparison of the Ig/TCR pattern identified in the study group with the pattern previously reported in a group of 58 B-cell precursor ALL (BCP-ALL) patients. *Methods*. Polymerase chain reaction with the use of standard BIOMED-1 and BIOMED-2 primers was used for detection of Ig/TCR gene rearrangements. Clonality of the rearrangements was assessed by heteroduplex analysis and confirmed by sequencing. *Results*. At least one clonal rearrangement was found in 68% of 19 MLL positive patients, in contrast to 97% in 58 BCP-ALL patients. The frequencies of rearrangements in the MLL positive group were as follows: 32% VH-(DH)-JH, 26% DH-JH, 53% total IGH, 11% Vκ-Kde, 21% Vδ2-Dδ3, 11% Vγ-Jγ, 5% Dβ-Jβ vs. 74%, 9%, 74%, 31%, 45%, 50%, 4%, respectively, in 58 BCP-ALL group (DH-JH and Dβ-Jβ rearrangements were studied in a subgroup of 23 BCP-ALL patients). In contrast to BCP-ALL group no Intron-Kde, Dδ2-Dδ3, Vδ2-Dδ3-Jα29 and Vβ-Dβ-Jβ rearrangements were found in the MLL positive patients. *Summary*. As compared to BCP-ALL group Ig/TCR gene rearrangements in MLL positive patients are characterized by more immature pattern (i.e. rearrangements are less frequent with predominance of IGH rearrangements, relatively frequent occurrence of DH-JH rearrangements and lower frequencies of IGK-Kde and cross lineage TCRD, TCRG, TCRB rearrangements; complete TCRB and TCRD/A rearrangements were not found in the study group). The study will be continued in a larger cohort of MLL positive patients. *Conclusions.* Immature Ig/TCR gene rearrangement pattern most probably reflects maturation arrest at early lymphoid developmental stages. It is postulated that early (in case of infant ALL- in utero) oncogenic transformation is determined by occurrence of the MLL gene rearrangements.

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DNA METHYLATION PATTERN IS ALTERED IN CHILDCHOOD T-ALL AS COMPARED TO T CELL SUBSETS AND HEALTHY CHILDREN

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Background. Therapy success rate for T-ALL is lower than in precursor B-ALL, which reflects higher T-ALL aggressiveness and its heterogenous biology. In many cancer subtypes it is possible to distinguish heterogenous DNA hypermethylation patterns. Identification of patient-specific CpG island methylator phenotype (CIMP) might facilitate treatment stratification. *Aim*. We aimed at assessing CIMP patterns in T-ALL. *Patients and Methods*. Methylation status of 20 genes was assessed by MS- PCR in 63 children with de novo, T-ALL treated at the centres of Polish Paediatric Leukemia and Lymphoma Study Group (PPLLSG). Additionally, 11 healthy bone marrow donors younger than 17 years of age and thymic subsets from healthy children were tested as controls. *Results*. Two groups of patients were delineated: CIMP- and CIMP+. Additionally, computational clustering of patients was concordant with CIMP groups (p<0,0001). Analysis of correlation between CIMP and traditional clinical risk assessment factors, EGIL T-ALL classification or NOTCH1 and FBXW7 mutation status, showed no significant association. *Conclusion*. Methylation patterns differ between controls and T-ALL patients. It is possible to divide patients into two groups characterized by two main methylation patterns. Results of our study indicate existence of CIMP phenomenon in childhood T-ALL, although its biological and prognostic significance needs further studies.

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CDKN1A-MEDIATED RESPONSIVENESS OF ACUTE LYMPHOBLASTIC LEUKEMIA TO AURORA KINASE INHIBITORS

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Background. The prognosis of acute lymphoblastic leukemia (ALL) is related to the cytogenetic status and age. Unfavorable cytogenetic abnormalities and old age in ALL will lead to dismal outcomes. Recent studies showed that Aurora kinases were therapeutic targets in cancer therapy, including acute myeloid leukemia, Philadelphia-positive leukemia and multiple myeloma. It is currently unclear whether the therapeutic activity of the compounds in leukemia is primarily due to selective Aurora kinase or multi-kinase inhibition. *Aim*. The aim of this research was to investigate the molecular mechanism regulating the differences in the responsiveness of ALL to Aurora kinase inhibitors. *Methods*. In this study, we used Aurora kinase inhibitor "VE-465". Nine ALL cell lines containing t(4;11) and non-t(4;11) ALL cell lines were used to evaluate the expressions of Aurora kinases by Western blot and RT-PCR and the treatment effect of Aurora kinase inhibitors by MTT assay. The effects of Aurora kinase inhibitors on the cell cycle were evaluated by flow cytometry. The expressions of CDKN1A (p21) in mRNA and protein levels were compared between the drug-sensitive and drug-resistant cell lines. *Results*. Cells treated with Aurora kinase inhibitors (VE-465) inhibited the phosphorylation of Aurora kinases effectively. Among nine ALL cell lines, RS4;11 was more sensitive to Aurora kinase inhibitors (IC50 <10 nM) and the treatment resulted in an increased G2/M and sub-G1 populations. RPMI-8402 and Raji were most resistant to Aurora kinase inhibitors (IC50 >1000 nM) and the treatment led to the polypoidy status. The different treatment efficacy was not related to the expression of Aurora kinases or their activators. The mRNA and protein expression levels of CDKN1A were up-regulated after treatment with Aurora kinase inhibitors in the drug-sensitive cell lines, but were still very low in the drug-resistance cell lines. Blockage of Aurora kinases activated CDKN1A in drug-sensitive cell lines, but not in drug-resistant cell lines. *Summary/Conclusion*. In this study, cell lines containing t(4;11) were more sensitive to Aurora kinase inhibitors. Expression of Aurora kinases and their activators did not correlate with the drug susceptibility in ALL cell lines. Activation of CDKN1A significantly increased the susceptibility to Aurora kinase inhibitors in ALL cell lines. Further investigation of the role of CDKN1A in the response to Aurora kinase inhibition is warranted in the future.

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EFFECTS OF FORMIN-LIKE 1 (FMNL1) SILENCING IN A HUMAN LYMHOBLASTOID CELL LINE

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Background. FMNL1 has a highly restricted expression in malignant lymphoid derived cells, including in cells from chronic lymphocytic leukemia and Non-Hodgkin lymphoma patients; and has been described

as a tumor associated antigen. The function and regulation of FMNL1 have not yet been well characterized; however its restricted expression suggests that FMNL1 represents an attractive target for novel immunotherapies in hematopoietic malignant disorders. Aims: Herein, we evaluated the role of FMNL1 in proliferation, colony formation and migration in the Namalwa cell line, a human B lymphoma cell line. Methods: Specific shRNA-expressing lentiviral vector targeting FMNL1 or LacZ genes (control) were used. Cell growth was measured using the MTT colorimetric reduction method. Colony formation was carried out in semisolid methyl cellulose medium and was detected after 8 days of culture by adding 1mg/mL of MTT reagent and scored by Image J quantification software. Both assays were carried out in lentiviral transduced cells, treated or not with different concentrations of rapamycin (10 or 100nM). Migration assays were performed in triplicate using 5-μm Transwells and the lower compartment was filled with 600 μL 0.5% BSA/RPMI containing 100ng/mL SDF-1. P value <0.05 was considered statistically significant. *Results*. The levels of FMNL1 mRNA and protein in FMNL1 knockdown cells were reduced by approximately 70%. Inhibition of FMNL1 resulted in a significant decrease of proliferation and clonogenicity by 40% and 32% respectively, when compared with control cells (P<0.001). Interesting, the combination of FMNL1 inhibition/rapamycin treatment showed higher reduction in both assays when compared with FMNL1 inhibited cells alone (P<0.01) or rapamycin treated cells (P<0.05). Moreover, FMNL1 silencing resulted in a significant decrease by 62% of cell migration when compared to control cells (P<0.01). *Conclusions.* Our findings indicate that FMNL1 participates in the regulation of proliferation, colony formation and migration of the Namalwa cell line, which suggests that FMNL1 represents an attractive therapeutic target. Interestingly, we observed a synergic effect on FMNL1 silencing and rapamycin inhibition in proliferation and clonogenicity, suggesting that they may act through different pathways. Based on this result, we hypothesized that combinatorial inhibition of these pathways would be effective for the treatment of lymphoid malignancies. Supported by FAPESP, CNPq and Instituto Nacional de Ciência e Tecnologia do Sangue-INCT do Sangue.

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AUTOMATED ANALYSIS OF CEREBROSPINAL FLUID (CSF)

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Background. Reference method for CSF cells count is cytometric chamber nowadays. However this method is quite slow; inter-observator variability has been reported and some errors are commonly committed. So, automated and standarized methods are desirable to simplify and to reach reliable results in the analysis. On the other hand, early detection of CSF neoplastic cells is very important in order to establish a suitable treatment in cancer diseases. New hematologic autoanalizers with biologic fluids software incorporated can help us to detect neoplastic and non neoplastic cells. Sysmex XE-5000 TM (SXE) are capable to carry out a leukocyte and differential counts and also to detect high fluorescence cells (HFBF) which show neoplastic, mesotelial and activated monocyte/macrophagic cells excluding them from leukocyte counts. *Aim*. Our aim was to evaluate SXE blood cell counts in CSF samples and to make a quantitative and qualitative analysis. We also compared SXE CSF results with those obtained by cytospin microscopic observation and flow cytometric studies. *Methods*. 232 CSF samples from patients with hematologic diseases (acute leukemia and NHL n=216), non hematologic cancer diseases (n=4) and infectious, inflammatory diseases and others (n=12) were analysed. CSF samples were firstly acquired in SXE counter and then manually processed for cytospin, May-Gründwald-Giemsa stain and microscopic observation. Flow cytometric analysis (FC) with four colour monoclonal antibodies according to pathology was also carry out. When initial diagnosis was unknown we used HLA-DRFITC /CD19PE / CD3PERCP/ CD45APC panel. All events from 0.5-1 ml sample were acquired in a FACScalibur (BD) machine. Paint- a- gate analysis software was used. Samples were classified as follows: non cellular, neoplastic or activated. *Results*. Sensibility for leukocytes detection was higher in SXE than cytospin interpretation (88% versus 51% respectively). 42.9% of neoplastic infiltrated samples showed more than zero HFBF, but only 8% of non cellular and 10% of activated CSF samples. We found statistic differences in HFBF percentages between all groups (Kruskal Wallis p<0.001). When we applied U de Mann-Whitney test, we could find higher HFBF % in infiltrated samples compared to non cellular (p< 0.001) and to activated ones (p=0.015). Differences in HFBF% between activated and non cellular samples (p>0.801) were not found. Infiltrated samples by FC had a tendency to have higher HFBF values. However, within the three classified types it was more frequent to find values close to zero. *Conclusion*. SXE autoanalyser could be a rapid, reliable and straightforward method to detect and differentiate SCF cells. High HFBF values in neoplastic samples might suppose a valid screening tool when flow cytometry is not available.

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MIR-155 INHIBITION EFFECT ON CELL PROLIFERATION AND APOPTOSIS INDUCTION OF JURKAT (ACUTE T CELL LEUKEMIA) CELL LINE

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Backgrounds. MicroRNAs are small non-coding RNA molecules with approximately 22 nt in length and cause inhibition of translation or degradation of mRNA. Mir-155 is a molecule with different functions, such as role in proliferation and immunity. Overexpression of this miRNA has been found in a number of cancers. One of its best known functions is apoptosis that affectsa on caspase-3 activity. *Aims*. The main aim of this study was evaluation of LNA mir-155 inhibitor effect in apoptosis. *Methods*. In this study, Jurkat cells were used and for evaluation of sensitivity to varied concentrations (25, 50 and 75 nmol) of mir-155 inhibitor using MTT assay. Mir-155 expression level was analyzed using the quantitative real-time polymerase chain reaction (QRT-PCR). Caspase-3 activity was measured by caspase-3 colorimetric activity assay kit. Unpaired t test test were used for analysis of the MTT and apoptosis results. Probability of 5% was assumed as statistically significant. *Results*. According to our results, the use of mir-155 inhibitor increased activity of caspase-3 by 2 fold in 75 nmol concentration. In this research, we found that the proper increase of mir-155 inhibitor concentration can inhibit mir-155 and consequently increase caspase-3 activity and induce apoptosis in the Jurkat cells leading to cell death ultimately. *Conclusions*. Apoptosis stimulation by miRNAs is probably one of the best and low risk ways of cell death induction in malignancies. Due to role of mir-155 in several cancer cells, it may be used as a therapeutic tool in future.

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META-ANALYSIS REGARDING THE RELATION BETWEEN ACUTE LYMPHOBLASTIC LEUKEMIA TREATED IN CHILHOOD AND THE APPEARANCE OF THE METABOLIC SYNDROME

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Background. Are there metabolic disorders that influence the apparition of ALL and finally the metabolic syndrome (MS) or the treatment of ALL favors MS? In the moment when ALL was diagnosed in children, a potentially atherogenic lipid profile was noted, that persists during the induction phase. The treatment of ALL can also be involved in the ulterior appearance of MS. Aims. Our objective is to study the arguments, existing in literature, that advocate for a connection between the MS and a pre-existing childhood ALL. Methods. The meta-analysis included all the 7 existent studies in MEDLINE, until January 2011, that refers to the relation between ALL and MS. The analyze studied: the period of time passed from the end of the ALL treatment, the type of treatment (chemotherapy and/or radiotherapy), the percentage of patients with MS, the percentage of the present criteria that defines MS and the relation with the growth hormone (GH). Results. The meta-analysis included 260 patients. The median period of time from the end of treatment was 13.04 years. The average proportion with patients having MS was 11.38%. There was only one study that compared the proportion of patients having both MS and ALL in antecedents (16.6%) with the one in general population of the same age and gender (17.5%); the proportion of MS was not significant different. In 2 of the studies the average percentage of obese subjects was 14.97% and the overweight subjects percentage was 42.10%. In a study 55.7% from the subjects had at least one of the criteria that define MS. In another study the criteria that define MS were present in the following proportions: hypertriglyceridemia - 17.63%, low levels of HDL - 10,08%, high fasting glucose - 6.01%, obesity - 24,71% and hypertension - 17,63%. Four studies noted that anterior cranial radiotherapy favors the decrease of the GH levels (more than

chemotherapy), decrease that correlate more frequently with an increase in total body fat, hypertriglyceridemia, hyper-LDL-cholesterolemia, hypo-HDL-cholesterolemia, a more frequent increase of fasting insulinemia, and a more frequent hypertension. In the study of a group of patients treated with both chemotherapy and radiotherapy, where the MS was present in a proportion of 33.33%, after 12 months of treatment with GH, the MS was present in only 5,56% of them, and after 24 months none of them had MS; they also noted an increase in left ventricular mass index and an improvement of cardiac systolic function. The cardiovascular toxicity of chemotherapy consists especially in left ventricular dysfunction induced by antracyclines and in endothelial dysfunction produced by the increase of homocysteine levels induced by methotrexate. Summary. The criteria that define MS are frequently present in the patients that were anterior treated for ALL. Anterior cranial radiotherapy favors a decrease in GH levels (more than chemotherapy), decrease that correlate more frequently with the presence of MS compounds. There is the necessity of studies that compare the metabolic dysfunctions present before ALL with those that appear after the treatment.

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ADOLESCENT AND YOUNG ADULT ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH PEDIATRIC PROTOCOL: A TUNISIAN MONOCENTRIC STUDY

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Background. Pediatric protocols give good results in childhood acute lymphoblastic leukaemia (ALL) comparing to adults treated with other protocol. *Aims*. We report in this study the results of pediatric protocol used for adolescent and young adult acute lymphoblastic leukemia *Method.* Between Januaty 2000 and December 2007, we retrospectively analyze the data of acute lymphoblastic leukaemia patients aged from 16 to 30 years treated according to the pediatric EORTC 58951 protocol. Patients were stratified in average and high risk groups according to white blood count at diagnosis, blasts phenotype, cytognenetics abnormalies and response to corticotherapy (cortico-sensitivity: blasts less than 1000/mm3 at day 8) and chemotherapy (complete remission: CR). Finally we analyzed the 5 years overall survival (OS), event free survival (EFS) and disease free survive (DFS). *Results*. Thirty three adolescent and young adult were treated according to the EPRTC 58951 pediatric protocol. Median age was 18 years (16 to 28) and sex ratio was 1.53. Median WBC was 53.000/mm3. B and T phenotype were observed in respectively 38 and 62% cases. Cortico-sensitivity was noted in 73% of patients. Three patient dead during induction and eight dead after, sepsis was the frequent cause of mortality (10 cases). Twinty nine patients achieved CR (96%). Four patients relapsed. Five years OS, EFS and DFS were respectively 48, 48 and 79%. *Conclusion*. This study showed that pediatric protocol can offer good results concerning CR and DFS to adolescent and young adult ALL. However OS an EFS, better than adult ALL treated during the same period by adult protocol (OS=18%, EFS=18% and DFS=24%) was not yet satisfactory because the high toxic mortality rate.

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ANTIOXIDANT STATUS IN EGYPTIAN CHILDREN WITH MALIGNANCIES

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Background. Combination of chemotherapy received by Children with malignancies make them prone to peroxidative injury. *Objectives*. The aim of this study was to evaluate antioxidant status in children suffering from different types of malignancies and to estimate the relation between antioxidants status and chemotherapy related side effects. *Patients and methods*. In a sample of 60 children having different childhood malignancies, antioxidant status was evaluated initially at diagnosis, after 3 and 6 months(6 ms) of therapy by measuring endogenous antioxidants (uric acid, albumin, bilirubin) , exogenous antioxidants (vitamin C , total antioxidant capacity [TAC]), and Malondialdehyde as oxidative marker. *Results*. Regarding exogenous antioxidants there was no statistical significant changes in albumin, bilirubin and uric acid, after 3or 6ms of therapy. Vitamin C and TAC showed significant reduction during follow up period when compared to baseline levels ($p < 0.001$ for all) while Malondialdehyde levels showed significant increase after 3 and 6 ms of chemotherapy when compared to baseline levels ($p<0.001$). Marked decrease level of endogenous antioxidants over 6 ms of chemotherapy treatment was associated with dose reduction and increase incidence of infection ($p=0.001$,$p=0.002$ respectively) but there was no significant changes in stoppage of chemotherapy and hospitalization throughout treatment when compared to baseline rate.No correlation between VIT C, TAC, and Malondialdehyde levels were found ($r=-0.17$ $P=0.37$,$r=0.09$ $p=0.63$,$r=0.00$ $p=0.99$). Levels of exogenous antioxidants and Malondialdehyde were not different among hematological or solid type of malignancies or in different age groups and both sexes .Patients with solid tumors showed significantly higher rate of chemotherapy stoppage after 6 ms compared to hematological tumors($p=0.03$). *Conclusion*. Children undergoing treatment for malignancy receive combination of chemotherapy which is associated with free radical production and increase oxidative stress in those children and related to complications of treatment.

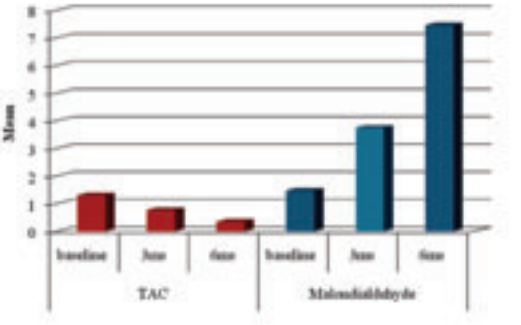

Figure 1. Mean values of TAC and MDA during treatment

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HOW TO TREAT ADOLESCENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Adolescents with acute lymphoblastic leukemia (ALL) have languished in the shadow of successful therapeutic outcome in childhood ALL. While 80% of children aged 1-15 years are long-term survivors, less than 40% of adults are cured with current therapies. Adolescents and young adults who may be eligible for both adult and pediatric protocols have continued to have an intermediate outcome, which has remained inferior to that in children. *Aims*. This article has summarized the recent and updated retrospective comparative analysis of adolescents treated with pediatric and adult trials. *Methods*. Data on ALL adolescents aged 16-18 treated with pediatric trial ALL IC-BFM 2002 were compared with literature reports on the treatment of ALL adolescents according to adult protocols. *Results*. During the 2002-2006 period, 143 ALL patients aged 16-18 were treated with pediatric trial ALL IC-BFM 2002. The 3-year event free survival (EFS) was 71%. During the 1997-2002 period, 67 ALL patients aged 15-17 were treated with adult trial UKALLXII/E2993, and 61 ALL age-matched patients were treated with pediatric trial MRC ALL97, with 5-year EFS of 49% and 65%, respectivelly. A similar 6-year EFS has been reported for the American pediatric regimen (CCG) that was by 26% higher in comparison with their adult regimen (Cancer and Leukemia Group B,CALCB). *Conclusion*. Therapeutic outcome in adolescents with ALL treated according to pediatric protocols is better (16 - 26%) in comparison with adult regimen.

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LONG-TERM OUTCOME AND PROGNOSTIC IMPACT RISK FACTORS IN 117 ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED WITH CHEMOTHERAPY WITH OR WITHOUT STEM CELL TRANSPLANTATION

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Background. Adult acute lymphoblastic leukemia (ALL) is often incur-

able despite intensive chemotherapy with or without hematopoietic stem cell transplantation (HSCT). Estimated overall long-term survival rate for adult ALL patients (pts) is about 20-50%. Aim. To present our results of treatment adult ALL and impact high risk factors on overall survival (OS). Patients and Methods. Since January 1989 till January 2011 we were treated 117 (male 83, female 43) pts with adult ALL, average age 30 years. Immunophenotype were determined in 85 (B-ALL 57, T-ALL 28) and cytogenetic/molecular analysis were succeeded in 55 pts. At the time of initial presentation: 29 pts had high white blood cell (WBC), 5 pts had CNS involvement and 19 pts had mediastinal mass. Pts treated with induction, consolidation and maintenance therapy under modified YU-ALL regimen for *high risk* (82), HyperCVAD (13), LALA 94 (8), CHOP, BFM, GMALL, EzOG UKCR, etc. (14). High risk pts was defined by the presence of at least one of following factors: age > 35 years, WBC > $30x10^9$/l (B-ALL) or $100x10^9$/l (T-ALL), CNS involvement, more than 4 weeks to achieve complete remission and finding Philadelphia chromosome (or bcr-abl+), t(4;11)+ or t(1;19)+. Medicament prevention (without radiotherapy) of CNS disease was applied to every pts under 50 years. Results. Complete remission (CR) was achieved in 104 pts (delayed CR in 18). Resistant to therapy were 5 (4.3%) pts, 6 (5%) pts have died (2 early deaths, 2 before evaluation of remission, and 2 in CR during the intensification due to infective complications). Maintenance therapy (MT) was applied for 24-36 months. HSCT was done in 44 pts: allo in 27 pts (CR1 in 15, CR2 in 8, and with partial response in 2 pts) and auto in 17 pts (CR1 11, CR2 5 and with molecular relapse 1 pts). Relapses have occurred in 67 pts (63%) with median time of 9 months. Frequency of relapses were higher in pts on MT (45/62 - 73%) comparing to HSTC pts (22/44 - 50%: auto HSCT 10/17 - 58% and allo HSCT 12/27 - 44%, respectively). The secondary allo HSCT was done in 5 pts (2 are still alive). Long-term survival without relapses had 19/106 (27.4%) pts; 10-years disease free survival (DSF) in MT pts was 13.65 +/- 5%, auto HSCT pts 16.4 +/- 11.5%, and allo HSCT pts 26.4 +/- 11%. Univariate analysis of impact high risk factors on overall survival showed significance in age (p=0.025) and CR achieved in 4 weeks (p=0.00028), marginal significance (p=0.070) in CNS involvement, while cytogenetics (p=0.90) and WBCs were without impact on outcome/overall survival in this cohort of pts. *Conclusion*. Results of our retrospective analysis are similar to the others and confirms that treatment of adult ALL is unsatisfactory. It is necessary to define precise prognostic factors, to stratify pts according to them and to use intensive chemotherapy with or without HSCT.

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PHILADELPHIA-POSITIVE ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: REAL LIFE OF TWO GEOGRAPHICAL REGIONS

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Background. Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ ALL) in adult patients is associated with a particularly unfavorable prognosis, although some progress has been made with the use of tyrosine kinase inhibitors (TKIs). Aims: Assessment of outcomes of adult Ph+ ALL patients treated in hematological centers in two geographical regions in the Czech Republic with a population of about 4.1 million between 1997 and 2010. *Methods*. Thirty-eight consecutive patients with newly diagnosed Ph+ ALL were included in the analysis, 26 (68%) patients were treated with chemotherapy and a TKI (imatinib or dasatinib), 12 (32%) with chemotherapy alone, mostly in the pre-TKI era. Sixteen (42%) patients underwent stem cell transplantation (SCT); 12 matched unrelated donor SCT, one HLA identical sibling donor SCT and 2 autologous SCT. Data were evaluated for complete hematological remission (CR), complete molecular remission (CMR) and relapse rates and for risk factors affecting overall survival (OS). We were also interested in the effect of a TKI added to chemotherapy. Results: Out of the total number of 38 patients, 29 (76%) achieved a CR (median time to CR 38 days, range 6-134 days), 17 (57%) of 30 evaluable patients achieved a CMR (median time to CMR 76 days, range 32-358 days). Ten (34%) patients experienced a relapse (median time to relapse 7.4 months, range 1.1-37.5 months) with a very short post-relapse survival regardless of the salvage therapy used. Molecular relapse was in all but one cases followed by a hematological relapse (median time to molecular relapse 2.9 months, range 1.4-13.1). At the end of the follow-up period with a median of 8.7 months, 14 (37%) patients were still alive in CR (only one treated in the pre-TKI era) and 24 (63%) died, mainly of infection (9 patients, 38%), multiple organ failure (7 patients, 29%) or disease progression (6 patients, 25%). Median OS was 9.0 months; the highest survival benefit was observed in patients achieving a CR (p<0.001) or a CMR (p=0.002) and treated with allogeneic SCT (p<0.001). The addition of a TKI to chemotherapy was associated with a higher CMR rate (p=0.026), but not with a higher CR rate (p=0.29), lower relapse rate (p=0.26) nor a prolonged OS (p=0.13). Summary/conclusions: In adult patients with Ph+ ALL the best outcome is achieved with the combination of chemotherapy, TKI and allogeneic stem cell transplantation. However, more than half of the patients are not eligible to undergo such intensive treatment. Although the investigated geographical area was wide, incidence of Ph+ ALL is very low and multicentric or international cooperation is therefore very helpful.

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LOW DOSE AMPHOTERICIN LIPID COMPLEX FOR PRIMARY PROPHYLAXIS AGAINST INVASIVE FUNGAL INFECTIONS IN TEENAGE AND YOUNG ADULT PATIENTS UNDERGOING INDUCTION CHEMOTHERAPY FOR ACUTE LYMPHOBLASTIC LEUKAEMIA

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Patients with acute lymphoblastic leukaemia (ALL) are at high risk of developing invasive fungal infections (IFI) during induction treatment with significant mortality and morbidity. The reported incidence of IFI is up to 40% and with an associated reported mortality of 20-50%. In Teenage and Young Adult (TYA) patients, there is increasing use of paediatric protocols, however the increased intensity of these regimens potentially increases the risk of developing IFI. There is no consensus regarding the optimal antifungal strategy in this population. In addition the interaction between vincristine and azoles restricts the choice of drugs. We have previously reported the efficacy and tolerability of low dose amphotericin B lipid complex (Abelcet) as anti-fungal prophylaxis in adult patients undergoing ALL induction. We therefore applied this regimen to our TYA population receiving treatment according to the UKALL 2003 protocol. 20 consecutive patients (aged 16-24) with newly diagnosed disease treated at a single centre (March 2007 - Jan 2011) were included in this retrospective series. 3 were non-evaluable as they received induction treatment elsewhere. Chemotherapy consisted of induction (35 days) comprising weekly vincristine (x5) and daunorubicin (x4), daily dexamethasone and 2 doses of pegylated asparaginase (days 4 and 18) followed by standard BFM consolidation comprising weekly cytarabine (4 days per week for 16 doses), daily 6-mercaptopurine and 2 doses of cyclophosphamide (days 1 and 15). 2 patients received augmented BFM consolidation. Patients were hospitalised during induction during which time they received antifungal prophylaxis using Amphotericicin. 17 patients received Abelcet (100mg 3 x per week by intravenous infusion). Only one patient was intolerant of Abelcet and was treated with Ambisome (5mg/kg once weekly by intravenous infusion). Consolidation chemotherapy was predominantly administered in the clinic and upon completion of vincristine, antifungal prophylaxis was switched to daily itraconazole liquid (200mg bd) or capsules (200mg tds). Fungal infections were categorised into possible, probable or proven according to the EORTC/MSG criteria. Four patients developed a possible or proven IFI during the study period with a cumulative incidence of 32.5% (95% CI 16.4-64.2%). All infections occurred during the consolidation phase with one death due to infection. A further 3 patients received empiric antifungal therapy (1 during induction and 2 during consolidation) due to suspected fungal infection (predominantly unresponsive fever), although they did not meet the EORTC/MSG criteria for a possible IFI. Our data indicate that low dose Abelcet is an effective and well-tolerated strategy for primary antifungal prophylaxis in TYA patients undergoing induction chemotherapy for newly diagnosed ALL. However, patients remain at high risk of developing fungal infections through subsequent consolidation treatment during which itraconazole prophylaxis appears ineffective. The optimal antifungal strategy during this phase of treatment has yet to be established although is of importance given the significant risks associated with IFI. Our results suggest that continued low dose intermittent amphotericin B lipid complex may be effective although is inconvenient in the outpatient setting.

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THE OUTCOME OF THE ADULT PH-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS FROM AN EAST EUROPEAN COUNTRY IN TKIS ERA - THE EXPERIENCE OF ROMANIAN WORKING GROUP FOR ADULT ACUTE LEUKEMIA STUDY (RWGALS)

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Background. Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (Ph+ALL) includes at least one-quarter of all adults with ALL. The conventional chemotherapy programs that are effective in other precursor B-cell ALL cases are unable to cure these patients. The availability of imatinib mesylate and other tyrosine kinase inhibitors (TKIs) changed the treatment programs and the prognosis for these patients. In Romania Imatinib combined with chemotherapy was approved as standard therapy for Ph+ALL in 2006. *Aims*. We studied the outcome correlated with treatment programs for 21 patients with de novo Ph+ALL. Materials and methods: There were 11 male and 10 female, aged between 17 and 71 years (median = 48y) diagnosed 2007-2009. Imunophenotypic diagnosis was ALL preB for 9 patients, ALL - common for 10 and biphenotipic acute leukemia for 2 patients. All patients (even at 71 years old) received curative chemotherapy for induction and consolidation and imatinib, 600 mg daily (except 1 patient who received 400mg/d). *Results*. Complete remission achieved 76% patients at on average of 44.8 days from diagnosis (limits 24 - 66 days). Complete molecular response was documented for 42.8% patients, at a median of 385 days (limits 177 - 485 days). Imatinib was started after second induction or after first consolidation. For 57.14 % of patients chemotherapy during imatinib was low dose or reduced intensity. Due to SCT policy in our country for ALL, no patient was transplanted in first remission. PFS was 13.8 months (limits 3 - 37 months). Relapsed treatment was: intensive chemotherapy for 4 patients, 1 patient switch to dasatinib and other 3 patients received tandem therapy with dasatinib and nilotinib. Only one patient was transplanted in the second CR, and received Dasatinib after transplant. At the end of our study, 5 patients were living at 8, 14, 16, 20 and 37 months respectively, 4 of them with CR, one relapsed. Disease/relapse related mortality was 27.7%. Treatment related mortality was 44.4%. Overall survival was on average 12.25 months. *Conclusions*. The prognosis of adult patients with Ph+ ALL treated only with chemotherapy is poor, with a less than 10% probability of long-term survival. The use of imatinib as part of front-line treatment in combination with cytotoxic agents has greatly improved the rates of complete hematologic and molecular remission and overall outcome in adult patients with newly diagnosed Ph+ ALL. However the allogeneic SCT is the only treatment option with definite curative potential. Our data show that only aggressive chemotherapy combined with imatinib would really change the outcome of Ph+ALL new diagnosed patients.

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EVALUATION OF THE CCG 1991 AND CCG 1961 PROTOCOLS OF THERAPY OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN TWO EGYPTIAN ONCOLOGY CENTERS: A PILOT STUDY

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Clinical trials in childhood ALL have yielded 5-year event-free survival (EFS) rates as high as 80%, yet we did not reach comparable results partly due the high mortality and morbidity rates associated with high dose methotrexate therapy .Since 2004, the Pediatric Oncology Centers in Ain Shams University and Menoufeya University shifted the protocol of therapy of standard risk ALL to the CCG 1991 , and the high risk ALL patients were shifted to the CCG 1961 protocol, being more cost effective and not including HDMTX like the previously used BFM 90 protocol.Aim of the work: This study aimed to evaluate the efficacy of the CCG protocols (CCG 1991and CCG 1961)in the treatment of childhood ALL in Ain Shams and Menoufeya University Pediatric Oncology Centers and the associated morbidity and mortality rates . Methods:A prospective study including 50 ALL patients less than 17 years old diagnosed in both University Centers in the period from April 1st 2004 to December 31st 2005 , recruited after having parental consent. Patients were classified into 3 risk groups, standard risk (SR), high risk standard arm (HR-SA) and high risk augmented arm (HR-AA) based on clinical and morphological data and response to therapy. Risk stratification used did not include cytogenetics or minimal residual disease. Protocol CCG 1991 (Arm OS with single delayed intensification) was used for standard risk patients. Protocol CCG 1961 (Arm A standard BFM arm of standard duration) was used for HR-SA patients , and CCG 1961 (augmented BFM arm using doxorubicin) was used to treat HR-AA acute lymphoblastic leukemia patients .Results: The mean age of the patients was 5.9 (+3.3) years, male to female ratio was 1.6:1. CNS leukemia was present in 6% of patients at diagnosis . 14 patients (26.9%) had T-ALL and 38 (73.1%) had preB-ALL. Initial total leucocytic count >50.000/cmm was present in 16 patients(30.8%). According to risk stratification : 25 patients were SR (48.1%), 16 HR-SA (30.8%), and 9 HR-AA (17.3%).The 5-year overall survival (OS) and 5-year EFS of the total number of patients was 83% and 67% respectively. The 5 year overall survival (OS) was 97% , 55%,88%, for SR , HR-SA, and HR-AA ,respectively. The 5-year EFS for the SR, HR-SA and HR-AA patients was 60%, 58% and 70% respectively. Grade 3-4 adverse events were reported in five patients. Relapse and death occurred in 3(12%) and 1(4%) in SR patients respectively, in 2(15.4%) and 4(30.8%) in HR-SA,and in 1(8.3%) and 1(8.3%) in HR-AA respectively ,(P=0.05). Conclusion: The outcome of SR and HR-SA protocols used was not satisfactory, accordingly intensification of protocols was done shifting SR ALL to CCG 1961 arm OD with double delayed intensification (DDI), and shifting HR-SA to CCG 1991 HR-SA ARM B (standard BFM with increased duration and DDI).Cytogenetics and Minimal residual disease assessment are mandatory for prediction of risky patients in SR ALL and in HR-SA groups for better risk stratification of therapy to achieve better childhood ALL survival.

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CHEMOTHERAPY RELATED ACUTE SIDE EFFECTS IN CHILDREN TREATED FOR ALL

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Background. The survival rate in childhood ALL has reached to 80% with the contemporary chemotherapy protocols and increased supportive care. However, chemotherapy related acute side effects may stil cause serious problems leading to morbidity and mortality during treatment. Although the reports about the late effects among the survivors are frequent, the data related to the acute adverse effects are scarce. *Aims*. This study evaluated the acute side effects of chemotherapy in children with ALL. *Method*. The acute toxicity according to the WHO criteria for each chemotherapy phase in 210 children treated as ALL between the years 1993 and 2009 were, retrospectively, evaluated from their hospital records. Children received either BFM-95 (76.5%) or BFM-2000 (%23.5) protocols. *Results*. The data of 187 children were evaluable. The mean age and male to female ratio were 62±51.7m and 1.7, respectively. The median follow-up period was 61.3±43.4m. Children were stratified into 3 risk groups as standart (19.8%), median (61.5%) and high (18.7%). General well being was similarly effected during the induction phase in all risk groups ($p>0.05$). However, poor condition was significantly more frequent in high risk group blocks ($p<0.001$). Bone marrow toxicity was more prominent during induction phase in each risk group, however, it was significantly depressed during high risk blocks ($p<0.05$). The frequency of nausea were found similar during all treatment phases. Vomiting, stomatitis, diarrhea were significantly occurred in high risk blocks along with elevated liver transaminases ($p<0.001$). MTX related skin changes (Grade 1/2) were observed in 3.2% (n:6) of the patients. Allergic drug reactions due to L-asparaginase including urticaria and/or anaphylaxis occured in 31% (n:58) of children. Seven out of 187 children (3.7%) developed tumor lysis syndrome with reversible acute renal insufficiency during induction phase. Grade 1 and 2 increase in serum creatinine level were significantly frequent in HRG blocks ($p<0.001$) and one of them required hemodialysis. Cardiac dysfunction and hypertension were determined in 6.9% (n:13) and 9.6% (n:18) of the children, respectively. Hyperglycemia related to steroid and/or L-asparaginase occurred in 13.9% (n:26) of the patients. CNS toxicity including, seizures, thrombosis, hemorrhage, infarct, PRES and encephalopathy was observed in 20 (10.6%) children. The number of febrile episodes per patient was 4.8. The primary site of infection could be determined in

%39.1(n:348) of these episodes. Causative microbiologic agent could be demonstrated in 23.3%. The frequency of grade 3 and 4 infections were significantly more in HRG (p<0.001). Overall and event free survival rates were found 86% and 82.9%, respectively. Twenty-six patients (%13.9) died of either infection (46.2%) or relapsed/refractory disease (53.8%). None of the childeren were lost due to drug related acute toxicity.

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9P21 DELETIONS IN ACUTE LYMPHOBLASTIC LEUKEMIA OF CHILDHOOD

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Background: The putative tumor suppressor gene called CDKN2/INK4A/p16 was also mapped to chromosome band 9p21. The gene codes for cyclin-dependent kinase inhibitors (CDKI). These CDKIs can bind and inhibit the kinase function of the cyclin-dependent kinase 4 (CDK4) and CDK6, resulting in the blockade of the cell cycle between G1 and S, leading to a suppression of cellular proliferation. For this reason, these CDKIs may act as tumor suppressors. Therefore, their inactivation might contribute to development of cancer. Deletion of the 9p21 chromosomal region are frequent in childhood acute lymphoblastic leukemia (ALL) but, the prognostic significance is controversial. In childhood ALL, CDKN2/INK4A/p16 inactivation is found in more than 20% of B-lineage ALL and 50% of T-ALL. Methods: We studied 85 children (aged 12 months to 17 years) consecutively diagnosed as ALL. Of the 85 children, 64 had B-ALL, whereas 21 had T-lineage ALL. All patients were treated according to the protocols of the BFM-ALL 2000 between January 2008 and December 2010. CDKN2A inactivation by deletion was studied using Fluorescence In Situ Hybridization (FISH). Results: Bi-allelic and mono-allelic deletion were found in, respectively, 4 (4.7%) and 8 (10.5%) of 84 children. At the time of analysis, the median follow-up was 1.2 years (range 6 months-3 years). Summary. A pathogenetic role for p16 gene deletion in the development or progression of those ALL, suspected because of the potentially antiproliferative properties of p16, will have to be clearly shown.

Table 1. Clinical outcome according to 9p21 status

	Total population studied (n:85)	9p21 subgrup	
		Bi-allelic deletion (n:4)	Mono-allelic deletion (n:8)
Immunophenotype			
T-cell	21	4	3
B-cell	64	0	5
No event	80	1	7
No CR	1	0	0
Relaps	2	2	1
Death in CR	2	1	0
Survival status			
Alive	79	1	8
Death	5	3	0

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GASTROINTESTINAL TOXICITY SECONDARY TO TREATMENT OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Acute lymphoblastic leukemia (ALL) is the most frequent cancer in childhood. Although intensive chemotherapy has improved survival in those patients, important side effects, including acute gastrointestinal (GI) toxicity are frequent. Although GI toxicity is frequently described in reported ALL series of patients, the different criteria used to define severe GI toxicity translates in very different incidence of such complications in the literature. *Aim*. To describe the incidence of severe acute GI toxicity in a series of ALL patients treated in a single centre during the last 11 years, and to report the short and long-term outcome of these complications. *Patients and methods*. Data from children diagnosed with ALL in Hospital Sant Joan de Déu from july 1999 to january 2011 were collected. Patients were treated according to two sequential trials of the Pediatric Hematology and Oncology Spanish Group (SEHOP), SHOP ALL-99 and SHOP ALL-2005 that include standard agents for ALL treatment. Severe (grade III-IV) GI complications were analyzed. *Results*. During this period 143 children were diagnosed with ALL at our institution. Sixteen of them (11.1%) presented enterocolitis-typhlitis and two (1.3%) appendicitis during the induction phase (n=14), consolidation (n=1) and intensification (n=1). The main clinical findings were abdominal pain (100%), defined as severe in 77% of cases, and mucositis (77%). Neutropenia was present in 83% of the patients (severe neutropenia in 55%), with a mean duration of 15 days. Blood cultures were positive in 4 children for S. aureus, P. aeruginosa, E. coli and S. epidermidis, respectively. Most patients (n=10) improved with medical treatment but 5 patients (3.4%) needed surgical treatment and one child died because of refractory sepsis (P. Aeruginosa). Twelve patients (8.3%) presented an elevation of pancreatic enzymes (amylase ranged from 114 to 1499 UI/L and lipase from 158 to 455 UI/L), related to L-asparraginase administration. Seven patients were asymptomatic and the rise in pancreatic enzyme was transient; however, five patients (3.4%) presented an acute pancreatitis with severe abdominal pain that needed analgesic treatment and delayed the scheduled chemotherapy administration. One patient died from fulminant pancreatitis and the other patients had a favorable outcome Three patients (2%) presented a grade III-IV increase in bilirrubin levels during induction phase. One of them developed a fulminant hepatitis and died, while the others improved after adjustment of the chemotherapy doses. Overall, the incidence of severe GI toxicity in our series was 16% and the mortality rate due to GI complications was 2%, similarly to other previously reported data. *Conclusions*. GI toxicity of standard chemotherapy regimens is frequent in ALL patients. Close monitorization of pancreatic enzymes is necessary during the L-asparraginase administration, but in most cases isolated rise in pancreatic enzyme levels in an otherwise asymptomatic patient will be transient and will not require treatment interruption. Although most GI complications were manageable with medical treatment, some patients eventually died due to GI toxicity.

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PEDIATRIC-LIKE INTENSIFIED THERAPY IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: A SINGLE CENTRE EXPERIENCE

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Background. Acute lymphoblastic leukemia (ALL) shows different outcome in children and adults, with event-free-survival (EFS) rates of 70-80% and 30-40% at 5 years, respectively. Recently, results improved in young adults/adolescents aged 15-21, with de novo ALL, when treated with pediatric intensive regimens rather than with typical adult regimens. Clinical studies are ongoing in older patients, toxicity related-therapy seeming the limiting issue. *Aims*. We report a single centre experience on adult ALL patients treated with an intensive pediatric-inspired schedule, aiming to assess its tolerability and efficacy. *Methods*. From 11/07 to 02/11 we treated 22 ALL patients (M/F=16/6) according to modified AIEOP-LAL2000 regimen. Treatment consisted of 7 days steroid pre-treatment, and four drugs 78-days induction (phase IA and IB) after which high risk (HR) patients were treated with three polychemotherapy blocks, while intermediate (IR) and standard risk (SR) patients went on 8-weeks consolidation and subsequent intensification. A 2 cycle consolidation therapy with nelarabine was planned for T-ALL patients. Patients with HLA-matched donor underwent allo-SCT; 2-years maintenance therapy was given to the others. Median age was 32 years (17-47). *Results.* 20/22 patients completed the phase IA, 2 being out for grade IV toxicity (intestinal occlusion and sepsis). 15 (68%) obtained a complete remission (CR), 5 (23%) were refractory. Three of the resistant patients subsequently achieved CR: one after polychemotherapy blocks, two after phase IB. Median induction duration (IA+IB) was 95 days (82-136); delays were mostly accumulated during the interval between phase IA and IB and were due to logistic reasons and extra-hematologic toxicity. A higher absolute number of adverse events during phase IA than during IB was registered (infections and gastrointestinal), without a significant prolongation of phase IA. After induction, 3 of the 15 CR patients received consolidation therapy, then 2/3 underwent allo-SCT. 5 patients received blocks: 3/5 undewent allo-SCT, 2/5 dropped out after the first and the second block for reversible grade II-III renal toxicity. 3 patients were treated with nelarabine, then 1/3 underwent allo-SCT. 3/15 directly underwent allo-SCT, while 1 patient completed the whole

therapeutic program because no suitable donors for allo-SCT were found. Median CR duration was 12 months (3+-44+); 6 patients relapsed, 3/6 after allo-SCT. With a median follow up of 13 months (3-49), 14/22 (63,6%) patients are alive, 7 in CR (4 underwent allo-SCT). 8 patients died, 4 for relapsed/refractory disease, 4 in CR (3 after allo-SCT). On the basis of pediatric management, minimal residual disease assessments on days 33 and 78 of induction were scheduled. Important technical and logistic problems were found: MRD was possible only for 7 of the 15 CR patients, due to no probe individuation, no sample collection or degradated sample at diagnosis. *Conclusions*. A pediatric-inspired therapeutic regimen seemed to be feasible in adult ALL patients, even if significant delays due to logistic reasons and extra-hematological toxicity caused a lack in dose-intensity maintenance. The overall outcome seemed to be lower than the one reached in other studies conducted on larger population: a longer follow-up and a larger population are needed to draw definitive conclusions.

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IMATINIB ASSOCIATED BONE MARROW FAILURE IN A PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA PATIENT

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Background. Imatinib is a tyrosine kinase inhibitor (TKI) that have improved substantially the outcome of Philadelphia-positive (Ph-positive) acute lymphoblastic leukemia (ALL) patients. Before the advent of TKIs, Ph-positive ALL carried a dismal prognosis and was characterized by a poor response to most chemotherapy combinations, short remission durations, and poor survival rates. Imatinib combined with chemotherapy induced complete remissions in >90% of patients with newly diagnosed Ph-positive ALL. *Aims.* Description of an unusual clinical case of bone marrow aplasia after imatinib treatment. *Methods.* Literature search from clinical cases reporting bone marow aplasia/failure/severe pancytopenia related to TKI. *Results.* A 44 year old man was diagnosed of Ph-positive ALL on March 2009. He started treatment according to the PETHEMA Group LAL-Ph-08 protocol receiving imatinib 600 mg/d in combination with chemotherapy. Complete response (molecular included) was achieved on day +29 since treatment initiation. No HLA-identical siblings were available; therefore he was offered an autologous bone marrow transplantation (BMT) according to the clinical trial. Neutrophils and platelet engraftments were documented on day +17 and +33 respectively. He never reached normal peripheral blood cell count due to a mild thrombocytopenia. On day +92 he started maintenance therapy including imatinib 600mg/d, 6-mercaptopurine 40mg/m2/d and methotrexate 15 mg/m2/w until two years from complete remission. Five months from maintenance initiation the patient was admitted to hospital facilities and diagnosed from interstitial pneumonitis assumed to be caused by an unidentified viral agent. He was discharged 3 weeks later without ALL treatment due to bicitopenia (anemia and thrombopenia). One month later hemoperipheral counts showed no improve. Bone marrow aspirate was normal and karyotyping showed cytogenetic response. Seven weeks after treatment discontinuation, imatinib was started at the same dose with progressive aggravation of citopenias. There were no peripheral causes involved. Bone marrow biopsy performed three months later showed non-severe bone marrow aplasia with no morphological evidence of disease progression and the patient stayed on molecular remission. Imatinib treatment was discontinued and peripheral counts recovered close to normal values after several weeks. *Summary.* The literature describes few cases of pancytopenia associated to TKIs use, mostly related to chronic myeloid leukemia (CML) probably due to the larger number of patients presenting the disease. We have found only one publication describing bone marrow failure in this clinical scenary. As in CML, Ph-positive ALL patients seem to have no other treatment options to preserve molecular remission. Bone marrow failure related to TKIs utilization is a potentially serious adverse event. We are not able to predict the clinical outcome but is reasonable to think that TKIs interruption could lead to disease relapse. In our opinion, some issues remain unclear, as if temporary interruption of TKI will be enough, when to restart medication or which dose should we use. Other items to address should be the possibility of using a different TKI or what to do if marrow failure is observed after drug reintroduction. Therefore an effort to collect and analyze data from this population should be made in order to offer the best therapeutic options available.

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EXPRESSION OF PIM-2 AND NF-KB IS INCREASED IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) AND ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AND CORRELATES WITH COMPLETE REMISSION RATE

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Background. PIM-2 is a proto-oncogene that encodes for serine/threonine kinase which interacts with various signalling molecules. PIM-2 is highly expressed in neoplastic tissues and in leukaemic and lymphoma cell lines which is consistent with a its role during oncogenic transformation. The nuclear factor kappa B (NF-κB) pathway appears to be deregulated in variety of tumors, with sustained activity of NF-κB leading to apoptotic resistance in tumor cells The aim. The aim of this study was to investigate whether the PIM-2 and NF-κB expression is altered in acute myeloid leukemia (AML) and acute lymphoblastic leukaemia (ALL). *Patients and methods*. One hundred fourty-three patients were included: 91 with AML and 52 with ALL (42 with B-ALL and 10 with T-ALL), aged 18-84 (median=41). Seventy-five patients reached complete remission (CR): 50 in AML and 25 in ALL. Bone marrow samples were collected at the time of diagnosis. Control samples were obtained from 24 healthy donors. We analysed PIM-2 and NF-κB expression by RQ-PCR analysis. *Results*. Expression of both PIM-2 and NF-κB in all leukaemic patients and in subgroups: AML and ALL was significantly higher than in controls. In AML group patients who reached CR expressed PIM-2 and NF-κB at significantly lower levels than patients with primary resistance to chemotherapy (with no CR, NCR). Moreover in AML, we have found the correlation between PIM-2, NF-κB expression and blasts in myelogram and PIM-2 and patients` age. Summary. Our data indicate that PIM-2 and NF-κB genes expression is increased in patients with AML and ALL and correlates with CR rate in AML patients.

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DYNAMICS IN SOME PARAMETERS OF IRON STATUS MARKERS AND ANTIOXIDANT SYSTEM IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background. Iron overload is a negative marker in patients with myeloid neoplasms as it associated with non-leukemic events including VOD, GvHD and fungi infection. The mechanism of tissue damage by free iron includes formation of NTBI and LPI with following excess generation of ROS. Intensive chemotherapy is possible way of NTBI formation in patients with AML and risk factor of infection in collaboration with neutropenia. Aim. To find out the association between dynamics in some parameters of iron status and AO system in AML patients after intensive chemotherapy. Methods. The serum of 14 AML patients with median age 46 y (34-68) was prepared before and after chemotherapy, at day of WBC level >1x10^9/L and day of the following hospitalization. The chemotherapy regimens were 7+3 induction therapy (3 patients), courses with Ara-C dose ≥1 g/m^2 (8 patients) and Bu+Cph before AutoSCT (2 patients) and AlloSCT (1 patient). The levels of transferrin saturation, malone dialdehyde concentration, and activity of superoxide dismutase, catalase and ceruloplasmine were detected by standards assays. *Results*. After chemotherapy the range of TS levels was 92.8%-101.5% and the median value was significantly higher than before therapy: 96.8% vs 41.9%; p<.0001. At the time of hematopoiesis restoration the level of TS was lower than after chemotherapy: 89.5% vs 96.8%; p=.003. Activity of antioxidant enzymes was changed differently, and the variability of MDA levels during and after chemotherapy was without any significant change. After chemotherapy and during the period of aplasia the activity of catalasa was significantly lower than before therapy: 3.8 and 3.3 vs 5.7; p=0.028 and p=0.011. Activity of SOD was decreased only at the time when WBC was ≥1.0x10^9/L: 21.0 vs 41.0; p=0.018. At the same time the activity of CP was significantly higher than before chemotherapy: 1.1 vs 0.8 g/L; p=0.029.Three episodes if infection complications were registered after chemotherapy. One patient was diagnosed with herpes simplex skin involvement after restoration of hematopoiesis. Two cases of sepsis were diagnosed during aplasia with one lethal outcome. *Summary*. As the level of TS exceeded ≥80% is associated with NTBI formation (*Sahlstedt L. et al. Br.J.Haematol*

2001;113:836) we conclude that there is a phase of free iron overload in AML patients during the period of postCT cytopenia. The data support the idea that the changes of AOS are the compensate mechanism which have to deteriorate the complications of non-transferrin-bound iron after chemotherapy. The prospective clinical trial will be design to compare the clinical effectiveness of antioxidants after intensive chemotherapy.

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METABOLISM OF MEMBRANE PHOSPHOLIPIDS IN ACUTE LEUKEMIA AND DURING OF CHEMOTHERAPY

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Background and aims. Membranes of blood lymphocytes were characterized with the significant phylogenetically stabilized phospholipids-phospholipids interrelations. It is well known that abnormalities of these interrelations play an important negative role in the development of pathogenic mechanisms, which condition inactivation of the membrane bound enzymatic systems, catalyzing the reactions transportation through membranes, as well as transduction of the external signals into the cell. The quantities and qualitative structure of phospholipids in the lymphocyte membranes, as well as lipids peroxidation processes and activity of phospholipase A2 have been studied in patients with acute leukemia and during of chemotherapy. *Materials and methods*. Experimental studies were carried out on 24 patients with first diagnosed acute leukemia. Biochemical analyses were done on first day and on tenth day: one group during traditional chemotherapy, second group during chemotherapy with additional antioxidant therapy. Fractionation of the individual phospholipids (PL) was realized by thin-layer chromatography with silica gel LC 5/40 m. After that the lipid phospholipids was defined. Lipids peroxidation activity has been determined by the reaction of malonic dialdehyde with tiobarbituric acid by the known method. Study of phosphlipasa A2 activity was accomplished by spectrophotometric assay modified. *Results and conclusions*. Our investigation showed to acute leukemia it is peculiar essential disturbance of the qualitative and quantitative contents of almost all representatives of lymphocytes membrane PL, mainly phosphatides. Under these conditions a pronounced increase of cytotoxic lysophosphatidylcholones (LPC) is accompanied by simultaneous pronounced of phosphatidylcholines (PC) concentration, which testifies about phospholipase A2 and lipid peroxidation processes activation. Hence, the acute leukemia is characterized by noticeable increase of the contents of diphosphatidylglycerides (DPG), while spingomyelins (SM), phosphatidylinosites (PI), phosphatidylethanolamine (PE) and phosphatidylserine (PS) decrease. While using chemotherapy alone in the treatment of acute leukemia is usually programmed for and followed by certain positive clinical outcomes, the combination of such treatment with additional antioxidant therapy results in more expressed biochemical changes. This method is bringing to normalization of phospholipase A2 and lipid peroxidation activity and balanced qualitative and quantitative structure of lymphocyte membrane PL. The changes of membrane PL can be qualitative characteristics of process activity. The same time level of balancing qualitative and quantitative structure of lymphocyte membrane PL is voluble index to appreciate treatment effectiveness.

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TRIPTASE: A NEW ACUTE MYELOBLASTIC LEUKEMIA MARKER

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Background. Triptase is the main protein of human mast cell secretory granules. Recent studies have shown that serum triptase levels are high in 40% of patients with acute myeloblastic leukaemia (AML), especially in those with favourable karyotype, inv 16 and t(8;21), with or without association of KIT mutations. Aims. To study the incidence of high triptase levels in acute myeloblastic leukaemia. To analize the correlation between high triptase levels and other markers like CBF alterations and KIT mutations. To analize its relevance as minimal residual disease (MRD) biological marker. *Methods*. The study was performed prospectively and retrospectively on a total of 58 patients with acute myeloblastic leukaemia (except promyelocitic M3): 29 men and 19 women (27-87 years old). Serum triptase levels were quantified by FEIA. Acute myeloblastic leukaemia subtype, karyotype and molecular biology were examined at diagnosis of every patient included. Results: Out of 58 patients, 17 showed high triptase levels (29%): 1 M0, 1 M1, 4 M2 (1 M2 Eo), 6 M4 (5 M4 Eo), 2 M5 and 3 unknown subtype. Taking into account molecular alteration, high triptase levels were observed in 3 of 4 AML1-ETO positive patients (one of them with KIT mutation), 1 of 2 patients with inv16 and 2 of 9 patients with FLT3-ITD mutations. Higher triptase levels (>200) were observed associated with inv 16 and t(8;21). Out of these 17 patients with high triptase levels, we have evolution data of 12. We observed that out of these 12 patients, 6 reached normal triptase levels concurring with complete remission of the disease and in 2 patients, high triptase level kept despite achieving CR (these patients had the highest triptase levels among all the samples processed, 165 and > 200) and levels came back to a normal range afterwards. Out of these 12 patients, 4 showed refractory disease; 3 of them kept high triptase levels and in the one left (with slightly high levels at diagnosis), triptase came back early to a normal range, despite having active disease. From the patients who achieved CR, just one relapsed. She has a central nervous system relapse and bone marrow relapse later in time; showing an increase in triptase levels with the bone marrow relapse. *Conclusions*. 29% of AML patients showed high serum triptase levels, which is lower than the incidence published so far. The higher triptase levels (> 200 µg/L) were found in patients with AML1-ETO and inv 16, there seems to be an association with CBF rearrangement, as it has been described before. Triptase levels tend to decrease when CR is achieved, so it might be useful as a MRD biological marker, taking into account that this should be confirmed studying a greater number of patients.

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PLATELET AGGREGATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROMES -CORRELATIONS WITH THE FLUIDITY MEMBRANE CHANGES AND ROS LEVEL

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Background. Patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) present severe alterations of platelet function. Platelets from these patients are dysplastic and have alterations in membrane and granular content. Aim The purpose of this study was to identify abnormalities in platelet function and a possible correlation with changes in platelet membrane fluidity and the reactive species level. Material and method We present a prospective study on 73 cases with MDS compared with 33 cases with AML admitted in University Emergency Hospital Bucharest. Three patients with AML were investigated in early stages and the phase of complete remission of the disease. Platelet function was investigated by platelet aggregation using as stimuli ADP, collagen, epinephrine and ristocetin. Membrane fluidity was assessed by fluorescence anisotropy measurements using TMA-DPH, the ROS level determination was performed using DCFDA method. Results. Platelet aggregation was altered in both groups of patients, more pronounced for AML patients. (AML vs. MDS: ADP 28.10 vs. 37.88, p=0.009; collagen 40.69 vs. 58.37, p=0.003; epinephrine vs. 16.48 24.16, p=0.14; ristocetin 43.73 vs. 50, p=0.37). No significant differences were obtained in the lag phase in the two groups of patients. The platelet aggregation response was ımproved in remission phase of AML for all reagents .The membrane anisotropy was ıncreased in AML patients compared with MDS patients(r = 0.1711 vs. 0. 1372, p = 0.002), this result correspond to low fluidity of membrane. There was not obtained a statistically significant correlation between the degree of membrane anisotropy and severity platelet function. Reactive species level is slightly higher in AML patients (0.0005280 vs. 0.0004051, p = 0.52). This level is significantly increased in advanced phase of disease.(LAM patıents 0.0006078 vs. 0.0001413, p= 0.003; MDS patıents 0.0005452 vs. 0.0001804, p=0.005). We could not establish a correlation of the fluidity changes depending on the level of ROS. *Conclusion*. AML patients have an advanced degree of alteration of platelet function and a low fluidity of platelet membranes compared to MDS patients. Achieving a clinical remission may improve platelet function, possibly due to a more appropriate expression of platelet receptors and / or improving cellular signaling, possibly correlated with low levels of reactive species.

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BENZENE - THE MOST IMPORTANT LEUKEMOGENIC FACTOR IN VOJVODINA

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Background. Benzene is the most toxic substance for hematopoesis. Hematotoxic effects are expressed as cytopenia, aplastic anemia, myelodysplasia, acute leukemia and other hemoblastoses, due to genotoxic effect or epigenetic modification. The most common consequence is acute myeloblastic leukemia (AML). There is no safe benzene concentration, no tolerance to benzene, and susceptibility is individual. Permissible concentration of benzene does not exist. Risk of AML is increased at cumulative exposure above 2 ppm-years and with intensity over 0.8 ppm. Inhalation is the most common exposure to benzene. During benzene metabolism, produced free oxygen radicals damage DNA, which could cause gene mutation or epigenetic modification with gene silencing. In Vojvodina, with petrochemical industry, agriculture with high pesticide usage and intensive road traffic, permanent increasing trend of AML was recorded during the last tree decades. On the basis of our clinical studies this can be explained by higher exposure to benzene. Of all adult patients with AML in Vojvodina 25% are from Novi Sad. *Aims*. The aim of this study was to analyze consequences of benzene exposure in residents of Novi Sad who are living near oil refinery. The second aim was to evaluate potential genotoxic damage in oil refinery workers occupationally exposed to benzene. *Methods*. Oxidative stress (OS) and DNA damage in the group of 60 residents environmentally exposed to benzene was investigated measuring 8-OhdG, which implies oxidation of DNA, in urine by gas chromatography-tandem mass spectrometry (GC-MS). Control group represented 60 residents from other parts of Novi Sad. Genotoxic biomarkers were investigated in peripheral blood lymphocytes in 313 refinery workers exposed to petroleum and its derivates using sensitive sister chromatid exchange test (SCE) and in minority of the workers by mononucleus test (MN). Control group represented persons without contact with benzene. *Results*. Examination of OS in environmentally exposed residents showed increased values of 8-OhdG. This result was significantly higher than in control group. Genotoxic biomarkers SCE and MN were significantly higher ($p<0.05$) in workers directly exposed to benzene in comparison to office workers. Cigarette smoking additionally increased SCE frequency. *Conclusions*. These results confirm our previous clinical studies which implicated that benzene is the most important leukemogenic factor. Low doses of benzene after inhalation can be enlarged by additional dermal contact or oral benzene intake with water. It is possible that low doses of benzene act in synergism with confounders (smoking, radiation, chemicals). Low doses of benzene and its metabolites could cause epigenetic modifications and immunodeficiency. On the basis of our research we would strongly support regulations for benzene concentration in Serbia and reduction of occupational full shift exposure in the range of 0.5-1 ppm, without high short exposures. Adequate individual protection, monitoring of biomarkers and hematologic examination are necessary. For ambiental air benzene concentration European limit is 5µg/m3. and benzene in gasoline is low (<1%). In agriculture biopesticides and biofertilizers are recommended. Persons with signs of oxygen stress, positive biomarkers for genotoxicity or epigenetic modification need chemoprevention by dietary polyphenols. Some AML patients with epigenetic modifications could be treated with hypomethylalting agents.

1114

ACUTE MYELOID LEUKEMIA WITH INV(3)(Q21Q26.2) OR T(3;3)(Q21;Q26.2): IMMUNOPHENOTYPIC CHARACTERISTICS IN 35 PATIENTS FROM A SPANISH RETROSPECTIVE MULTICENTRIC SURVEY

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Background. The 2008 WHO classification recognizes acute myeloid leukemia (AML) with inv(3) (q21q26.2) or t(3;3) (q21;q26.2) as an independent clinicopathological entity, with an aggressive course and short survival. Its incidence is very low (1% of all AML) and immunophenotypic studies of this type of AML are limited. *Aims*. The purpose of the study was to analyze retrospectively the immunophenotypic features of leukemic blasts in patients with inv(3)(q21q26) or t(3;3)(q21;q26.2), through collecting a significant number of cases within the Spanish Group of Hematological Cytology (a working group into the Spanish Hematology and Hemotherapy Society). *Methods.* We collected a total of 35 cases [23 patients with AML inv(3) and 12 with AML t(3;3), mean age 50 years, range 14-84, males 54%], diagnosed between 1983 and 2010, in 13 national hospitals. During the time they were diagnosed, 2-, 3- or 4-color flow cytometric analysis was performed on peripheral blood or bone marrow aspirate specimens collected in EDTA. Although the panels of MoAb were not exactly similar in different centers, all included the most frequently used at diagnosis in acute leukemias. Cases were considered positive if 20% or more of the cells expressed the specific antigen. We compare our findings with those reported by Medeiros *et al*. in the most important series published to date (*Leuk Res, 2010*). *Results.* The following table compares the findings in our series with those published by Medeiros *et al*.

MoAb	Medeiros et al. (N = 15)	Spanish Group of Hematological Citology (N = 35)
CD34	13/15 (87%)	27/28 (96%)
CD33	15/15 (100%)	28/30 (93%)
CD13	15/15 (100%)	26/28 (93%)
CD117	9/13 (69%)	20/23 (87%)
CD38	3/14 (21%)	9/11 (82%)
HLA-Dr	11/14 (79%)	17/21 (81%)
CD7	7/15 (47%)	14/23 (61%)
CD123	NE	6/10 (60%)
CD11b	NE	9/18 (50%)
CD36	NE	5/11 (45%)
CD15	NE	8/19 (42%)
CD4	NE	7/18 (39%)
MPO	3/13 (23%)	7/19 (37%)
CD14	5/14 (36%)	7/20 (35%)
CD65	NE	3/9 (33%)
CD56	4/13 (31%)	4/20 (20%)
CD41/CD61	5%	3/19 (16%)
CD64	4/13 (31%)	1/7 (14%)
CD11c	7/14 (50%)	NE

Table 1. NE: not evaluated.

In our experience, more than 80% of the patients were positive for CD34, CD33, CD13, CD117, and HLA-Dr. Of note, contrary to Medeiros *et al*. and similarly to the revised 2008 WHO classification, we find a considerably high proportion of cases showing CD38 positive blasts. But also a relatively high rate of patients (>40%) were positive for CD123, CD11b, CD36 or CD15. The aberrant expression of CD4 is found in more than one third of cases. In our study, positive expression for CD3, CD8, CD10, CD20, TdT, or glycophorin (all antigens not analyzed in the other study) was never observed. *Conclusions.* Basically our results are consistent with those found by Medeiros *et al*., and blasts exhibit an immature myeloid phenotype with a frequent aberrant expression of CD7. However, an expanded panel can also find cases

positive for CD123, CD11b, CD36, CD15 and CD4. For the best of our knowledge, this is the largest series of patients with AML with inv(3)/t(3;3) that specifically studies the immunophenotypic characteristics of leukemic blasts.

1115

THE CHLOROQUINE DIPHOSPHATE CHANGES PROTEIN PNAS-2'S SUBCELLULAR LOCATION IN LEUKEMIC CELLS AND INDUCE THEM TO APOPTOSIS

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Background. Previous studies in our laboratory had found PNAS-2 was an anti-apoptosis gene and might participated in leukemogenesis, this was confirm by scholars in Germany. When we studied Pnas-2-GFP fusion protein, we found Pnas-2's subcellular location in leukemic cells might be abnormal with instable membrane of multivesicular body. Some articles had reported chloroquine diphosphate (CQ) could stabilize membranal structures. *Aims*. We hypothesized that CQ might normalize Pnas-2's subcellular location in leukemic cells and by this way induce them to apoptosis. In this study, we explored whether CQ could induce leukemic cells to apoptosis or stabilize membrane of multivesicular body in leukemic cells. *Methods.* Both leukemic cell lines such as HL60, U937 and leukemic primary cells were studied. All samples were obtained with informed consent. *De novo* samples of 12 patients with acute leukemia (AL) were used in this study, including 1 M3, 6 M4, 5 M5 and 1 ALL patients. Seven samples of relapsed acute myeloid leukemia (1 M2, 4 M4 and 2 M5) and 4 CR patients (1 M3, 2 M4, 1 M5) were also assessed. Immunofluorescence method were applied to investigate Pnas-2's subcelluar location by using monoclonal antibody of PNAS-2 which was prepared by our laboratory. Annexin -V-APC/PI kit was used, after that, laser scanning confocal microscope and flow cytometery were applied to study apoptosis. U937 cells was study in each experiment and severed as a calibration to avoid experimental error. *Results*. We found just like we suspected previously, Pnas-2 protein was not only located in vesicles nearby the nucleus but also distributed throughout the cytosol in leukemic cells, instead of in vesicles which was nearby the nucleus in 293T cells and cells from healthy volunteers (*data not shown*).

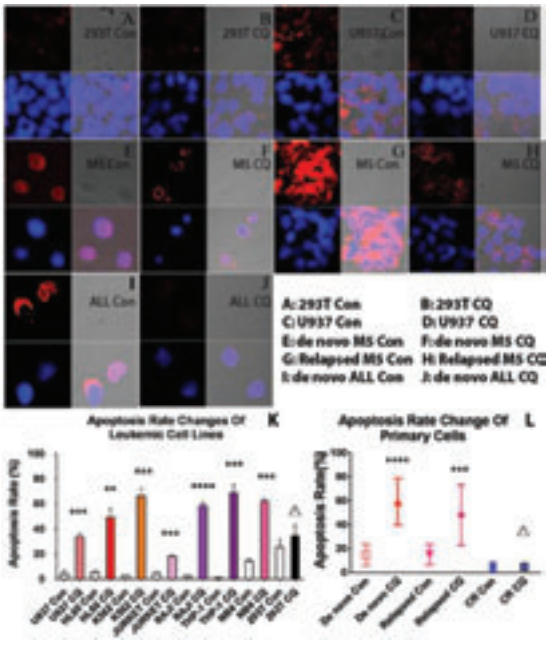

Figure 1. Pnas-2's subcellular location and apoptosis rate.

After the treatment CQ, Pnas-2 protein rehabilitated its subcellualar location: in vesicles nearby the nucleus.(Figure 1, A 293T Con, B 293T CQ, C U937 Con, D U937 CQ, E de novo M5 Con, F de novo M5 CQ, G Relapsed M5 Con, H Relapsed M5 CQ, I *de novo* ALL Con, J *de novo* ALL CQ) A remarkable increasing of apoptosis rate in leukemic cell lines and primary cells when treated by CQ was detected by both laser scanning confocal microscope and flow cytometery. But there was not statistic difference after the treatment CQ in cells from 293T and CR patients. (Figure). *Conclusions*. Pnas-2's subcellular location might be abnormal in leukemic cells. Pnas-2 protein was just in the multivesicular body nearby the nucleus in non-leukemic cells, but it was distributed throughout the cytosol in leukemic cells, this phenomenon might indicate the membranal structures of multivesicular body in the leukemic cells were instable. So we presumed that instability of membrane structures in leukemic cells might participate in leukemogenesis. CQ could induce leukemic cells to apoptosis when Pnas-2's subcellular location was recovered，as we mentioned, some experts had reported that CQ could stabilize membranal structures, so this phenomenon might indicate the mechanism of chloroquine diphosphate treatment for leukemia: stabilizing membrane of multivesicular body, then inducing leukemic cells to apoptosis.

1116

MEMBRANE MOLECULE PROFILE AND MUTATIONAL STATUS IN ACUTE MYELOID LEUKEMIA

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Background. Flowcytometric characterization of membrane molecule expression is important for the characterization of the leukemic cells in patients with acute myeloid leukemia (AML). The membrane molecule profile is useful mainly as a diagnostic tool, e.g. to distinguish between myeloblasts and lymphoblasts and to detect lineage-associated differentiation markers, whereas mutational analyses (especially FLT3 and NPM1 mutations) are important in the prognostic evaluation of AML patients. *Aims*. We wanted to use a limited number of well-characterized differentiation markers to characterize the membrane molecule profile of primary human AML cells derived from an unselected cohort of AML patients, to use bioinformatic tolls to subclassify the patients based on this profile, and finally to investigate whether there was an association between these patient clusters and biological or clinical patient characteristics. *Methods*. The membrane molecule profile was investigated by flow cytometry for 162 consecutive AML patients. Unsupervised hierarchical clustering methods were used to identify distinct immunophenotypic clusters. *Results*. The markers CD11c, CD13, CD14, CD15, CD33, CD34, CD45 and HLA-DR were used in an unsupervised hierarchical cluster analyse (Persons correlation, with complete linkage), and six major different patient clusters (I-VI) were then identified based on the expression levels. The figure describes the major characteristics for each cluster. The left panel describe the immunophenotypic characteristics. Black symbols indicate that the patients in that cluster mainly stained positive for the respective membrane molecule. Likewise white squares represent mainly negative expression of the respective marker.

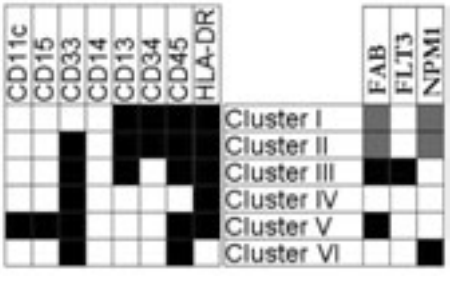

Figure 1. Characteristics of the immunophenotypic clusters.

The French-American-British (FAB) classification was used for a morphological evaluation of leukemic cell differentiation. Grey squares represent either (i) FAB subclasses M0/M1/M2, or (ii) FLT3/NPM1 wild type being present in at least 80% of the patients in the cluster. In contrast, black squares represent monocytic differentiation (FAB-M4/M5) or mutated FLT3/NPM1 alleles in at least 80% of patients in a specific cluster. White squares indicate no predominant morphology or mutational status in the respective cluster. The six clusters did not differ with regard

to age of the patients, hemoglobin levels or platelet count at diagnosis; in contrast to the morphology as well as frequency of genetic abnormalities which differed between the clusters. *Summary/Conclusions*. Our results suggest that there is an association between mutational status and morphological as well as molecular signs of differentiation in primary human AML cells. Our bioinformatical analysis suggests that the membrane molecule profile can be used to identify different subsets among patients with similar genetic abnormalities. Only future studies can clarify whether the patient clusters differ with regard to chemosensitivity and prognosis, and the potential role of specific mutation in for the phenotypic characteristics.

1117

MONITORING OF MRD BY NPM1 MUTATIONS FROM MRNA AND GENOMIC DNA AND ITS COMPARISON WITH EXPRESSION OF WT1 GENE IN AML PATIENTS

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Mutations of nucleophosmin 1 (NPM1) gene are the most frequent mutations in acute myeloid leukemia (AML) patients. In our Institute 20% AML patients have a mutation in NPM1 gene. Mutations in NPM1 gene were suggested as useful molecular markers for monitoring minimal residual disease (MRD) in AML patients. We compared monitoring of MRD by NPM1 mutations from both mRNA and genomic DNA with those of Wt-1 gene expression. The mutations of NPM1 gene and Wt-1 expression was estimated by a quantitative (RT)-PCR. MRD was estimated in 28 AML patients with mutation A of NPM1 gene and with a high Wt-1 expression at diagnosis. The results showed a very good correlation between NPM1 mutations and Wt-1 expression and a clinical course of the disease. Comparison of results of MRD monitoring by NPM1 mutations from mRNA and genomic DNA showed in 10 AML patients a utility of both approaches. The results obtained from genomic DNA seem to fit better clinical data. Molecular relapse estimated by NPM1 mutations and Wt-1 expression preceded hematological relapse by 36 (median) days. The advantage of the NPM1 gene as the marker of MRD is his negativity in control healthy persons and in AML patients in permanent remission. The presented results showed a utility of both NPM1 mutations and Wt-1 expression as molecular markers of MRD in AML patients.

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1118

ACQUIRED COPY NUMBER ALTERATIONS AND COPY NEUTRAL LOH IN ADULT AMLS WITH NORMAL KARYOTYPE IDENTIFIED USING SINGLE-NUCLEOTIDE POLYMORPHISM MICROARRAY

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Background. FLT3, ITD, and NPM1 are recently discovered biomarkers dissecting prognostic subgroups of AML with normal karyotype (AML-NK). Genome-wide single-nucleotide polymorphism (SNP) analys has devoted to reveal the previously unrecognized microdeletions and copy neutral LOH (CN-LOH). *Aims*. In the present study, we aimed to identify significant acquired CN-LOH and copy number alterations in AML-NK. *Methods*. Total 26 adult AML-NK cases were subjected. Exclusion criteria were any recurrent gene rearrangements of PML/RARA, BCR/ABL1,RUNX1/RUNX1T1, MLL, and CBFB/MYH11 or mutations in FLT3, ITD, and NPM1 genes. DNA was extracted from the bone marrow cells collected at the time of diagnosis. Parallel study was available with the bone marrow samples at complete remission state from 3 cases. Genome-wide SNP analysis was performed with HumanCytoSNP-12 bead chip (Illumina Inc.,USA). *Results*. Additional chromosomal copy changes were detected in 5 cases (19.2%); gains at 3p12.1-p14.1 (14.9Mb), 7q33-q36.3 (25.4Mb), 13q12-q34 (92.9Mb), and deletion at 11q13-q25 (61.1Mb), 13q14.11-q21.31 (21.2Mb). The parallel study with CR samples showed 9 germ line CN-LOH regions larger than 2Mb, all of which were smaller than 3.2 Mb. Overall 16 CN- LOH regions larger than germ line ones (>3.2 Mb) were observed in 14 cases, which were discriminated to two subgroups of different sizes. Eight CN-LOH regions observed in 7 cases were smaller than 6.0 Mb. While, the other 8 regions observed in 8 cases (30.8%) were distinctively large, including 3p12.2-q11.22 (17.3Mb), 4q22.1-q35.2 (102.2 Mb), 7q11.23-q36.3 (85.2Mb), 9p24.3-p13.3 (35.6Mb), 11p14.3-p11.12 (28.9 Mb), 11p15.5-p11.2 (45.7 Mb), 19q12-q13.43 (28.6 Mb and 31.3 Mb). CN-LOH in 19q12-q13.43 was recurrently observed in two different cases. *Summary/conclusions*. SNP microarray revealed additional copy number alterations at 19.2%, which were not detected in conventional karyotyping. We found significantly large CN-LOHs in 30.8% of AML-NK patients. Prognostic genes harbored in the large CN-LOHs, especially recurrent LOH region at 19q12-q13.43, need to be identified in further studies.

1119

EXPRESSION OF APOPTOSIS-ASSOCIATED GENES IN ACUTE MYELOID LEUKEMIA (AML) - CORRELATION WITH PROGNOSIS AND DISEASE OUTCOME

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AML is a heterogeneous group of malignant disorders characterized by deregulation in differentiation, proliferation and apoptosis. Abnormalities in apoptotic pathways determine survival advantage and clonal domination of leukemic cells. The aim of this study was to determine expression pattern of the key genes involved in two distinctive apoptotic pathways. In extrinsic, receptor-mediated pathway, we analyzed the expression of Fas-receptor (Fas) and Fas-ligand (FasL) genes. In intrinsic pathway, mediated by Bcl2 gene family, we analyzed the expression of anti-apoptotic Bcl2 and pro-apoptotic Bax gene. We also examined the differential expression of membrane (mFas) and soluble (sFas) Fas transcripts, because up-regulation of sFas expression is implicated in pre-receptor blockage of Fas-induced apoptosis. Using RQ-PCR method we analyzed the m-RNA expression of Fas, FasL, Bcl2 and Bax gene in PBMCs collected from 39 AML patients and 6 healthy individuals. On the basis of cytogenetic findings patients were divided into 3 prognostic groups (Grimwade D, Blood 1998). Relative expression level for Fas and FasL gene was calculated using standard curve method, and for Bcl2 and Bax we used ddCt method. Abl gene was used as endogenous control and as a calibrator we used median expression from the healthy individuals. The mFas/sFas transcript ratio was determined on 2100-Bioanalyzer(Agilent). The expression of Fas (0.0-10.13, median 0.67) and FasL (0.00-17.00, median 0.045) among patients was very heterogeneous. In the case of FasL, the expression was significantly lower compared to the healthy individuals (0.41-1.32, median 1.00)(p=0.004). Using median expression level for Fas and FasL as a cut-off-value, the patients were divided into two groups with high or low expression. We didn't find any significant association between the expression levels of these genes and prognosis/outcome of the disease. Range of the obtained mFas/sFas ratio values among patients was very wide (0.39-17.33, median 2.28) and it wasn't significantly different from the values found in healthy individuals (1.34-2.19, median 1.82). The domination of sFas transcript (mFas/sFas<1,00) was found in 18.6% of AML cases, and it wasn't associated with an adverse outcome of the disease. The expression of Bcl2(0.14-1.29, median 0.48) and Bax(0.04-3.72, median 0.41) among patients was very heterogeneous, with no significant difference compared to the healthy controls. Using median expression level of Bcl2 and Bax as a cut-off-value, the patients were divided into two groups with high or low expression. High Bcl2 expression was associated with CD34+status (p=0.029), bad prognosis, and adverse outcome. Contrary to expectations, we found significant correlation between reduced expression of Bax and favorable prognosis (p=0.039). Based on the heterogeneous Fas and FasL gene expression pattern in AML patients, we concluded that the disruption of Fas-induced apoptosis is not essential for leukemogenic process. The domination of sFas transcript that was found in 18.6% of cases, further led to the conclusion that this mechanism of resistance to Fas-induced apoptosis is not dominant in AML. Our findings that patients with high Bcl2 expression level are in high risk of fatal outcome indicate that application of new therapeutics (antisense-Bcl2 oligonucleotides) could contribute to better survival of this group of patients.

1120

THE EFFECT OF CURCUMIN ON TELOMERE LENGTH AND CASPASE ACTIVITY IN ACUTE MYELOID LEUKEMIC U937 CELL LINE

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Introduction. Apoptosis is a controlled cell death mechanism of eradicating irreversibly damaged or unwanted cells from the body. Activation of this process is achieved by the shortening of telomeres at chromosomal endpoints to a critical length. Apoptosis is then induced by either caspase-dependent or -independent pathways. Highly activated telomerase, a reverse transcriptase may prevent shortening of telomeres to the critical length needed to activate apoptosis. This may result in uncontrolled proliferation of cancerous cells and lead to disorders like acute myeloid leukaemia. Curcumin, commonly known as turmeric, has been reported to have anti-tumour and apoptotic potential. It may influence telomerase activity and cause apoptosis via a caspase dependent pathway. *Aim*. To determine the effect of Curcumin on telomere lengths and caspase 3 and -4 activity in U937 acute myeloid leukaemia cells. *Methods*. The U937 cell line was cultured and treated with different Curcumin concentrations for 24- and 48 hours respectively. The relative telomere lengths were determined using Flow FISH. This assay was performed to determine how Curcumin influences telomerase. ELISA's on caspase 3 - and caspase 4 activity, involved in the endoplasmic reticulum apoptotic pathway were performed. This was done to determine whether Curcumin influence the endoplasmic reticulum apoptotic pathway. *Results*. An increase in telomere lengths were observed with increasing Curcumin concentrations at 24 and 48 hour treatment periods. At a concentration of 10μM for the 24 hour treatment period, the telomeres were shortened. Caspase 3 - and caspase 4 activity mostly increased with increasing Curcumin concentrations. *Conclusions*. Curcumin might influence telomerase and cause telomere shortening, however an alternative way of telomere elongation have to be considered in that case. Curcumin does influence the endoplasmic reticulum pathway of apoptosis as illustrated by increasing caspase 3 - and caspase 4 activity and decreasing cell viability with increasing Curcumin concentrations.

1121

EXPRESSION OF THE STEM CELL MARKERS CD133 AND CD90 ON BLASTS IN ACUTE MYELOID LEUKEMIA AND IN MYELODYSPLASTIC SYNDROMES IS ASSOCIATED WITH POOR PROGNOSIS

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Background. CD133 and CD90 are surface markers expressed on various types of normal and cancer stem cells. Thus, we hypothesised that the expression of these two molecules could be of prognostic value in acute myeloid leukemia (AML) and in myelodysplastic syndrome (MDS), as describe in some other cancers. *Aims*. In this study, we analysed the expression of CD133 and CD90 on blasts in AML and MDS cases. The aim was to evaluate the stemness of the blasts and to correlate the expression of CD133 and CD90 to clinical outcome and to classical prognostic factors. *Methods*. The expression of CD133, CD90, CD34, CD38 and CD33 was evaluated by 8-colors flow cytometry on bone marrow samples collected at diagnosis for more than one hundred AML and MDS. On the blast population, we assessed the level of expression and the different co-expression profiles of these markers. Clinical and biological data were also collected. Results. Blasts expressed CD133 in approximately 40% of cases, generally at a high level. CD90 was detected in 10 to 40% of cases, depending on the cut-off chosen (3 to 20% of cells stained) with a level of expression fainter than CD133. Significant correlations exist between the stem cell markers assessed. As expected, CD133+ blasts are more immature than CD133-, and express CD34 higher. The expression of CD90 is correlated to that of CD34 too. Interestingly, in several cases, we could isolate two distinct subsets in blasts: CD34+ and CD34- displayed different patterns regarding the expression of CD133 and CD90. In most cases CD133 was present on CD34+ blasts while CD90+ was preferentially expressed on CD34-. Co-expression of CD133 and CD90 in the same subset is very rare. We assessed the correlation between the expression of CD133 or CD90 and usual prognostic factors, and clinical outcome. The expression of CD133, as that of CD90, was significantly different between patients with a favourable, intermediate or unfavourable karyotype. Expression of CD133 correlated also with the molecular prognostic profile. Moreover, CD133 and CD90 were associated with a higher risk of relapse. *Summary/conclusions*. CD133 and CD90 are expressed on normal haematopoietic stem cell (HSC) and other stem cells. They are markers of poor prognosis in several cancers but few studies analysed their expression in hematologic diseases. In this study we demonstrate that their expression correlates with prognosis factors as strong as cytogenetics. Interestingly, we observed different co-expression patterns for CD133, CD90 and CD34 within blasts. That heterogeneous population contains several subsets with different degree of stemness, as described in its normal counterpart. This could explain the relationship between CD133 and CD90 expression and clinical outcome. The prognostic value of the expression of CD133 and CD90 on blasts in AML and MDS must be confirmed and refined. Nevertheless, these encouraging results provide new insights into the leukemic stem cell characteristics. Moreover, as CD133 and CD90 correlate with robust prognostic factors, they may represent novel prognostic markers in AML and MDS, particularly for patients with intermediate risk karyotype.

1122

INVESTIGATING THE IMPORTANCE OF MSI2 IN MLL-FUSION INDUCED ACUTE MYELOID LEUKEMIA

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Chromosome translocations that disrupt the mixed lineage leukaemia (MLL) gene are associated with a unique subset of Acute Myelogenous and Lymphoblastic Leukaemias. MLL translocations are most prevalent in infant leukaemia, where they comprise 80% of cases of acute lymphoblastic leukaemia and 60% of cases in acute myeloid leukaemia. Expression of MLL-fusion proteins is known to induce malignant transformation of normal haemopoetic progenitor cells. To identify transcriptional target genes required for the immortalisation, previous work in the lab involved generating constitutively and conditionally immortalised primary mouse haemopoietic progenitor cells. Global gene expression analysis, upon loss of MLL-fusion protein, identified a number of genes that are differentially expressed. One of these genes was Msi2, an RNA binding protein, which is found to be expressed in haemopoetic stem cells. MSI2 prevents cell differentiation by binding to the mRNA of the cell fate determinant protein, Numb, and repressing its translation. Recent reports identified MSI2 to be highly expressed in both Acute Myeloid Leukaemia and Chronic Myeloid Leukaemia. Using the conditionally MLL-ENL immortalised cell lines, the expression of Msi2 and Numb was analysed by qPCR. Loss of MLL-ENL fusion protein resulted in significant reduction in Msi2 expression and an increased level of Numb mRNA. shRNA mediated knockdown of Msi2, in the constitutively immortalised MLL-ENL cell lines, led to decreased cell proliferation in culture and reduced colony formation in methylcellulose. Interestingly Numb over-expression, in these immortalised cells, had no effect on proliferation but reduced their colony forming capability. In vivo studies, examining the importance of Msi2 and Numb in MLL-fusion induced leukaemia, are currently under investigation.

1123

REGULATION OF THE MTG16 LEUKEMIA ASSOCIATED NUCLEAR CO-REPRESSOR GENE

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The MTG16 gene, a member of the highly conserved ETO homologue family of corepressors also containing ETO and MTGR1, is implicated in hematopoietic development, in controlling erythropoiesis/megakaryopoiesis and is the 3´partner of t(16;21) generating the leukemic AML1-MTG16 fusion gene. We examined MTG16 gene promoter regulation to shed light on hematopoietic functions. A TATA- and CCAAT-less promoter containing a GC box close to start site was identified. Mutation of an evolutionary conserved GATA -301 consensus binding site repressed promoter function. Furthermore, results from in vitro antibody-enhanced electrophoretic mobility shift assay and in vivo chromatin immunoprecipitation indicated specific binding of GATA-1 to the GATA -301 site. The leukemia associated AML1-ETO fusion gene strongly suppressed all the ETO homologue promoters. In conclusion an evolutionary conserved GATA binding site is critical in transcriptional regulation of the MTG16 promoter. ETO has been shown previously also to be regulated by GATA-1. The third ETO homologue member, MTGR1 was found to be a *housekeeping* gene with a TATA-less promoter possibly regulated by the ubiquitous transcription factor SP1. Our results show ETO homologue promoters to be regulated differently consistent with hematopoietic cell type-specific expression and function.

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THE PRESENCE OF NPM1 MUTATIONS AS AN UNFAVORABLE FACTOR FOR OVERALL SURVIVAL AND FOR ACHIEVING COMPLETE REMISSION IN ACUTE MYELOID LEUKEMIA (AML)

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Background. Mutations in nucleophosmin (NPM1) gene are the most frequent abnormalities found in AML. Based on current findings, the presence of NPM1 mutations is associated with increased probability of complete remission (CR) and better overall survival (OS). *Aims*. To evaluate the incidence and the prognostic relevance of NPM1 mutations, their association with FLT3 mutations and other clinical characteristics. *Methods*. Bone marrow or peripheral blood samples from 92 adult de novo AML patients (median age 50 years, range 16-75; M/F: 53/39) were studied. NPM1 mutations were detected by PCR method and the products were directly sequenced (*Falini B, N Engl J Med 2005*). FLT3 mutations were detected as previously described (*Kiyoi H, Leukemia 1997; Yamamoto Y, Blood 2001*). *Results*. NPM1 mutations were detected in 17/92 patients (18.5%). Three types of mutation were detected; type A in 15/17 patients (88.2%) while the remaining two patients were carriers of type D and type K mutations, respectively. NPM1 mutations were closely associated with normal karyotype in 85.7% of cases, and with CD34- status (p=0.003). In 9/17 NPM1+ patients (53%), NPM1 mutations were associated with the presence of FLT3 gene mutations; in 6 (66.7%) patients with FLT3/ITD mutations, and in 3 (33.3%) patients with FLT3/D835 mutations. Complete remission was achieved in 7/17 NPM1+ patients. CR rate in NPM1+ patients was significantly lower than in NPM1-/ FLT3- patients (p=0.02). When double positive NPM1+/ FLT3+ patients (9 patients) and single positive FLT3+ patients were excluded from the calculation, CR rate was even lower (p=0.008) and CR was achieved in only 2/8 NPM1+ patients. No significant difference between NPM1+ and NPM1- patients was found concerning median duration of disease-free-survival (DFS) (10.5 vs. 12 months, p=0.38 Log-rank). Surprisingly, median OS among NPM1+ patients was significantly lower compared to NPM1- patients (4 vs. 10 months, p=0.015 Log-rank). *Conclusions*. The frequency of NPM1 mutations (18.5%) found in our study was slightly lower than in other previously published reports. The explanation for this may lie in the fact that 30% of our patients were younger than 40 years and only 13.5% were older than 60 years, while NPM1 mutations have been shown to be more frequent in older patients. Young age structure of our cohort may also be the reason for unexpected finding that NPM1 mutations had an unfavorable impact on the CR rate and OS. It was previously shown that NPM1 mutations had favorable prognostic impact in older patients, especially those age ≥ 70 years, while in some other studies carried out on cohorts with different age structure, NPM1 mutations were associated with a higher relapse rate and poorer DFS. Association of NPM1 mutations with FLT3/ITD mutations, which have dominant adverse prognostic effect (also found in our study), may explain this unfavorable impact of NPM1 mutations. Recent data show that even in the presence of prognostically favorable NPM1 mutations, some AML patients may have an adverse outcome, suggesting that some other secondary genetic lesions may cooperate with NMP1 mutations influencing prognosis.

1125

This abstract has been withdrawn<<<.

1126

CCAAT/ENHANCER BINDING PROTEIN ? EXPRESSION IN T(15;17) ACUTE PROMYELOCYTIC LEUKEMIA

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Background. Given the role that CCAAT/enhancer binding protein α (C/EBP α) plays in myelopoiesis, we anticipated that their expression might be disrupted in myeloid neoplasms including acute promyelocytic leukemia (APL). *Purpose*. To estimate the expression of C/EBPα in patients with APL for evaluation of its role in the pathogenesis of the disease, and to assess response of these patients with variable expression levels of CEBPα to therapeutic regimen combining all-transretinoic acid (ATRA) with chemotherapy. *Patients and Methods*. Forty APL patients with t(15; 17), detected by FISH analysis, and 10 age and sex matched healthy controls were included in the study. Blood samples of patients and controls were analyzed for CEBPα expression by quantitative RT-real time PCR using TaqMan technology. *Results.* Thirty six (90%) patients out of the 40 showed low expression levels of CEBPα below the cutoff value with median of 0.51 (range:0.0007-0.96). Following therapy, full maturation of myeloid series was seen in 31 patients (77.5%). These patients had higher median level of CEBPα of 0.6 (range: 0.22-26.7) compared to other 9 patients (22.5%) who showed partial response to therapy (median level: 0.03; range: 0.0007-0.15; p<0.0001). *Conclusions*. Our findings are highly suggestive that C/EBPα may have a role in the pathogenesis and prognosis of APL.

1127

CORRELATION OF WT1 EXPRESSION WITH THE BURDEN OF TOTAL AND RESIDUAL LEUKEMIC BLASTS IN BONE MARROW SAMPLES OF ACUTE MYELOID LEUKEMIA PATIENTS

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Background. WT1 gene encodes a transcription factor that is physiologically expressed in hematopoietic stem cells. It is related to cell development and survival. It is overexpressed in different haematological malignancies like acute myeloid leukemia. Hence, it can be used as minimal residual disease marker. *Aims*. Given that WT1 is expressed in developing not aberrant hematopoietic stem cells, we want to check if its level of expression is more accurately related to the total blast burden than to leukaemic blast burden in bone marrow samples of *de novo* acute myeloid leukaemia patients (non M3). *Patients and methods*. We have collected 18 minimal residual disease values measured at different time points in 11 different patients. Median age was 52 years (17-75). All of them had a basal diagnostic determination of WT1 expression and others following chemotherapic treatments. WT1 expression was determined by RT-qPCR. A retrotranscription reaction was performed and WT1 transcripts were measured using a relative quantification with GUS as a housekeeping gene. Four colours flow cytometry (FC) was used to quantify residual leukaemic blasts. Total blast burden was determined by bone marrow cytology. Flow cytometry (FC) and bone marrow cytology values were transformed into percentages referred to basal diagnostic ones to mimic the way WT1 measurements are expressed. Three samples were not evaluable by bone marrow cytology. Statistical analysis was carried out using Spearman test correlating 18 sample values of FC and RT-qPCR and 15 values of RT-qPCR and bone marrow cytology. *Results*. We have found a mild correlation (0.56, p< 0.01) between leukemic blasts count and WT1 expression and a stronger correlation (0.86, p< 0.0001) between WT1 expression and total blast count. Conclusions. The stronger correlation found between total (leukaemic and regeneration) blast count and WT1 expression levels confirms that it is related to normal hematopoietic stem cell development. It also suggests that this way of determining minimal residual disease in bone marrow samples of acute leukaemia patients after receiving chemotherapy or G-CSF is not reliable.

1128

ACUTE MYELOID LEUKEMIA IN UNSELECTED COHORT OF ELDERLY PATIENTS: RESULTS OF INDUCTION VERSUS NONCURATIVE TREATMENT

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Introduction. The aim of our study was to analyze the results of treatment in elderly group of patients with AML diagnosed at the Institute of Hematology in Belgrade over a period of 6 years. We wanted to identify prognostic factors important for making decision how to treat elderly patients, with induction chemotherapy or with supportive or palliative therapy only. *Material and results*. A retrospective analysis of 210 consecutive patients aged ≥ 65 years (median age 69, range 65-88) with acute myeloid leukemia (AML) diagnosed at a single center over the period of 6 years from January 2001 to December 2006 was performed. All patients who were included in this study provided informed consent

according to Helsinki Declaration. There was 179 (85.2%) patients with de novo AML and 31 (14.7%) with secondary. The patients' morphological FAB type equivalents were M0 23 (11%), M1 36 (17.15%), M2 57 (27.1%), M3 8 (3.8%), M4 45 (21.4%), M5 31 (14.8%), M6 1 (0.5%), M7 1 (0.5%) and 8 (3.8%) unclassified. The patients with M3 FAB type were excluded from the further analyses. Cytogenetic analysis was performed in 172/202 (85%) patients,. Normal karyotype was found in 81/172 (47%), favorable in 13/172 (7.5%), complex karyotype in 32/172 (18.6%) and analysis was unsuccessful in 28 (16.2%) patients. The whole cohort of patients was divided in NIC group (no induction chemotherapy but palliative and supportive therapy) which consisted of 115 (56.9%) patients and 87 (43.1%) patients in IC group (patinets who received induction chemotherapy). Complete remission (CR) was achieved in 45/87 (51.7%) and in 5 patients in NIC group which were treated with low doses cytosine arabinoside. After median follow up of four years deaths occurred in 194 patients (96%). In univariate analysis, significant factors for longer OS were age, ECOG performance status (p=0.000, CI 95.0%, 1.358-2.049), serum LDH (p=0.000, CI 95.0%, 1.465-2.946), number of white blood cells, (p=0.011, CI 95.0%, 1.102-2.100), splenomegaly (p=0.015, CI 95.0%, 1.082-2.102), hepatomegaly (p=0.008, CI 95.0%, 1.125-2.171). In multivariate analysis significant factors for OS were induction chemotherapy, sLDH, ECOG performance status and achievement of CR. *Conclusions*. Elderly AML patients belong to different prognostic subgroups which can benefit from different therapeutic approaches. Treatment decision in elderly AML patients is complex and the results of intensive versus palliative treatment should be investigated in randomized prospective studies and this group of patients representing the vast majority of AML patients should not be neglected.

1129

PRIMARY RESISTENT ACUTE MYELOID LEUKEMIA

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Aim of the study. Standard combination (anthracyclines and ARA-C) as remission induction chemotherapy for de novo acute myeloid leukemia (AML) results in complete remission (CR) rates of 55-70%, the rest showing a partial response, absolute resistance or leukemic regrowth after two courses of remission-induction therapy. These rates had not improved for the last 20 years with. We analyzed prognostic factors in 53 patients with AML who failed to achieve CR after the first-line standard-dose remission-induction therapy. *Results*. Over a study period of five years, fifty three patients with AML (48 with de novo and 5 with a secondary) received two cycles of the MRC AML10 protocol as a first-line standard-dose remission-induction therapy [ARA-C, days 1-7 and daunorubicin, days 1-3]. The HDAC (5 pts), MiDAC (7 pts), and FLAG-IDA protocols (3 pts) were given as salvage. All patients who were included in this study provided informed consent according to Helsinki Declaration. None of the patients achieved CR. There were 27(49%) males and 26 (51%) females (median age, 55.7 years (range 28-76). None presented with the central nervous system involvement. Six (11.1%) patients had M0, 9 (16.9%) M1, 20 (37.7%) M2, 4 (7.5%) M4, 11 (20.8%) M5, and 3 (5.6%) the mixed phenotype AML (FAB Classification). Thirty one (57.4%) patients had the intermediate risk karyotype changes and 12 (22.6%) the unfavorable ones. The medians of WBC count were 53 (range 0.9 -350) $x10^9$/L, platelets 55 (range 1-204 $x10^9$/l) and the bone marrow blasts, 67%. Two (3.8%) patients scored 3, 11 (20.8%) scored 2, 12 (2 2.6%) scored 1 and 16 (30.2%) scored 0 by Sorror comorbidity score. Median overall survival (OS) was 5 months (range 1-14). There was a significant correlation between OS and ECOG PS . Patients with ECOG 0-2, suvived longer than patients with ECOG 3 and 4(p<0.001). Presence of hepatomegaly influenced OS (p<0.01) while splenomegaly was of no consequence (p>0.05). Patients with the WBC ≤ $25x10^9$/l had a longer OS (p<0.001). The platelet count had no impact on OS ((p>0.05). The serum level of LDH had no influence on OS. The OS significantly differed between patients with intermediate-risk and unfavorable cytogenetics (p<0.001) and between patients with and without trilineage dysplasia (p<0.01). The comorbidity score influenced OS, the patients with score 1 surviving significantly longer than those with score 2 (p<0.01). *Conclusions*. The subgroups of patients with AML show distinctive clinical characteristics suggesting a different biology of disease. A higher prevalence of comorbidity and a sharp diversity in biological features account for the poor response to therapy in this cohort of AML patients.

1130

AGE AND KARYOTYPE ARE THE MAIN PROGNOSTIC FACTORS IN PATIENTS WITH DE NOVO ACUTE MYELOID LEUKEMIA

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Background. The main method to determine the intensity of chemotherapy of patients with acute myeloid leukemia is the stratification according to prognostic factors. There are many markers of prognosis including clinical, morphological, cytogenetic and genetic lesions. It has been shown previously that the survival of older AML patients is worse than younger patients. Another principal marker is karyotype which aloud to stratify AML patients into three prognostic categories. *Aim.* To determine whether age and karyotype are associated with overall survival of patients with de novo AML. *Methods*. The retrospective analysis of history of 214 patients with de novo AML was performed. The patients were treated with different kinds of induction and postremission therapy including AlloSCT and AutoSCT during the period from 1990 till 2009. The median age of patients was 57 y. (16-84). The patients were separated into 3 age groups: 16-40, 41-60 and ≥61. As there was no difference in OS between first two groups they were united. Four cytogenetic groups were designed in every age categories. The cases without cytogenetics abnormalities were separated into independent group. The group with balanced karyotype includes t(8;21), t(15;17) and inv(16). Cases with ≥3 chromosomal aberrations were united into complex karyotype. All others cases were joined in group with unbalanced aberrations. *Results*. The difference in the median OS of patients aged ≤60 years and ≥61 years was statistically significant: 18.6 mo. vs 7 mo.; p<.001. The 5-y. OS in the aged groups was 31.5% vs 0%. The difference in the median of survival between patients' groups with balanced, normal, unbalanced and complex karyotype was statistically significant, too: 15 mo., 13 mo., 10 mo. and 6.0 mo, respectively; p<.001. The 5-y. OS in the groups with different karyotype was 40.5%, 26.7%, 12.4% and 3.6%. In multivariate analysis the age (≤ 60 y. vs ≥ 61 y.) and karyotype (4 groups) were independent prognostic factors: p<.001 and p=.004; respectively. When patients with different karyotype were grouped according to the age (≤60 y. and ≥61 y.) the difference in median survival was significant only in the younger patients. The median OS in younger patients with balanced, normal, unbalanced and complex karyotype was 16, 15, 12 and 8 mo, respectively; p=0.006. Estimated 5-y. OS of patients ≤ 60 y. with different karyotype was 44.4%, 34.6%, 15.2% and 1.4%, respectively *Summary*. The data aloud to use age (≤60 y. vs ≥61 y) and karyotype (in patients ≤60 y.) as prognostic factors for stratification AML patients at the time of diagnosis of the disease.

1131

RESPONSE TO 5-AZACITIDINE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND SECUNDARY AML: RESULTS

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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. *Material and Methods*. we evaluate the efficacy and safety in all MDS groups and secondary AML patients, non candidates to aggressive therapy. Results. In our institution, a total of 27 patients were treated with AZA since 2006. We evaluated 13 patients diagnosed according to WHO criteria as low/intermediate-1 International Prognostic Scoring System (IPSS) risk MDS, 10 patients as high/int-2 IPSS risk MDS and 4 secondary AML diagnosed patients. At baseline, median age was 73.2 years (range 46-86), male/female ratio 15/12. Median time from diagnosis was 29.4 months (range 1-192). 85% patients were transfusion-dependent, 89% had received a prior treatment (rhu-EPO+G-CSF 55%, only rhu-EPO 34%). High risk MDS and AML patients received AZA dose of 75mg/sqm/d subcutaneously during days 1-7, in a 28-day cycle; and low-risk received same schedule by 5 days. The median number of monthly cycles was 10.0 (range 1-39), and 70.3% completed at least 6 cycles. Grade 3-4 adverse events documented in these patients were neutropénica (11%), anaemia and thrombopenia (7.4%), injection site reaction (18.5%), 25.9% constipation, 11% diarrhea and 3.7% nauseas. Response duration ranged from 1 to 8 months. There were no significant differences in response rate according to age, previous treatment, transfusion requirements, basal EPO and Hb pre-AZA. 5 patients were trans-

formed to AML after median 10.4 cycles of AZA (2-13). *Conclusions*. 1.- 90% 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, although more studies are necessary. 4.- In secondary AML patients, AZA is a excellent alternative therapy in patients with co-morbidity and poor performance status.

1132

ASSOCIATION OF CD34 CELL SURFACE ANTIGEN EXPRESSION WITH CYTOMORPHOLOGICAL CHARACTERISTICS OF ACUTE PROMYELOCYTIC LEUKEMIA BLASTS AND CLINICAL CHARACTERISTICS OF PATIENTS: ONE CENTER EXPERIENCE

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Background. Acute promyelocytic leukemia (APL) is characterized by leukemic cells blocked at the promyelocytic stage of granulocytic differentiation (APL-blasts). Two main cytological subtypes are recognized: classical hypergranular promyelocytic leukemia (M3) and the microgranular promyelocytic leukemia variant (M3v). A 3-parameter classification system (nucleus, granularity and Auer rods) leads to the distinction of 12 categories of APL-blasts with 3 additional categories (with basophilic granules, Chediak granules and Pelger-like maturing cells). Low or negative CD34 expression in addition to absent HLA-DR used to be the paradigm of the APL immunophenotype. However, higher CD34 expression can occur in APL and appears to be associated with leukocytosis, hypogranular morphology and poorer clinical outcome. AIMS: to investigate association between cytomorphology and immunophenotypic expression of CD34 cell surface antigen of APL-blasts and their relationship with clinical and laboratory characteristics of patients with acute promyelocytic leukemia. *Patients and Methods.* Sixteen consecutive patients diagnosed with APL at Department of Hematology, University Hospital Merkur, between August 1998 and December 2010, were included in this study. Patients' clinical and laboratory features, cytomorphological characterstics of APL-blasts and their immunophenotype determined by flow cytometry were analyzed. Patients were grouped into 2 groups, CD34(-) and CD34(+), and were then compared according to clinical and laboratory characteristics. Expression in more than 20% blast cells was required to define antigen positivity. RESULTS: Mean age of patients at diagnosis was 43.9 years (range: 18-78, SD14.9), 69% of patients were male. All evaluable patients had high CD13 and CD33 expression, with low HLA/DR expression, except one who had higher HLA/DR expression (HLA/DR(-) blasts: 27.6%). There was no statistically relevant difference between patients grouped according to CD34 expression according to gender, age or WBC counts. Mean value of hypogranular/agranular APL-blasts in CD34(+) group was 34% (range: 9-60, SD 24.4), markedly higher than in the CD34(-) group, 11.5% (range 0-38, SD 13.7), with borderline statistical significance (Mann Whitney, p=0.055) (fig. 1). CD34(-) patients had significantly better overall survival than CD34(+) ones (p=0.02). *Conclusions*. Our results are consistent with the results of other published studies and point to the fact that higher CD34 expression and lower cytoplasmic granularity of APL-blasts are factors that seem to define a specific subgroup of patients with APL. Together with other diagnostic tools they could be of value in planning treatment of patients with acute promyelocytic leukemia.

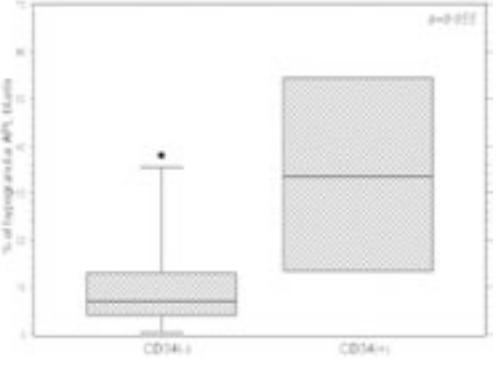

Figure 1. Hypogranular APL-blasts and CD34 expression.

1133

COMPLETE REMISSION AND EARLY DEATH AFTER INTENSIVE CHEMOTHERAPY IN PATIENTS AGED 60 YEARS AND OVER WITH ACUTE MYELOID LEUKEMIA: RESULTS OF A POPULATION-BASED APPLICATION OF A WEB-BASED SCORING SYSTEM

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Background. Acute myeloid leukaemia (AML) in patients aged ≥ 60 years at diagnosis is difficult to treat, with median survival typically < 12 months. Recently, the German AML Cooperative Group and the Study Alliance Leukaemia Investigators published a predictive scoring system using data from clinical trials of intensive induction chemotherapy (IIC) (*Krug et al., Lancet 2010; 376: 2000-8*). This scoring system can potentially predict the probability of complete remission (CR) and early death (ED) amongst patients considered fit for IIC. Used alongside clinical assessments, this could be a valuable tool when counselling patients regarding potential risks and benefits of treatment. *Aims*. To assess the utility of the published web-based scoring system when applied retrospectively to a population-based cohort of AML (excluding acute promyelocytic leukaemia [APL]) patients aged ≥ 60 years, diagnosed January 2007-December 2010. *Methods*. Records of our team's multidisciplinary meetings (MDM) were reviewed to identify the study cohort. The regional population studied was that of Northumberland, Newcastle upon Tyne, Cumbria and County Durham (population approximately 1.5 million). Diagnostic material was centrally reviewed. Well recognised prognostic information was collected including age, haemoglobin concentration, platelet count, serum lactate dehydrogenase concentration, *de novo*/secondary disease, plasma fibrinogen concentration and cytogenetic risk group (low, intermediate or high, as per Krug et al). Treatment intention, response, dates of relapse, death and cause of death were recorded. CR was defined as marrow blasts <5% with peripheral blood count recovery. ED was defined as death within 60 days of chemotherapy commencement. Risk scores were calculated at *www.AML-Score.org*. *Results*. 46 patients (median age 65, range 60-75years) were diagnosed with AML and deemed fit for IIC, during the study period. Cytogenetic information was available for 40 patients: 2 low risk, 29 intermediate risk and 9 poor risk. FLT3 and NPM1 mutation status was available for 41 patients. CR rate was 28/43 (65%) on intention to treat analysis; 4 patients were non-assessable due to early death, data missing (n=2). There were 9 early deaths (21%) with data missing for 1 patient. Median disease free and overall survivals were 9 and 10 months respectively. Median probabilities of ED predicted by the model for patients who experienced ED or not were 22.6%(range 15.3-37.5%) and 20.6%(range 10.4-50.1%) respectively. Median probabilities of CR predicted for patients who attained CR or not were 55.9%(range 20.7-80.3%) and 55.6%(range 25.2-73.7%) respectively. Of patients with scores predicting >50% probability of CR, 18/22 entered CR (82%), for those with predicted CR <50%, 10/15 (66%) entered CR. *Conclusion*s. In this population-based cohort the AML scoring system was not a useful predictor of individual's ED or attainment of CR. Patients in this cohort were younger than those of Krug et al where median age was 67 years (range 60-85); we may have a more conservative approach to the use of IIC than the German Group. This may explain the slightly higher CR rate in our study (65% vs 53%). It would be useful to reassess this model prospectively in a larger cohort.

1134

OUTCOME OF FIT AML OLDER PATIENTS IN A SINGLE CENTER: MAJOR ROLE OF CYTOGENETICS

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Background. Acute Myeloid Leukemia (AML) is most common in older patients and they are often thought to be unfit for chemotherapy. Patients with poor prognostic karyotype are even not recommended for treatment outside clinical protocols. In this context prognostic factors are important tools for clinical judgement, decision making and pt information. Objectives. We retrospectively reviewed whether this assessment is validated in our AML population older than 60 yo. Population and methods. 89 AML pts, 60 yo and older, PS < 2, were referred to our centre between 1997 and December 2009. Pts requiring treatment were

treated according to the EORTC (LAM 13 and LAM 17) protocols for pts above 60 yo. AMLs were classified according to the old FAB classification. Cytogenetic data were obtained by routine karyotype and additional FISH analysis since 1995. Complete remission was defined by morphology and flow cytometry on bone marrow smear. Karyotypes were stratified according to good and intermediate 1 versus intermediate 2 and poor prognosis (ELN recommendation). Statistical analyses using age and Karyotype were performed for the following outcomes: complete remission (CR), overall survival, median survival and disease-free survival. *Results*. 82 files were evaluable for cytogenetic data and outcome. Median age was 70 (60-86) yo. Because we are a referral tumour centre, all the pts had a PS <3 and no geriatric syndrome (falls, dementia, incontinence). The median follow up time was 40 (1-180) months. Overall survival for the whole population was 17% at 180 months with a median survival of 7 months. Median survivals of pts below 70 yo was significantly better ($p < 0.0001$) than pts above 70 yo (38 vs 7 months). Whatever the age, median survival was significantly better for pts in CR after the induction (48 vs 6 months) and presenting with a favourable Karyotype (35 vs 9 months). Taking into account the cytogenetic data and age, the median survival of AML pts below 70 yo with a favourable Karyotype was 64 months with 40% of CCR, very similar to the younger population Conclusions. In our series of selected fit elderly AML pts, we confirm that cytogenetic data have a major impact of OS and CCR in pts between 60 and 70 yo. For patients above 70 yo, median survival remains unsatisfactory and these pts should be offered new alternative treatment approaches after an extended geriatric assessment.

1135

CLOFARABINE IN THE TREATMENT OF POOR PROGNOSIS ACUTE MYELOID LEUKEMIA PATIENTS. A SINGLE INSTITUTION REVIEW

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Introduction. The incidence of Acute Myeloid Leukemia (AML) increases with age, and Cytarabine plus Antracycline based treatments usually unfit for older patients, therefore worsening their prognosis and increasing the relapse rate. Clofarabine is a purine nucleoside antimetabolite indicated in refractory pediatric Lymphoblastic Leukemia, but with demonstrated efficacy in AML patients in combination with Cytarabine, with tolerable side effects, becoming an option for those patients with relapse/refractory AML, as well as for those non candidates for aggressive approaches. *Aims*. To evaluate the effectiveness and toxicity profile of Clofarabine in AML elderly patients. *Methods*. This is a single-center retrospective review of patients with AML treated with Clofarabine based regimens in patients aged 60 and above. Main points were complete response as IWRv2003 criteria and toxicity as CTCAE v3.0 del NCI criteria. *Results*. In the period between January 2007 and December 2010, 6 AML patients above the age of 60 began treatment with Clofarabine (30 or 20 mg/m2/day x 5 days) plus AraC (500 mg/m2/12h x 8 doses). The mean age was 68.5 (63-77), and the ratio male/female 4/2. AML *de novo*: 2 patients. Salvage therapy: 4 patients. Five of the 6 patients (85%) presented poor prognosis cytogenetic alterations. *Effectiveness*. Two patients (33%) obtained complete remission, with a progression free survival of 7 and 4 months (mean 5.5), and an overall survival of 9 and 10 months (mean 9.5), respectively. No effective data of 30 mg/m2/day dose patients are collected, because they were exitus within the first cycle. *Toxicity*. Hematologic toxicity was observed in all patients, grade 4 Neutropenia, grade 4 trombocytopenia and anemia transfusion-dependent.

Age	Status	Cytogenetic	Previous lines	Clofarabine Dose (mg/m2)	Response	PFS (months)	Overall Survival
63	Refractory	Poor	1	30	Not evaluated	--	1
66	Relapse	Poor	1	20	CR	7	9
68	Relapse	Poor	1	30	Not evaluated	--	1
65	Relapse	Normal	4	20	Refractory	--	7
72	De novo	Poor	0	20	Refractory	--	10
77	De novo	Poor	0	20	CR	4	7
							Mean 5.8

The 2 patients treated with 30 mg/m2/day x 5 days were exitus because of septic shock during the induction cycle. There was no correlation between number of previous lines received and toxicity. No relevant extrahematologyc toxicity was reported in patients treated with 20 mg/m2/day x 5 days. *Conclusions*. Our experience confirms that Clofarabine at 20mg/m2/day (instead of the initially proposed 40 mg/m2 , or the 30 mg/m2 we have tried before) x 5 days plus AraC 500 mg/m2/12 x 8 doses, is effective, obtaining a complete remission rate of 33% in these poor prognosis settings, and well tolerated. These preliminary results encourage the testing of Clofarabine in AML patients, and reveal the need of post remission consolidation or maintenance to increase PFS and overall survival.

1136

HIGH-DOSE DAUNORUBICIN VERSUS STANDARD-DOSE IDARUBICIN REMISSION-INDUCTION THERAPY FOR NEWLY DIAGNOSED PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Continuous infusion of cytarabine with an anthracycline has been the mainstay of therapy in acute myeloid leukemia (AML). Recent studies suggested that intensification of anthracycline dose might improve complete remission rates in AML patients. Results of high-dose daunorubicin induction studies demonstrated improvements not only in complete remission rates but also overall survival. In this retrospective study we investigated the efficacy of remission-induction therapy for adult patients with newly diagnosed AML either as high-dose daunorubicin (90 mg/m2 daily for 3 days) or idarubicin (12 mg/m2 daily for 3 days) in combination with 100 mg/m2 cytarabine by continuous infusion daily for 7 days. Patients achieving complete remission received intensive postremission therapy that consisted of 4 courses of high-dose cytarabine. Nine of the 20 patients had high-dose daunorubicin. Remaining 11 patients received standard-dose idarubicin as a part of induction therapy. The median age of all patients was 40.5 years (range, 19-67). Cytogenetic risk groups were similar in both groups. There were no significant difference in complete remission rate between two induction groups (P= 0.2). The median (? SEM) follow-up time was shorter in daunorubicin induction group (7 ? 1.1 months) compared to idarubicin induction group (13 ? 5.2 months) (P=0.04). Relapse rates were not different between two groups (P=0.4). The incidence and the intensity of adverse effects were comparable. Our data revealed that high-dose daunorubicin and standard-dose idarubicin therapies were equally effective as remission-induction treatment however longer follow-up with larger series is definitely needed.

1137

ADAPTIVE DESIGN OF VALOR, A PHASE 3 TRIAL OF VOSAROXIN OR PLACEBO IN COMBINATION WITH CYTARABINE FOR PATIENTS WITH FIRST RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA

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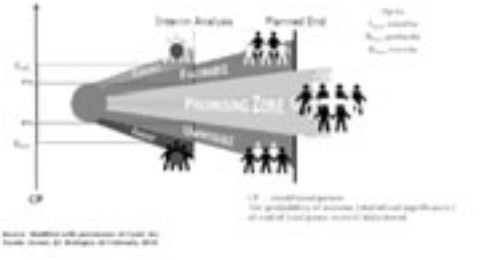
Background. Patients with relapsed or refractory AML have a short median overall survival (OS) of 3 to 6 months. Vosaroxin (formerly voreloxin), a first-in-class anticancer quinolone derivative, showed promising activity in combination with cytarabine in a single-arm phase 2 trial in this patient population (N=69). Median OS was 7.1 months, combined complete remission (CR) rate (CR+CRp+CRi) was 29%, CR rate was 25%, median leukemia-free survival is currently 14.5 months, and 30-day all-cause mortality was 3%. Aims: Given the uncertainty of basing study design on historical data, and the inherent challenges of extrapolating from a phase 2 dataset to a larger, multinational study, an adaptive design was incorporated into the VALOR phase 3 trial to mitigate the risk of negative outcome where statistical significance is not reached at the final analysis, yet where a truly clinically meaningful benefit could be detected if sample size adjustment were made at interim analysis. An adaptive study design allows staged commitment of patients and

resources compared with a conventional design that assumes a smaller difference in OS between treatment arms, requiring a larger initial commitment of patients. *Methods*. Adaptive Trial Design: VALOR is a phase 3, randomized, controlled, double-blind, multinational clinical study of the efficacy and safety of vosaroxin and cytarabine versus placebo and cytarabine in patients with first relapsed or refractory AML. Vosaroxin (90 mg/m2) or volume-equivalent of placebo is administered on days 1 and 4 by short, ≤ 10-minute IV infusion. Cytarabine (1 g/m2) is administered on days 1 through 5 by 2-hour IV infusion. The primary endpoint is OS; key secondary endpoints include CR rate and safety parameters. Base case assumed 40% improvement in median OS, from 5 months for the placebo plus cytarabine arm to 7 months for the vosaroxin plus cytarabine arm (hazard ratio, HR=0.71); 375 OS events are required to provide 90% power at a 2-sided alpha of 0.05 with one interim look allowed at 50% OS events. The interim result is partitioned into zones based on conditional power (Figure 1).

Figure 1. Adaptive Design – Possible Outcomes at Interim Analysis



The Data and Safety Monitoring Board (DSMB) can recommend stopping early for futility or efficacy, or if the interim result is in either the unfavorable or favorable zone, completing per base case. However, if the result falls in the promising zone, the DSMB can recommend a one time sample size adjustment, increasing the final number of events and sample size by 50% (from 450 to 675 evaluable patients) in order to detect a smaller but relevant treatment effect (HR >0.71) (Mehta, Pocock, Stat Med 2010). For example, if the HR is 0.77 rather than 0.71, the 50% increase in sample size boosts the conditional power within the promising zone from approximately 70% to slightly greater than 90%. *Results*. VALOR is enrolling patients as of December 2010. *Summary/Conclusions*. The key advantage of VALOR's adaptive design is that the 50% increase in patients is recommended only if the interim result demonstrates that the increase substantially reduces the risk of failing to detect a clinically meaningful OS benefit.

1138

EFFICACY OF PETHEMA LPA-99 PROTOCOL: A SINGLE CENTER EXPERIENCE

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Background. In acute promyelocytic leukemia (APL) a cure rate of 75-80% can be anticipated with a combination of all-trans retinoic acid (ATRA) and anthracyclines. *Aim*. To evaluate the efficacy of PETHEMA LPA-99 in adults with newly diagnosed APL, managed in the Clinic of Hematology. *Method*. From 2004 to 2010, 42 consecutive APL patients (pts) confirmed either by t(15;17) or PML/RARA were treated with PETHEMA LPA-99. The median follow-up was 32 months (range: 1-78). *Results/Patients characteristics*. Median time from the first symptoms to diagnosis was 23 days (range: 5-90). Pretreatment pts characteristic were as follows: median age 42 years (range: 21-69), 22/42 male; mean WBC 25.6 x10^9/L (range: 0.6-183); mean platelet count 34x10^9/L (range: 4-101); hypergranular form in 39/42 (93%) pts; PETHEMA risk stratification: high 17/42 (40%), intermediate 17/42 (40%), low 8/42 (20%); mean D-dimer 3606 µg/L (range: 996-11340). DIC was confirmed in 32/42 (86%) pts, mean score 6 (range: 3-7). Additional cytogenetics abnormalities were detected in 8/42 (19%) pts (trisomy 8 in 4/8). Therapy results: 33/42 (76%) pts achieved complete remission (CR). Induction death occured in 9/42 (21%) patients due to: differentiation syndrome (DS)- 4/42 (9.5%), central nervous system hemorrhage- 4/42 (9.5%) and infection- 1/42 (2.3%). Early died pts had higher WBC (55.9x10^9/L vs. 14.1x109/L, p=0.014), higher percentage of peripheral blood (PB) promyelocytes (15.0% vs. 9.59% vs., p=0.03) and higher DIC score (6.32 vs 5.85, p=0.014). All died patients were CD15 negative and with a DIC score of >5. DS occured in 11 pts (26%) with a median onset time of 4 days (range: 1-24) and a median WBC of 25.9x10^9/L (range: 1.4-87.7x10^9/L). DS was severe in 9 cases/ 4 fatal and moderate in 2. Pts with DS had significantly higher percentage of PB promyelocytes (48% vs. 28.68%, p=0.04). Microgranular subtype was predictive for DS (p=0.048). Three relapses occured after the third consolidation, during a maintenance and 6 months after the maintenance termination, respectively. Mean time to relapse was14 months (range: 2-31). The 26/42 (62%) pts are alive in first continuous CR. Three-year overall survival (OS) is 76% and disease free survival (DFS) 82%. Tolerance and toxicity according NCI: Leuko-thrombocytopenia grade 3-4 was registered in 30 pts in induction (mean duration 15 days), and in 28 (13 days), 26 (10 days), and 13 (8 days) pts during consolidation, respectively. Hepatotoxicity grade 2-4 (2 with MTHFR) developed in 10 pts; severe cardiotoxicity in 2 pts; genital ulcer one pts; headache grade 3-4 2 pts; pneumonia 7 pts (3/7 Aspergilosis); thrombosis in 6 pts. Patients with thrombosis were older (52 vs. 41 years; p=0.04). *Conclusions*. PETHEMA LPA-99 proved effective in our APL patients concerning CR rate, OS, DFS, toxicity and realpse rate. High rate of early death could be attrubuted to the numerous high-risk pts and increased rate of DIC. WBC >10x10^9/L, DIC score >5 and CD15 negativity were poor prognostic factors for early death. Reduction of the incidence of early deaths is mandatory and requires a timely diagnosis and better management of both DS and bleeding.

1139

CHEMOTHERAPY WITH LOW-DOSE MERCAPTOPURINE IN ELDERLY, UNFIT PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

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Background. Acute myeloid leukemia (AML) is a disease affecting especially elderly patients with a median age at onset of 68 years. AML in older adults seems to be a distinct entity due to specific characters: resistance of leukemic cells to standard chemotherapy; comorbidities; impaired bone marrow stem cell reserve; high percentage of poor prognosis cytogenetics. The current chemotherapy-based treatment has disappointing results: CR rate <45% (, high treatment related mortality, short median overall survival, high relapse rate is. Aim of the study: to evaluate the efficacy of low-dose 6 Mercaptopurine (6 MP) therapy and outcome in elderly, unfit patients with AML. *Patients and methods*. 18 elderly patients with AML followed between Sept. 2002 - December 2010; median age 76 years (range 66 - 84 years); sex ratio: M/F: 7/11. Diagnostic was established after bone marrow aspiration examination, and in 4 cases with confirmation of trephine bone biopsy. Ten patients had AML following myelodisplasia (MDS), 2 after Ph-negative chronic myeloproliferative diseases (1 postpolycythemic myelofibrosis, 1 unclassified myeloproliferation - both were positive for JAK2 (V167F) mutation) and 6 with *de novo* AML. Cytogenetics was available in 6 patients - 3 normal, 1 with (-Y), 1 with (20q-), 1 complex ((-6,del(9),der(17), t(6;17)). All patients had performance status ≥ 2 at diagnosis and at least 2 comorbidities. Chemotherapy consisted in 3+7 courses in 5 cases, 2+5 regimen in 2 cases and low - dose Ara-C regimens for 11 cases. Due to failure of conventional therapy or after severe infectious complications during induction the therapy was changed to low-dose 6MP oral palliation.The dose of 6MP was 100 - 150 mg/week. Treatment decision was made after consulting the patients and their families. Results: Five patients had complete remissions ranging from 12-66 month (3 AML secondary to MDS, 1 de novo AML, 1 postmyelofibrosis). Other 6 patients achieved partial responses with duration of 4 - 15 month. The patient with unclassified chronic myeloproliferation achieved partial remission with bone marrow blast count< 7% but had persistent thrombocytosis and leucocytosis (therapy with hydroxiurea and methotrexate had to be associated) and relapsed to overt AML after 15 months. The CR rate was 27,7% with a median duration of 24 month; partial response was obtained in 33,3% of cases. Overall response rate was 61 %. The 6 MP therapy was well tolerated; in 7 cases the treatment was temporary (5 -10 days) discontinued due to neutropenia (<1000/µl); 2 patients had severe thrombocytopenia, no infections were recorded dur-

ing the treatment period. All patients with cytogenetic abnormalities were poor responders. The patients who failed to obtain response had survivals of 1 - 5 months. In our opinion there might be a group of elderly patients with AML that would have a real benefit in terms of survival and quality of life using low-dose 6 mp therapy. This hypothesis has to be confirmed by further studies on larger patient population combined with cytogenetic and molecular analysis.

1140

EVALUATION OF TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA (APL) BY A PROTOCOL INCLUDING ALL-TRANS RETINOIC ACID (ATRA)

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Introduction. The APL is a rare form of acute myeloid leukemia (AML) but potentially serious because of the risk of bleeding involving immediate threat to life. The introduction of all-trans retinoic acid (ATRA: Tretinoin), whose first results were published in 1997, has completely revolutionized the treatment and prognosis of this disease *Material and methods.* From January 2000 to December 2009, 50 patients (pts) with APL were diagnosed from a total of 308 AML (16%). The diagnosis was made on the morphological study of blood and bone marrow smears according to FAB classification criteria. Including ATRA treatment was applied in 37 pts. Among the remaining 13 pts, 8 died before any treatment of hemorrhagic syndrome and 5 had received only chemotherapy (CT). The median age of 37 pts was 30 years (12-66), 26 were female and 11 male (sex ratio: 0.3). At diagnosis, all pts but one, have an hemorrhagic syndrome, 22 pts/37 (56%) were febrile, no tumor syndrome was observed. On haematological, thrombocytopenia is consistent with a median of 21.10^9/L (2-63), 16 pts (43%) have leucocytosis with with blood count (WBC) more than 10.10^9/L part of the high-risk prognostic group (Sanz, Blood 2000). Symptomatic treatment involving transfusions of platelets to maintain a platelet count greater than 30.10^9/L and fresh frozen plasma is associated with specific treatment protocol follows: ATRA 45 mg/m2/day only if rate WBC ≤ 5.10^9/L. The 3+7 protocol is started at day 5 if WBC ≥ 6.10^9/L, at day 10 if WBC ≥ 10.10^9/L or after obtaining a complete remission (CR) and 2 courses of consolidation followed by maintenance therapy comprising ATRA. Among the 37 pts, eight received ATRA alone for induction and 29 of ATRA + CT; all pts received consolidation therapy and maintenance therapy. The median follow-up was 60 months (15-112). *Results*. CR was achieved in 30 pts/37 (81%), 6 pts (16%) died during induction within 5-19 days (23 septic shock, an acute respiratory distress, 1 hemorrhage cerebrospinal meningitis, 2 of unknown cause), 1 pt died in failure at 3 months. During follow-up among the 30 CR, 5 pts died (3 due to septic shock after a course of consolidation, after an allograft and 1 after early relapse at 5 months). In total, 25 pts/37 (67%) are in persistent CR. The actuarial overall survival (OS) and event-free (EFS) are 68% to 112 months (9 years). *Conclusions*. Our CR rate of 81% is lower than that reported in the literature where it is in good standing over 90%, wich can be improved by strengthening the symptomatic treatment by deletion of ARA-C in induction CT as it is shown that ATRA-anthracycline combination is sufficient and an easing of consolidation treatment in the forms of good and intermediate prognosis

1141

CLINICAL AND BIOLOGICAL CHARACTERISTICS OF ADULT BIPHENOTYPIC ACUTE LEUKEMIA (BAL): A SERIE OF 49 ALGERIAN PATIENTS

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Background. BAL is a rare disorder that can be difficult to diagnose. The first published reports mentioning the entity of BAL can be found in the 80's. By definition BAL displays features of both myeloid and lymphoid markers in one leukemia cell lineage, there are several classification criteria for BAL diagnoses, the most commonly used is the EGIL classification. *Design and Methods.* Between January 2002 and December 2010, 480 pts with acute leukemia (AL) were diagnosed in our unit. The diagnoses of AML and ALL were performed according to the FAB classification; BAL were performed according to the EGIL score. All the pts have morphology and cytochemical analysis on blood and bone marrow aspiration. Only 20 pts have a genetic profil. All of the pts have an immunophenotyping performed on fresh bone marrow or blood specimen. All pts with negative peroxydase, received ALL treatment (Daunorubidomycine, vincristin, asparaginase and prednisolone), and all pts with positive ones received AML treatment (Daunorubicine and cytarabine). *Results.* Of 480 pts with AL, 49 cases of BAL were diagnosed (10,2%), among them 37 pts (75,5%) were Lymphoid and Myeloid, 12 pts (26,6%) were T Lymphoid and Myeloid, no cases of B/T or trilineage differentiation. The median age of the series is 28 years old (15-81), the sex ratio is 1,6 (30/19), the median count of white cells is 32000/mm3 (1100-239000), the median rate of Hb is 8,1 g/dl (4,4-15,9), the median rate of platelet is 64000/mm3. The median prevalence of blast in the bone marrow is 94% (27-100), The extra medullary infiltration concerned 20% of the pts (10 pts). (Mediastinum: 19 pts (40%), lymph nodes: 19 pts (40%), spleen: 14 pts (28,5%), both:12 pts (24%). The incidence of CD34 antigen were 73,3%. Above 20 pts with genetic profil, 6 pts (30%) have t(9-22). Forty five pts (91,8%) received an ALL treatment, with a complete remission rate of 83%, only 2 pts (6,9%) received an AML treatment and 2 pts do not have any treatment. The median follow up is 6 years (1-96). Among the 47 pts who were treated, 26 pts are alive (55,3%) in complete remission. Two pts are alive in relapse, 19 pts (40,4%) dead. The relapse concerned 9 pts/47 (19,1%). The overall survival and the disease free survival are 41,1% and 38,1%. *Conclusions.* The prognosis of BAL seems less poor than other acute leukemia however the type of treatment of induction (ALL mainly in our series) may have a serious influence on the rate of the complete remission (good in this trial) and on the occurrence of the relapse. The rate of death in complete remission is too high, because of the remoteness of sanitary structures. The place of the allograft may be discussed in this indication.

1142

TREATMENT OF ELDERLY, UNFIT PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) WITH VALPROIC ACID AND LOW-DOSE MERCAPTOPURINE

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Background. Acute myeloid leukemia (AML) is a disease affecting especially elderly patients with a median age at onset of 68 years. Because of specific characters of AML in older adults (resistance of leukemic cells to standard chemotherapy; comorbidities; impaired bone marrow stem cell reserve; high percentage of poor prognosis cytogenetics) the current chemotherapy-based treatment has disappointing results: low CR rate, high treatment related mortality, short median overall survival, high relapse rate. These disappointing results are a major challenge in the therapy of this patients and call for more effective and less toxic treatment strategy. Valproic acid is a inhibitor of histone deacetylase activity and we already had cases treated with low dose mercaptopurine showing encouraging results in some cases of AML in elderly patients Aim of the study: to evaluate the efficacy of the association of valproic acid and low-dose 6 Mercaptopurine (6 MP) therapy and outcome in elderly, unfit patients with AML. *Patients and methods*. 8 elderly patients with AML followed between Jan. 2005 - December 2010; median age 76 years (range 65 - 85 years); sex ratio: M/F: 4/4. All patients had performance status ≥ 2 at diagnosis and at least 2 comorbidities. Diagnostic was established after bone marrow aspiration examination. Six patients had AML following myelodisplasia (MDS), 2 after Ph-negative chronic myeloproliferative diseases (1 postpolycythemic myelofibrosis, 1 unclassified myeloproliferation). All patients did not responde to initial chemotherapy (2+5 regimen in 2 cases and low - dose Ara-C regimens for 6 cases) and all patients had severe, life-threatening complications during chemotherapy (severe infections - 6 cases, hemorhage - 2 cases, severe anemia associated with heart failure or cardiac ischemia - 3 cases). After failure of conventional therapy the therapy was changed to low-dose 6MP oral treatment (100 - 150 mg/week) in association with valproic acid (Convulex 900 mg/day, orally). Treatment decision was made after consulting the patients and their families. Results: Two patients had complete remissions with duration of 6 and 36 month, respectively (both AML secondary to MDS, 1 with AML6 subtype and t(12;15)). The other 6 patients did not achieve hematologic responses, but 2 of them had stable disease with survival of 14 and 20 month despite the lack of remission. For the other 4 patients the survival varied between 3 - 7 month. In our opinion that some elderly with AML could respond to the association of valproic acid and mercaptopurine and even in the absence of remission there might be some benefit in terms of survival. This hypothesis has to be confirmed by further studies on larger patient population combined with cytogenetic and molecular analysis.

1143

CLOFARABINE-BASED REGIMENS AS SALVAGE CHEMOTHERAPY FOR ADULT ACUTE MYELOBLASTIC LEUKEMIA - EXPERIENCE OF ROMANIAN WORKING GROUP FOR ADULT ACUTE LEUKEMIA STUDY (RWGALS)

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Background. Clofarabine (2-chloro-20-fluoro-deoxy-9-b-D-arabinofuranosyladenosine) is a second-generation nucleoside analog, which was developed as a hybrid molecule to combine the most favorable pharmacokinetic properties of both fludarabine and cladribine. Aims: The encouraging clinical response rate in the heavily pretreated study group, tolerable toxicity profile, and associated pharmacokinetic and pharmacodynamic parameters led us to investigate clofarabine in relapsed or resistant acute myeloblastic leukemia (AML). Materials and methods: We had studied 9 patients with relapsed AML (7 patients) or resistant AML (2 patients) diagnosed in 2010. Three patients had relapsed secondary AML post SMD, other 5 had relapsed high risk AML. One patient had relapsed AML after allotransplant, matched related donor (twin brother). There were 3 female and 6 male, median age 53 years. Clofarabine based regimens was variable: 4 patients received Idarubicine 12 mg/sm 3 days combined with Clofarabine 32 mg/sm 5 days, 3 patients received monotherapy with clofarabine, 32 mg/sm/d 5 days, 1 patients received Clofarabine 32 mg/sm/d 5 days combined with low dose AraC (7 days) and 1 patient received Clofarabine 32 mg/sm/d 4 days combined with AraC 1g/sm/d 4 days. Results: Clofarabine, given at 32 mg/sm daily for 5 days per course combined with idarubicine had a significant antileukemic activity in heavily previously treated relapsed AML, with CR an average duration of 3 months. Nonhematological side effects were tolerable and mostly reversible. Hematological (thrombocytopenia and neutropenia) grade III-IV toxicity had 89% patients, with an average duration of 23 days. Summary: We found that patients younger than 40 years were much better tolerated treatment with clofarabine than older people. Also, treatment toxicity in patients with early relapse was shorter in duration than those with full relapse. These observations could lead to reconsider the indication of treatment with clofarabine in adult AML

1144

DE NOVO ACUTE MYELOID LEUKEMIA WITH TRILINEAGE DYSPLASIA. HEMATOLOGICAL AND BIOLOGICAL FEATURES. SINGLE CENTER EXPERIENCE

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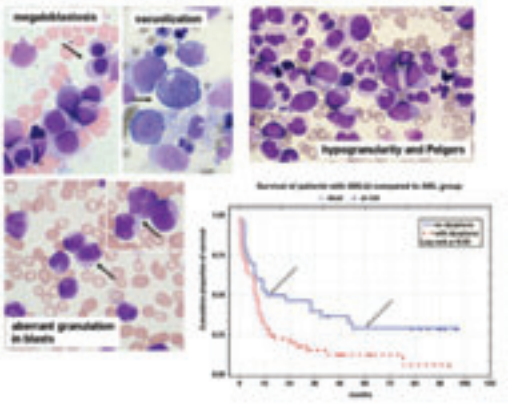
Background. Current WHO classification of AML decribes the entity of AML with trilineage dysplasia. This type of leukemia is hard to diagnose since many patients lack enough morphological details due to expansion of leukemia. Thus, not many biological features were described in selected group of patients. *Aim.* The aim of our study was to analyze patients diagnosed with AML with dysplasia according to WHO (2000/2008) classification and to compare their features with similar patients from the same period within single academic institution. *Patients*. Between 1998-2003, we have identified 50 pts with de novo AMLtd among 270 leukemia cases diagnosed. Comparisson was made with 30 patients without dysplasia (AML) treated in the same period, with same chemotherapy based on MRC AML 10 trial. We analyzed hematological, morphological, cytogenetic and biological data such as proliferation and spontaneous and drug-induced apoptosis (in vivo apoptosis after 48h of therapy), bcl-2 positivity by immunohistochemistry. Also we analyzed response to treatment.



Results. The incidence of this type of AML is roughly 18% of all AML. Patients with AMLtd are elder than others (50.3 vs 42.2). They had in general more myeloid leukemia (M2) than monocytic forms. The incidence of aleukemic type is much more common in AMLtd (54 vs 16%), meaning that WBC and blast counts are also lower. In morphology, when enough myeloid tissue besides leukemia was present, all morphological dysplastic changes in all lineages were found, with prominent dysplastic changes in eryhtroid and granulocytic series (megaloblastic E lineage, dysplastic nuclei; pseudopelgers, disturbed or hypogranulated G lineage). Karyotype revealed that AMLtd patients had typical cytogenetic lesions affecting chromosomes 5, 7, 8, multiple aberations (52%) but also normal karyotype, no prognostically favourable karyotype was found. Remisison rate was lower 42.5% vs 73%, with higher induction death 22.5% and longer aplasia (19 days). Median OS was shorter (9 vs 15 mths) with 11 vs 28% at OS5y. Patients with AMLtd had higher number of proliferative cells (Ki67+, 13.5 vs 6.5%), and this gropu also had higher incidence of patients with Ki67+>10% (43% vs 16%). There was no difference in spontaneous apoptosis rate 3.7 vs 3.5%, but hemotharapy induced apoptosis was higher in patients without dysplasia 5.8±5.2% vs 3.6±3% in AMLtd. Bcl-2 positivity (IHC) revealed that 65% with AMLtd were positive compared to 43% in AML group (p=0.07). *Conclusions*. Patients with AML with trilineage dysplasia had charateristic form of disease, with lot of poor prognosis features, and low response to treatment. In case that they are elegible, they probably will benefit from more intensive treatment approaches.

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GENE MUTATION IN PAIRED INITIAL PRESENTATION AND RELAPSE SAMPLES FROM ACUTE MYELOID LEUKEMIA

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Background and Aims. Several gene mutations were found in acute myeloid leukemia (AML) and were expected to be used as prognostic factors and minimal residual disease (MRD) markers. We have studied Flt3, NPM1, CEBPA, IDH1/2 gene mutations in paired samples at initial presentation and relapse of AML to ascertain the biological meanings of these mutations and to evaluate whether they can be used as MRD marker. *Methods.* We analyzed paired samples at initial presentation and relapse from 30 adult patients with de novo AML who diagnosed at Nippon Medical School from 2000 to 2010. Bone marrow or peripheral blood samples containing 20% or more blast cells obtained were used for mutation analyses. Mutation analyses were performed for Flt3ITD by PCR amplification, Flt3TKD by PCR-RFLP, and NPM1, CEBPA, IDH1/2, mutations by direct sequence. To validate sequencing results, PCR products were inserted into the pCR2.1-TOPO vector using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Recombinant plasmids isolated from 8 to 12 white colonies were sequenced. *Results*. Flt3ITD, Flt3TKD, NPM1, CEBPA, IDH1/2 mutations were detected in 9 (30.0%), 3 (10.0%), 10 (33.3%), 1 (3.3%), 2 (6.7%) samples at initial presentation samples, and 8 (27.6%), 0 (0%), 7 (23.3%), 1 (3.3%), 2 (6.7%) samples at relapse, respectively. Among 24 available samples for chromosomal analysis at relapse, 16 (66.6%) showed additional chromosomal aberrations, 6 (25.0%) changed to be complex chromosomal aberrations. Chromosomal instability at relapse was observed in many cases, but frequency of gene mutations at relapse was lower than those at initial presentation. About 40% of AML patients at relapse did not have these gene mutations detected at initial presentation. CEBPA mutation

was found in one paired sample. IDH1/2 mutations were detected in 2 paired samples of patients. Interestingly, 3 of 9 patients with Flt3ITD mutation at initial presentation had no Flt3 ITD mutation at relapse. Flt3/TKD mutations were found in 3 patients at initial presentation, but all of them were lost in these three cases at relapse. Among the 10 patients with NPM1 mutation at initial presentation, 3 had no detectable mutation at relapse. These results indicate that Flt3ITD, Flt3TKD, and NPM1 mutations should be used carefully for the detection of MRD. Furthermore, among the 6 patients with NPM1 mutation without Flt3ITD mutation that is known as favorable factor, Flt3ITD mutations were detected in 2 patients at relapse. There was a possibility that detection sensitivity for Flt3ITD is not sufficient by PCR amplification method. *Conclusions*. Our study showed that about 65% of the patients with Flt3ITD mutation had the same mutation at relapse, which means that these mutations may contribute to expand resistant clones. On the other hand, detection of Flt3ITD mutation only at relapse suggested that a minor clone harboring Flt3ITD mutation might be overlooked at diagnosis. We have developed a highly sensitive mutation detection method (15th. Congress of European Hematology Association, 2009, Barcelona, Spain) and studies to clarify these findings are ongoing.

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GEMTUZUMAB-OZOGAMICIN AS POST-CONSOLIDATION THERAPY IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA: A PILOT STUDY

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Background. The optimal post-remission treatment for elderly patients with acute myeloid leukemia (AML) is currently unknown. Autologous transplantation is not always feasible in this setting because unsuccessfull mobilization and comorbidities. The high relapse risk of the disease raise the issue of exploring alternative consolidation regimens. Aims. We evaluated safety and efficacy of low dose Gemtuzumab-Ozogamicin (GO) as post-remission late consolidation therapy in a cohort of 19 consecutive patients, failing mobilization, enrolled in a pilot prospective study. *Methods*. Between June 1999 and February 2010 we observed 152 elderly patients, aged more than 60 years and affected by AML. One hundred and two patients (67%) were considered fit for aggressive therapy and received intensive induction and consolidation therapy including HD Ara-C, Idarubicine and Amifostine. Thirty-nine patients (38%) were over 70 years, 38% had a secondary disease, 35% unfavourable, 45% intermediate and 4 % favourable prognosis Karyotype. Those achieving successful mobilization received autologous transplant while poor mobilizers received a second consolidation with three monthly courses of GO 3 mg/sm i.v. followed by three other courses of GO administered every 3 months. A third group received chemotherapy alone for patient decision. The three groups showed similar disease and patient characteristics. *Results*. Seventy-two patients achieved CR (69%) after induction: one patiente died in CR, 5 patients had a PS ≥2 and the remaining 66 were elegible for first consolidation. Fifty of these maintained the CR status and received a second consolidation: 19 with GO, 20 with Autologous Transplant, and 6 with chemotherapy alone for patient decision. Five patients with a family donor received Allogeneic Transplant and were not included in this analysis. GO was well tolerated: no major adverse events were seen. We overall observed 18 WHO grade III/IV adverse events were all transitory and included hematological toxicity (n = 17), hypertransaminasemia (n = 1). Eight patients (42%) relapsed after GO consolidation and received a GO reinduction: five eventually died after a median follow-up of 13 months while three are still alive with a median follow up of 10 months. Five out of 20 patients died of transplant related toxicity (25%) and 9 of the remaining 15 relapsed (60%) after autologous transplant. All nine patients died of progressive disease. Five of the 6 patients receiving chemotherapy (83%) relapsed and died. Five years Overall survival was 22% and Disease Free Survival 29.5% in the whole cohort of patients (median follow-up: 50 months). The landmark analysis showed a superior outcome in patients receiving GO with a 60% 5 yrs OS and DFS (median follow-up: 60 months) in comparison with patients receiving autologous transplant (28% 5 yrs OS and DFS with median follow-up 70 months) and chemotherapy (17% 5 yrs OS and DFS with a median follow-up of 77 months) (p= 0.009). *Conclusions*. Patients receiving GO had a better outcome in comparison with patients receiving autologous transplant and chemothearapy. High percentages of poor mobilizers and transplant related mortality seem to jeopardize autologous transplant feasibility and safety in elderly patients. GO showed to be an alternative and feasible choice in this setting.

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BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASIA (BPDCN): REPORT OF 2 CASES

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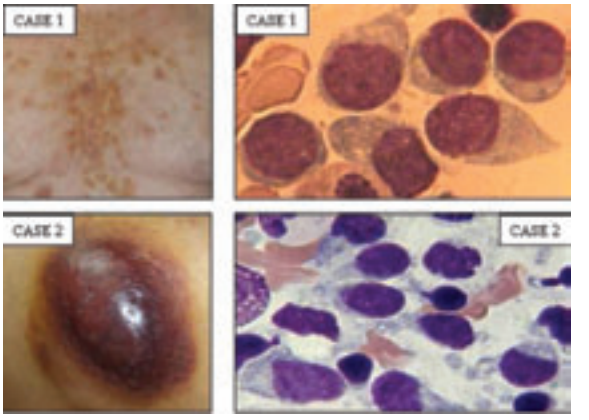
Background. BPDCN is a rare hematopoietic disease that typically involves the skin, lymph nodes, peripheral blood and bone marrow. Prognosis is dismal with a median overall survival of only 12-14 months despite aggressive chemotherapy. We report 2 cases of BPDCN associated with dysplasia. CASE 1: A 68-year-old man consulted for asymptomatic, infiltrated, disseminated skin lesions that had appeared during the previous 6 months, with progressive onset of anemia (hemoglobin 112 g/L) and leukopenia (2.8 x10^9/L). Platelet count and lactate dehydrogenase were normal. A skin biopsy revealed a dermal infiltration of monotonous medium-sized mononuclear cells with expression of CD4 and CD56 but not CD3 or CD20. Bone marrow aspirate was infiltrated by 48% medium-large sized blasts with a peculiar morphology. The nucleus was irregularly shaped and the chromatin was lacy with a blastic appearance. The cytoplasm was large, grey and agranular, with pseudopodium-shaped expansions and occasionally vacuoles. Myeloperoxidase was negative and esterase was not conclusive. Trilineage dysplasia was observed. Flow cytometry immunophenotyping confirmed the diagnosis of BPDCN (positivity for CD4, CD56, CD123, and HLA-DR; negativity for B, T and myelo-monocytic markers). Fluorescence in situ hybridization (FISH) revealed +12, del (13q14.3) and del (13q34). The karyotype was not available. FLAG-Ida (fludarabine, cytarabine, idarubicin and G-CSF) was administrated as induction therapy. Consolidation therapy with FLAG-Ida is ongoing and the patient is scheduled for allogeneic hematopoietic stem cell transplantation. He is currently in remission, with negative minimal residual disease documented by flow cytometry and FISH although with persistence of trilineage dysplasia and cytopenias. CASE 2: A 78-year-old man was monitored for a medical history of ischemic heart disease and refractory anemia that progressed to refractory anemia with excess blasts type 1 (RAEB-1). His clinical course over the previous 4 years has been stable. He presented with a purple asymptomatic cutaneuos lesion on his left shoulder and weakness. A blood count revealed neutrophils 0,4 x 10^9/L, platelets 16 x 10^9/L, and hemoglobin 76 g/L. Cytological examination of bone marrow showed 79% blast cells with the same morphology and cytochemistry as case 1, as well as myelodysplasia of the 3 cell lines.



Immunophenotyping revealed BPDCN (positivity for CD4, CD56, CD123, HLA-DR, negativity for B, T and myelo-monocytic markers). A cytogenetic study revealed a complex karyotype. Cutaneous biopsy showed massive dermal infiltration by BPDCN. In view of the patient´s age and comorbid conditions, supportive care was provided and he died 4 months later. *Conclusions*. The clinical presentation of both patients was very similar, with cutaneous involvement and cytopenia. Morphologic and cytochemical features were characteristic and similar. Immunophenotyping was diagnostic. Both patients had chromosomal changes. Survival is usually poor without treatment. This justifies aggressive protocols with bone marrow allotransplantation.

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ACUTE LEUKEMIA: HOW WELL DO HISTOLOGY AND FLOW CYTOMETRY CORRELATE AT DIAGNOSIS?

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Background. Acute leukemia is a common haematological malignancy, which comprises acute myeloid leukaemias (AML), acute lymphoblastic leuaemias (ALL) and acute leukaemias of ambiguous lineage. Diagnosis is by bone marrow trephine biopsy (BMTB), flow cytometry (FC), bone marrow aspirate, peripheral blood investigations and cytogenetics. Review of the available literature showed correlation rates between FC and histology of 88% when sampling lymph nodes in non-Hodgkin lymphomas (*El Sayed et al. 2008*) and 87% in marginal zone lymphoma when sampling bone marrow (Boveri et al. 2009). No literature could be found on this parameter in acute leukaemias. Aim: To compare BMTB with FC in patients with suspected acute leukaemia at diagnosis. Methods: Diagnostic BMTB and FC reports, from May 2004 to December 2010, from patients suspected to have an acute leukaemia on either multicolour FC or BMTB were reviewed. Results were included if the FC and BMTB samples were sent within two weeks of each other. FC panels included antibodies to CD10, CD117, CD13, CD34, CD33, myeloperoxidase, HLA-DR, CD45, TdT, CD79a, CD19, cCD22, CD2, cytCD3, CD7, CD14 and CD15. Immunohistochemical panels on BMTB included CD34, CD117, myeloperoxidase, HLA-DR, TdT, CD68, CD68R, CD33, glycophorin C, CD61/CD42b, CD20, CD79a, PAX5 and CD3. *Results*. A total of 46 cases were studied. In 44 cases (96%) both FC and BMTB reported an acute leukaemia. In the remaining 2 cases - 1 was reported as AML on FC and as myelofibrosis with dysplastic megakaryocytes on BMTB, the other was reported as suspicious of AML on BMTB and as lymphocytosis on initial FC, though the subsequent FC in this patient did diagnose AML. Of the 44 cases where both investigations reported acute leukemia: Both FC and BMTB reported precursor B-cell ALL in 5 cases and T- cell ALL in 1 case. In 35 cases, both FC and BMTB reported AML. Of these 4 were reported as acute promyelocytic leukemia; 2 by both FC and BMTB, 1 by FC only and 1 by BMTB only. A further 8 were reported as having monocytic differentiation; 5 by both FC and BMTB, 2 by FC only and 1 by BMTB only. In 1 case, the BMTB suggested erythroblastic differentiation which was not confirmed by FC, this sample was reported as being haemodilute. In 1 case, the BMTB suggested megakaryoblastic differentiation which was not confirmed on FC.In 2, FC reported poorly differentiated acute leukemia, and in 1 of these cases BMTB reported acute leukaemia - not otherwise specified (NOS), in the other case BMTB reported pro B-cell ALL. In 1, FC reported mixed phenotype acute leukemia and the BMTB reported AML-NOS. *Conclusions*. BMTB and FC correlated in 96% of cases of acute leukemia (44/46). A concordance of 95% was seen in the diagnosis of the subtypes of acute leukemia (42/44). Within AML, where lineage/subtype-specific subtype differentiation was attempted, BMTB and FC correlated in 50% of cases (7/14). This study emphasizes that FC and BMTB are complementary in the diagnosis of acute leukemia and shows an excellent correlation rate between the two investigations.

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TREATMENT OF ACUTE MYELOID LEUKEMIA IN THE ELDERLY - A SINGLE CENTER EXPERIENCE

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Background. Treatment of the elderly with Acute Myeloid Leukemia (AML) is a challenge, considering the pre-existing comorbidities that could limit the therapeutic spectrum in a patient suffering from a biologically aggressive disease. While overall survival in patients with AML decreases with age, most studies performed in elderly patients show Complete Remission (CR) rates between 40 and 60%, but only if they are treated aggressively, potentially limiting their quality of life and prolonging the duration of hospitalization. *Aims.* This study aims to characterize an elderly population with a diagnosis of AML (considering age subgroups, cytogenetic risk groups, type of intended treatment and use of consolidation) and to analyze the impact of different treatment approaches on their overall survival (OS). *Methods*. Retrospective analysis of clinical and laboratory data from 131 patients aged over 59 years, with the diagnosis of AML (excluding acute promyelocytic leukemia, according the 2008 World Health Organization classification), treated in our institution between 1st January 2005 and 31st December 2010. Statistical analysis performed using the 19.0 version of SPSS. *Results*. Both genders were equally represented (M:F=1). The median age at diagnosis was 70 years and 70% of patients were over 65. The majority of cases (55%) were secondary to a pre-existing hematological disease (myelodysplastic or myeloproliferative syndromes) or to chemotherapy for other malignancy. According to the French-British-American classification, there was a predominance of AML with monoblastic features (40%), followed by the M1 subtype (26%), M2 (22%) and M0 (12%). We obtained conventional karyotype cytogenetic results in 60% of our patients, of whom 6% were of low risk, 71% of intermediate risk and 23% of high risk. OS was 35% at 12 months and 10% at 36 months with a statistically significant difference in survival between the 60-65 and over 65 age groups at 12 months (50% vs 20%; p<0.05), and an equally significant difference between the palliative and aggressive treatment groups at 12 months (10% vs 45%; p<0.05). We opted for an aggressive treatment strategy in 75% of our patients, pre-selected according to age, performance-status and comorbidities, attaining a CR rate of 53%. The use of consolidation therapy after CR in this age group didn't improve overall survival (60 vs 58% at 12 months, p=N.S.) or progression-free survival (25% vs 24% at 12 months, p=N.S.). *Conclusions*. Although the decision to treat is based on the patient's co-morbidities and performance status, we conclude that the outcome of aggressively treated patients is better, with prolonged overall survival and no significant increase in treatment-related complications. On the other hand, the use of consolidation didn't improve OS or PFS, in our series. While treatment decisions should be individualized to each patient, according to risk factors, performance status and co-morbidities, the recommendation to, whenever possible, enroll the patient in a clinical trial still prevails.

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MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA WITH INV(3)(Q21Q26.2) OR T(3;3)(Q21;Q26.2) ABNORMALITY. SINGLE CENTER EXPERIENCE

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Background. Recent World Health Classification (WHO) has incorporated acute myeloid leukemia (AML) with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) as a new entity in the category of AML with recurrent genetic abnormalities, that accounts for 1%-2% of AML. This type of AML can arise de novo or from pre-existing MDS. Prognosis of patients with MDS/AML with 3q abnormality (3q abn) is very poor with low complete remission (CR) rate and short long-term overall survival (OS) with standard chemotherapy. P*atients and methods*. We report baseline characteristics and outcome of 6 cases of patients with MDS or AML with 3q abn from our center. Demographics, blood counts, bone marrow features and evolution are shown in Table 1. *Results*. Four out of 6 pts were aged <65y. All of them received standard intensive chemotherapy (IC). No complete remission was achieved followed by progression in all 4 cases. One pt underwent allogeneic stem cell transplant with subsequent early relapse. Other 2 pts (#5 and #6) have been recently diagnosed. Treatment received were non-intensive chemotherapy (NIC): one of them low-dose AraC and 5 azacitidine the other, carrying additional chromosomal abnormality (monosomy 7 in the context of monosomal karyotype). The only treatment offering stable disease with OS over 1 year was 5 azacitidine (16 months after diagnosis). No serious adverse events with this approach occurred and treatment is ongoing. Our results compare with data recently reported in this group of very-poor prognosis MDS/AML pts in which treatment options are still unclear.

	Sex	Age	Hb	ANC	Platelet	BM blast	Dysplasia	Treatment	Status
Patient #1	M	57	84	0.53	1653	49.25	E	IC	Death
Patient #2	W	34	76	2.4	367	22	E/WC/P	IC, AlloSCT	Death
Patient #3	M	48	89	0	25	75.5	No	IC, AlloSCT	Death
Patient #4	W	45	76	0	12	40	W/B	IC	Alive
Patient #5	W	68	76	0.4	36	29	E/WC/P	IC, 5-aza (17 courses)	Alive
Patient #6	W	78	81	0	45	59	E/WC	NIC (LD-AraC)	Alive

Table 1. Baseline characteristics of pts. Treatment and outcome. M: Male. F: Female. Hb: Hemoglobin (g/L). ANC: Absolute neutrophil count (x10 9/L). Platelets (x10 9/L). E: Erythroid. WC: White-cell. P: Platelet.

*Conclusion*s. It is well known poor prognosis and decreased survival in patients with AML and genetic abnormalities on chromosome 3. Most are refractory to intensive chemotherapy and generally not well tolerated when dealing with elderly patients with frequent comorbidities. However, certain drugs as hypomethylating agents (5 azacitidine) have incorporated to treatment strategies after approval for low-blast count (20-30%) AML with promising results even in older AML pts when compared to conventional care regimens. Achievement of CR was not needed to extend survival in this pts. Whether this could be effective in this subset of pts with 3q abn must be addressed in larger prospective studies.

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WT-1 EXPRESSION LEVEL FOR MINIMAL RESIDUAL DISEASE MONITORING IN ACUTE MYELOID LEUKEMIA

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Monitoring of minimal residual disease in AML is an important part of patient management which potentially can increase overall survival by early detection of relapse. Many targets has been proposed for MRD monitoring in AML, but all have known limitations as being useful only for a small subset of AML cases. WT-1 was shown to be overexpressed in most AML patients and is a useful target for MRD. *Materials and methods:* we used a previously described method which standardized the use of RQ-PCR for WT-1 expression monitoring. A total 140 patient samples along with normal controls were analyzed. Samples were taken from 40 patients at presentation and different time point during follow-up. *Results*. Most of patients presented elevated levels of WT-1 expression at presentation (above 50 WT-1/10^4 ABL) ranging from 1 to 1,5*10^4 WT-1/10^4 ABL. During and after the therapy levels of WT-1 transcripts steadily decreased reaching normal levels. In 5 patients raise in WT-1 transcript level was predictive of a relapse as confirmed by fusion gene expression monitoring (4 cases with PML-RARA and 1 with AML-ETO). *Conclusions*. Due to expression of WT-1 in normal hematological tissue sensitivity of this assay is somewhat reduced, but in comparison of other methods of MRD monitoring it permits monitoring of the majority of AML patients regardless of other genetic abnormalities.

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AZACITIDINE FOR ACUTE MYELOID LEUKEMIA TREATMENT. ENCOURAGING RESULTS OF THREE SPANISH HOSPITALS

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Introduction. The potential and limitations of the standardized cytotoxic chemotherapy (AraC plus Antracycline) for Acute Myeloid Leukaemia (AML) have been assessed by different trials. In the search for novel treatment strategies, different trials have evaluated, with promising results, the role of Azacitidine (AZA), a DNA methyltransferase inhibitor, in the treatment of AML de novo or relapsed, before or after transplantation, or as maintenance after complete remission. *Aims.* To evaluate effectiveness and toxicity profile of Azacitidine in the treatment of AML patients. *Methods.* A retrospective study was carried out at 3 Spanish institutions including patients diagnosed with AML, defined as bone marrow blasts count >20%, receiving Azacitidine either as front line, salvage therapy or maintenance. Main points were progression free survival (PFS), overall survival (OS), complete response (CR) according to the IWG v2003 criteria; and toxicity, graded according to the CTCAE v3.0 of NCI criteria. *Results*. Data from 26 AML patients treated with AZA 75 mg/m^2/day x 7 days every 28 days, were analyzed. Baseline characteristics were: mean age 67.6 years old (38-80), median 68y. Ratio male/female 17/9. Clinical history of previous Myelodysplastic Syndrome (MDS) 54% (14 /26). *Effectiveness.* AZA as front-line therapy: 13 patients, mean age 72.6y (62-80). Previous MDS 8/13 (62%) and poor prognosis cytogenetic 3/13. Five patients achieved cytological complete response (CR). At the time of report, with a mean follow up of 12.1 months (5-43), 8 patients are alive, 4 of them remain in CR, and 5 patients have died because of leukemia progression (mean OS 10.6 months), all of those with previous MDS. AZA as salvage therapy: 8 patients, mean age 63.6y (38-77). Previous MDS in 4 patients, mean of previous lines received: 1.5 (1-2). 2 patients achieved CR, one of them was consolidated with allogeneic transplant and died because of leukemia progression. At the time of report 1 patient is alive in CR with a follow up of 12 months, and 7 have died (OS 3.5 months). Hyperleucocytosic (>20,000/uL) presentation was correlated with a poor response rate and shorter OS; at the time of report there has been 4 deaths out of 5 hyperleucocytosic patients. On the other side, pancytopenic patients presented high response rate, often associated to a significant increase in platelet count, which allowed most patients to become transfusion-independent. AZA as maintenance after CR: 5 patients, mean age 61.1y (48-71). Previous MDS 2 patients. The mean of cycles received was 9.8 (2-21). With a mean follow up of 22.6 months (5.7-33.3), 3 patients are alive in CR, and 2 died with an OS of 22.9 months. *Toxicity.* Hematologic toxicity was observed in all patients, neutropenia grade IV in 23 patients, febrile neutropenia in 12 patients and thrombocytopenia grade IV in 18 patients. All deaths were related to leukemic progression. No relevant extrahematologyc toxicity was reported. *Conclusions*. This study shows Aza is effective in AML and safe with no serious adverse event reported.

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5-AZACYTIDINE IN REFRACTORY/RELAPSE OR UNFIT TO INTENSIVE CHEMOTHERAPY ACUTE MYELOID LEUKEMIA PATIENTS

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Background. 5-Azacytidine (AZA) prolongs survival in higher-risk MDS patients including those with 20-29% marrow blasts, currently diagnosed as Acute myeloid leukemia (AML). However, no large AML series, especially with BM blasts >30%, treated with AZA have been reported. *Patients and Methods*. We have retrospectively analyzed results in patients having received at least 1 cycle of AZA in 7 centers for refractory/relapsed AML or not eligible for intensive chemotherapy (CT). 63 patients were included between Feb/07 to Feb/11; M/F: 42/21; median age 69 years (range 31-84). Median WBC was 2,2x10^9/L (0,1-17,5). Median BM Blasts: 29% (20-94). 30 cases had >30% BM blasts. Karyotype (MRC classification), was adverse in 18 patients (including 5 Complex karyotype; 5 del5q; 6 -7/del7q, and 2 patients with 3q26), Intermediate in 37 (Normal karyotype 31, NPM1mut/FLT3wt in 4 and FLT3-ITD in 3 patients) favorable in 2 cases (1 t(8;21) and 1 t(16;16) and failed in 6 patients. Eighteen patients were treated for refractory/relapse AML after intensive CT or SCT (Auto-SCT=4; Allo-SCT=3); 22 had prior MDS y 23 as first line therapy. AZA doses were 75-100 mg/m^2/day x 7 days (5+2). First evaluation was made after 4-5 cycles according to the International Working Group criteria (AML-IWG-2006). *Results.* With a median follow-up of 9,5 months, patients had received a median of 5 AZA cycles (1-24). In first evaluation made after 4-5 cycles, an overall response rate (ORR) was observed in 17 evaluated patients (30%) including 9 CR (16%) and 8 PR (14%). Additionally, 16 (28,6%) patients achieved hematologic improvement (HI, according to MDS-IWG 2006 criteria). No pretreatment characteristics as age, prior MDS, karyotype, % BM blasts were statistically correlated with response. Eight responder patients progressed within a median time of 8 months. The 12 mo Overall Survival (OS) was 40,9%, and 24 mo OS 15% and median OS of 11 months. In our series, pre-treatment characteristics as higher WBC (p=0,042), adverse vs intermediate cytogenetics (26% vs 54%, p<0,001) and >30% BM blasts (27% vs 50%, p=0,095) showed negative prognostic significance for 12 mo-OS. However, prior MDS (55% vs 31% 12 mo-OS, p=0,13) and disease status (AZA as first line or advanced disease: 42% vs 35%, p=0,5) did not influence OS. Achievement of ORR was associated with improved OS (12 mo-OS 57% vs 33%, p=0,0013). In patients with no AML-IWG response, HI also predicted better survival (p=0,03). *Conclusions*. Patients diagnosed with relapsed AML and those not eligible for intensive CI have limited treatment options. AZA can be a well-tolerated and effective alternative option in this group of patients. In our experience, these patients can achieve OOR of 30% with a 12 mo OS of 40.9%. Higher WBC counts, adverse karyotype and >30% BM blasts were associated with poorer OS but did not preclude response to AZA. Moreover, achievement of ORR or even HI is significantly associated with improved overall survival.

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PEDIATRIC AML IN LEBANON: BIOLOGIC FEATURES AND OUTCOME

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Background. AML is a disease with marked heterogeneity in clinical and biologic features, response to therapy and survival. Despite major achievements in the treatment of AML, long term survival remains poor. About half of pediatric AML patients relapse and die internationally. There is no published data on pediatric AML in Lebanon. Aims: We aimed to identify clinical, cytogenetic, molecular findings and outcome data in the Lebanese population in comparison to international and regional data and to identify specific needs in order to improve outcome. Methods: We underwent a retrospective chart review of children with AML diagnosed at 3 institutions in Lebanon in the past ten years. We filled and analyzed data collection sheets for each patient including demographic information, clinical, laboratory, molecular, therapeutic and outcome data. *Results*. We identified 24 patients with AML, 12 girls and 12 boys. Two had Fanconi anemia, one had Down syndrome, one had myelodysplastic syndrome with monosomy 7, and one had secondary AML after treatment for Burkitt lymphoma. Mean age was 8.6 years (range 1 to 24 years). Mean WBC at diagnosis was 86,500 (Range 2,100-376,000). Two were diagnosed as M0, 4 as M1, 2 as M2, 6 as M3, 5 as M4, 0 as M5, 2 as M6, and 1 as M7. Karyotype was normal in 37.5 % of patients. 25% had t(15;17), 4.1% had t(8;9), 4.1% had t(7;11), 8.3% had t(8;21), 8.3% had inv 16, 4.1% had t(9;11) and 4.1% had complex abnormalities. FLT-3 was positive in 3 patients and was associated with high WBC at presentation and a poor outcome. NPM1 was tested in one patient and was negative. Death in induction was observed only in 3 patients with APML, hyperleucocytosis and bleeding and one child with M6 AML and Fanconi (16.6%). BMT was indicated in 17pts and was performed in 8 pts in Europe or the United States: 1 had a cord blood transplant, 2 had a haploidentical BMT, 5 had matched related sibling BMT. Survival after transplant was 37.5%. Median survival for patients who died from disease progression was 25.8 months. Overall disease-free survival was 30.4% *Summary/Conclusions.* This is the first report looking at pediatric AML in Lebanon. Overall survival in this cohort was 30.4% . Areas of improvement would be initial support of patients with APML and hyperleucocytosis as high mortality was observed in these patients, and availability of BMT in a timely fashion for high risk patients. FLT3 positive patients did poorly with or without transplant. Further data collection to include the entire country is in process.

1155

ANTICANCER ACTIVITY OF 2-AMINOPHENOXAZINE-3-ONE (PHX-3) AGAINST HTLV-1-POSITIVE LEUKEMIA CELLS

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Background. It is very difficult to treat patients with adult T cell leukemia (ATL), a malignant tumor of human CD4+ T cells infected with a human retrovirus, T lymphotropic virus type l (HTLV-1). Moreover, ATL is recognized to precede by oligoclonal expansion of HTLV-1-infected T cells, where the virus expands via cell-to cell transmission. Therefore, the agents, which cause apoptosis of this malignancy and prevent the cell-to-cell transmission of the virus, are urgently required. *Aims*. Present research aimed at studying the cytotoxic and proapoptotic effects of 2-aminophenoxazine-3-one (Phx-3) on HTLV-1-positive leukemia cells, as well as its preventing effect against the cell-to-cell expansion of the virus. *Materials and Methods*. HTLV-1-positive leukemia cells, MT-1 cells, HUT-102 cells and MT-2 cells and HTLV-1-negative rat sarcoma cell line, XC cells were treated with different concentrations of Phx-3, which was prepared by the reaction of o-aminophenol with bovine hemoglobin solution, and were incubated in a humidified incubator containing 5% CO2 and 95% air at 37°C. The syncytium formation assay for detecting the cell-to-cell transmission of HTLV-1, consisted of a coculture of HTLV-1-bearing MT-2 cells and indicator XC cells. *Results*. Phx-3 (10 μg/ml) suppressed the viability, arrested cell cycles at sub G0/Gl phase, and induced apoptosis of MT-1, HUT-102 and MT-2 cells, but did not suppress the viability of XC cells and phytohemagglutinin-activated human peripheral blood mononuclear cells. In addition, Phx-3 extensively inhibited the syncytium formation between HTLV-1-positive cells (MT-2 cells) and HTLV-1-negative cells (XC cells), indicating that the transmission of HTLV-1 from HTLV-1-positive cells to HTLV-1 negative cells was prevented by Phx-3. *Conclusions*. Phx-3 may be useful as a therapeutic agent for the treatment of patients with ATL.

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OXIDATIVE STRESS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA EARLY STAGE. ASSESSMENT AND CORRELATION WITH PROGNOSTIC FACTORS

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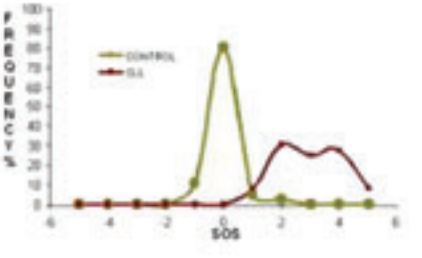
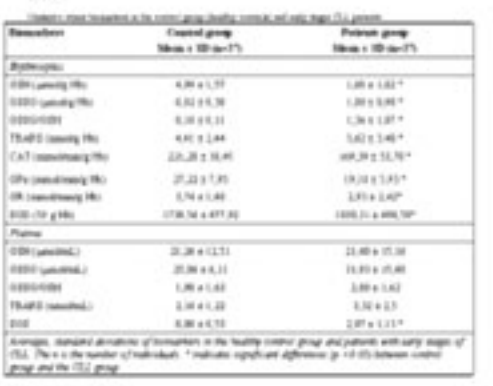
Background. An important area of research of CLL is the identification of markers that are useful for predicting the likelihood of disease progression. Measurement of oxidative stress (OS) makes reference to the imbalance in favour of prooxidating state in front of antioxidative state. Aims. In a population diagnosed of early stage CLL and in a matched group control, we describe the values corresponding to different OS biomarkers. Its weight was set in a model of overall score (SOS) to assess the degree of OS in patients in early stages of CLL. Finally, we assessed whether there were differences in markers of OS in relation to different recognized prognostic factors. *Methods*. Informed consent was obtained. In a group of 37 patients, the values of different recognized prognostic factors were collected. In patients and in a matched group control, we determined the following markers of OS: superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, reduced and oxidized glutathione, thiobarbituric acid reactive substances and global antioxidant capacity through the ORAC method. Then, we propose an SOS based on the analysis of these biomarkers. SOS was related with the presence of poor prognosis factors, in an attempt to introduce SOS as a prognostic marker of CLL early stage. Results. The SOS of patients did not follow a normal distribution. The score moved significantly towards more positive values, indicating OS (Figure 1). Higher oxidative state was evidenced through each patient's biomarkers compared to control group (Table 1).

Figure 1
Frequency distribution of the SOS in the control groups and the patients group





We found correlations between biomarkers of OS and analyzed prognostic markers. We created the variable 'number of prognostic factors'

(NFPP) that groups patients without poor prognostic factors, patients with 1-2 factors and patients with 3-4 prognostic factors. Most of the biomarkers of and the SOS have values indicative of greater OS in the group of 3-4 prognostic factors. The frequency distribution suggests a tendency to classify patients into patients without poor prognostic factors for CLL that have low SOS, a second group with maximum NFPP (3 or 4 poor prognosis factors) and SOS (five points), and intermediate group regarding NFPP (1 or 2 poor prognostic factors) as the SOS (2 to 4 points). *Summary/Conclusions*. Patients with early stage CLL have greater oxidative distress in all biomarkers analyzed. There is a positive relationship between biomarkers of oxidative distress and most CLL prognostic factors. The presence of poor prognostic factors confers greater OS. The information from the SOS is more accurate than that obtained with each of the biomarkers studied individually and it represents more accurately the state of prooxidant-antioxidant balance of the individual. The SOS is a possible clinical parameter to consider when evaluating the oxidative distress routinely and individually.

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This abstract has been withdrawn.

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PROGNOSTIC SIGNIFICANCE OF TELOMERE LENGTH IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS IN EARLY STAGE DISEASE

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Background. Chronic lymphocytic leukemia (CLL) is a genetically heterogeneous disease with a variable outcome. The identification of factors that could predict the clinical course of early-stage CLL represents a crucial objective in this malignancy. Although previous studies indicated that telomere length may be a useful independent prognostic factor in the risk stratification of CLL patients, limited information has been reported in asymptomatic early stage patients (Binet stage A). *Aims*. The present study was aimed at investigating the association of telomere length with the major biological and cytogenetic markers known to predict clinical outcome in CLL. The global DNA methylation levels of Alu and LINE sequences, was also investigated. Correlation with disease progression, measured as the time elapsed from diagnosis to first treatment, was evaluated. *Methods*. We measured relative telomere length (RTL) by real-time PCR in a panel of highly purified (>90%) peripheral mononuclear CD19+ cells from 7 healthy donors and 77 untreated CLL patients. All cases were characterized by FISH for the most frequent chromosomal aberrations, namely trisomy 12 and 17p13.1, 11q22.3 and 13q14.3 deletions (*Fabris et al. GCC, 2008*). Molecular markers including mutation status of the heavy chain variable regions of immunoglobulin genes (IGVH), the expression of the 70-kd zeta-chain T-cell receptor-associated protein kinase (ZAP-70) and CD38 cell surface antigen protocols were previously reported (*Cutrona et al. Haematologica, 2008*). A quantitative bisulfite-PCR Pyrosequencing method was used to evaluate methylation of Alu and LINE-1. *Results*. We found a significantly lower RTL values in CLLs (median RTL=0.4 IQR 0.3-0.6) as compared with controls (median RTL=1.0 IQR 0.9-1.3) ($P < 0.001$). A progressive and significant RTL decrease in low (13q- and normal karyotype), intermediate (+12) and high (11q- and 17p-) cytogenetic risk categories (P for trend =0.008) was observed. Patients with IGVH mutated genes had longer telomeres than patients with unmutated genes ($P<0.001$). No significant association between telomere length and either CD38 or ZAP-70 expression was found. Telomere shortening was significantly correlated with hypomethylation of Alu (P=0.048) and LINE-1 (P=0.001), indicating a contribution to chromosome instability. Finally, follow-up analysis, available for 63 patients, showed a significantly higher risk of starting treatment for patients with shorter telomeres (P=0.037). *Conclusions*. Our results extended previous evidence that telomere length could be used as marker for the identification of CLLs with a different prognostic risk.

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MULTIPLE LIGATION-DEPENDENT PROBE AMPLIFICATION AND FLUORESCENCE IN SITU HYBRIDIZATION TO DETECT CHROMOSOMAL ABNORMALITIES IN CHRONIC LYMPHOCYTIC LEUKEMIA: A COMPARATIVE STUDY

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Background. Chronic Lymphocytic Leukemia (CLL) is a clinically heterogeneous disease characterized by recurrent chromosomal aberrations of prognostic significance. Although Fluorescence in situ Hybridization (FISH) is the most common technique used to detect these abnormalities, it still remains a quite expensive time-consuming method. *Aims*. We aimed to evaluate the potential of the novel Multiplex Ligation-dependent Probe Amplification (MLPA) technique, to detect genomic alterations in CLL. *Methods*. Highly purified (>90%) peripheral mononuclear CD19+ cell populations from 100 untreated CLLs in early stage disease (Binet stage A) were included in this study. All samples were investigated by fluorescence in situ hybridization (FISH) for the presence of trisomy 12 and 17p13.1, 11q22.3 and 13q14.3 deletions. For MPLA analysis, DNA was amplified by means of 2 commercially available probes sets allowing the simultaneous screening of 56 genomic sequences. *Results*. Overall, a high degree of concordance (95%) between MPLA and FISH results was found provided that abnormal clone was present in more than 30% of leukemic cell population. The use of multiple MPLA probes allowed the fine the mapping of the 13q14 deletion and the identification of intragenic or small alterations undetected by FISH. Moreover, additional alterations in 2p24 (MYCN) (3pts), 8q24 (C-MYC) (1pt), 9p21 (CDKN2A-2B) (1pt), 1q21 (LMNA) (1pt), and 6q25-26 (1pt) regions not covered by a standard FISH assay were detected and all confirmed by FISH. *Conclusions*. Our data extend previous limited evidence that MLPA may represent a useful technique to characterize well-known lesions as well as to investigate additional genomic changes in CLL.

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THE POTENTIAL ROLE INKT CELLS IN THE DEVELOPMENT AND PROGRESSION OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Backgroud and Aims. Chronic lymphocytic leukemia (CLL) is characterized by multiple and complex immune disorders. It was recently demonstrated that in addition to conventional T and NK cells, an important role in tumor immunity, plays a third type - natural killer T (NKT) cells. Invariant NKT (iNKT) cells are CD1d-restircted T cells which express invariant T-cell receptor (TCR) incorporating Vα24 chain paired with Vβ11 chain and NK cell markers. Human Vα24+ NKT cells play crucial roles in various immune responses including autoimmune and antitumor responses. Little is known however about the role of iNKT in patients with CLL. The aim of the study was to evaluate iNKT cells numbers in peripheral blood of CLL patients and their correlations with the other populations of immune cells and prognostic factors. Methods: We investigated iNKT cells numbers in peripheral blood of 60 untreated patients with CLL and 20 healthy individuals matched for age. iNKT cells were labeled with anti-Vα24 and anti-CD3 antibodies and analyzed using flow cytometry. *Results*. Analysis of Vα24 surface expression on CD3+ T cells revealed significantly lower median percentage of iNKT cells in peripheral blood of CLL patients (0.57%) than in healthy donors (0.80%; p=0.04) and significantly lower in patients in 3-4 stages according to Rai (0.08%) compared to the stage 0 (0.39%; p=0.04). There were also correlations between iNKT cells and populations of CD4+ (R=0,362; p=0,026) and CD8+ cells T (R=0,324; p=0,047), and inverse correlations between the percentage of iNKT cells and the percentage of CD19+CD5+ leukemic B cells (R=-0,372; p=0,021) and percentage of CD4+CD25high T regulatory cells (R=-0.506; p=0.03). Analysis of the relationship between iNKT cells and selected prognostic parameters showed inverse correlations between the percentage of iNKT cells and peripheral blood WBC (R=-0.378; p=0.03)

and lymphocyte count (R=471; p=0.04), β2-microglobulin serum level (R=-0.479; p=0.04) and the percentage of leukemic cells with the expression of ZAP-70 (R=-0.436; p=0.0.008) and CD38 (R=-0.374; p=0.004). The median treatment free survival (TFS) was significantly longer in patients with iNKT cell percentages higher than the median in the whole group (0,57%) as compared to patients with the lower percentages. *Conclusions*. Patients with CLL have lower percentages of Vα24 cells in peripheral blood as compared to age-matched healthy control. Moreover iNKT percentages decrease along with disease progression, correlate with CD4+ and CD8+ T cells and NK cells, and adversely correlate with Tregs as well as will negative prognostic factors. These results suggest an important role of iNKT cells in the development and progression of CLL, as well as their potential prognostic significance. Further studies involving larger groups of patients, and an assessment of iNKT cell function in patients with CLL are ongoing.

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TH17 CELL FREQUENCIES IN PERIPHERAL BLOOD OF PATIENTS WITH CLL ADVERSELY CORRELATE WITH T REGULATORY CELLS AND POOR PROGNOSTIC FACTORS

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Backgroud and Aims. Th17 cells have been defined as a subset of CD4+ lymphocytes, characterized by their production of IL-17 and expression of the transcription factor ROR? T. They have been implicated in inflammation and autoimmune diseases but still little is known about their prevalence and function in human cancer. In contrast to T regulatory cells, there are data showing both beneficial and harmful implication of Th17 cells in tumor development. In patients with chronic lymphocytic leukemia (CLL), many abnormalities in T cells populations have been described, but the data on TH17 cells are very limited. The aim of the study was to evaluate Th17 cells in peripheral blood (PB) of CLL patients and their correlations with the other populations of immune cells, such as: T regulatory cells, NK cells, iNKT cells and prognostic factors. Methods: Frequencies of Th17 cells among CD3+CD4+ T cells, were measured in 56 patients with CLL, and 20 healthy individuals matched for age. Peripheral blood CD4+ T cells were analyzed for intracellular expression of IL-17A, FoxP3, IL-2, IL-4, IL-10, IFNγ and TNF using flow cytometry. Th17 cells were defined as CD3+CD4+/CD17A+, T regulatory cells (Tregs) as CD4+CD25+FoxP3+; NK cells as CD3-/CD56+CD16+ and iNKT cells were counted as CD3+ Vα24+ cells. *Results*. Analysis of intracellular expression of IL-17A in CD4+ T cells revealed significantly higher median percentage of Th17 cells in peripheral blood of CLL patients (18.4%) than in healthy donors (3.28%; p=0.003) and significantly lower in patients in 2-4 stages according to Rai (4.48%) as compared to the stage 0 (36.16%; p=0.04). There were significant correlations between the percentages of Th17 cells and iNKT cells (R=0.556; p=0.04), and inverse correlation between the percentage of Th17 cells and T regulatory cells (R=-0.280; p=0.04), CD3+CD4+/IL-4+ (R=0.545; p=0.04) and CD3+CD4+/TNF+ T cells (R=0.592; p=0.01). The percentage of Th17 inversely correlated with β2-microglobulin serum level (R=-0.293; p=0.04) and the percentage of leukemic cells with the expression of ZAP-70 (R=-0.244; p=0.04) and CD38 (R=-0.333; p=0.01). In patients requiring therapy during observation period, median percentage of Th17 cells was significantly lower comparing to untreated ones (5.74% vs 10.97%, p=0.02). *Conclusions*. Percentage of Th17 cells in peripheral blood of patients with CLL was significantly higher than in age-matched healthy control, it decreased along with disease progression, correlated with iNKT cells, and adversely correlated with Tregs, CD4+ T cells expressing IL-4 and TNF as well as with negative prognostic factors. These preliminary results suggest that Th17 cells might be involved CLL pathogenesis, and higher percentage in patients with early stage CLL, may suggest that they take an important part in the control the leukemic cells growth in early, stable phase of CLL. Further studies are required to confirm these observations.

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IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH STEREOTYPED IGH4-39/IGHD6-13/IGHJ5 REARRANGEMENT PROGRESSION MIGHT BE CONSIDERED AS RICHTER'S TRANSFORMATION INDEPENDENTLY OF HISTOLOGIC CONFIRMATION

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Background. Among factors predictive for CLL transformation to diffuse large B-cell lymphoma (Richter's syndrome, RS), there is the usage of the IGHV4-39 gene with a stereotyped HCDR3 belonging to subset 8 (according to *Murray et al, 2008*). *Aims*. This study describes 3 CLL cases carrying IGHV4-39 with stereotyped HCDR3-subset 8 in whom transformation presented with a sudden and impressive increase in peripheral B-cell count. *Methods*. Diagnosis of CLL was based on clonal B-lymphocytosis > 5.00x10^9/L (phenotype score >3); ZAP70 and CD38 expression was assessed by flow cytometry; FISH analysis was performed using commercialy available probes (LSI13, LSID13S319, CEP12, LSIp53, LSIATM, and BCL3 split signal probe [Dako]); cytogenetic analysis was performed after CpG-oligonucleotide DSP30 and IL2 stimulation. IGHV mutational status was evaluated according to IMGT. Results: Among 408 unselected CLL patients, 15 (3.7%) carried the IGHV4-39 gene and among them 3 showed the IGH4-39/IGHD6-13/IGHJ5 rearrangement. In these pts a stereotyped HCDR3-subset 8 was detected. No other pts displayed this specific stereotyped HCDR3. At diagnosis, these 3 pts (all Binet stage A) showed similar biologic characteristics: IGHV unmutated status, ZAP70 and CD38 positivity, surface IgG (λ light chain in 1 and k in 2), and isolated trisomy 12. Two pts carried BCL3 translocation, occurring as a consequence of t(14;19)(q32.3;q13.2). First-line treatment was administered at 20, 23 and 8 mos from diagnosis: pt 1 and 2 (age >70 yrs and comorbidities) received mono-chemotherapy and achieved a partial response, while pt 3 (a 63-year-old female) received a fludarabine-cyclophosphamide combination and achieved a complete response with low-level minimal residual disease. Transformation (at 65, 43, and 45 mos from diagnosis) was characterised by a sudden increase in lymphocyte count to > 150.00x10^9/L after a period of stable disease. Morphologic evaluation revealed the predominance of large, lymphoma-like cells; enlargement of retroperitoneal lymph-nodes was detected in pt 2, but a biopsy could not be performed because of poor performance status. Splenomegaly was absent. LDH value was extremely high in all cases (from 6,300mU/ml to 42,900mU/ml). Bone marrow was extensively infiltrated by large B-cells. Cytogenetic re-evaluation detected del17p in pt 1, no clonal evolution in pt 2 and a highly complex karyotype (defined as ≥3 abnormalities) in pt 3. CD23 expression was reduced in pt 2 and 3, while CD5 and CD20 expression was maintained. In all cases, analysis of IGHV-D-J rearrangement confirmed that B-cells were clonally related to the CLL phase. Pt 1 died 2 mos after transformation while on treatment with Alemtuzumab; pt 2 is still receiving treatment; pt 3 experienced an incredibly poor clinical course and died of respiratory failure caused by multiple pulmonary infiltrates in a few days after admission. *Conclusions*. (i) In our experience, HCD3-subset 8 stereotypy was associated with an aggressive outcome characterised by a predominant involvement of peripheral blood and bone marrow. Independently of a pathologically-proven shift to lymphoma, progression in pts with this peculiar HCD3 stereotypy might be considered as a transformation to Richter's syndrome. (ii) In CLL the t(14;19) may cooperate with the stereotyped IGHV4-39/IGHD6-13/IGHJ5 rearrangement in causing aggressive evolution.

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EVALUATION OF TP53 MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA USING RESEQUENCING MICROARRAY PLATFORMS

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Background. TP53 abnormalities in CLL are currently considered to be a very important prognostic and predictive factor. In addition to the 17p deletion (10-15% patients), which is typically assessed, but also TP53 mutations have been confirmed to have negative impact on disease progression and response to chemotherapy. However, the most comprehensive method for mutational analysis is being discussed here. *Aims*. The aim of this project was to compare mutation detection by two microarray resequencing assays - Roche AmpliChip p53 Test (Roche Molecular Systems) and CLL custom resequencing Microarray (Affymetrix), with the functional analysis of separated alleles in yeast (FASAY) coupled to sequencing of colonies with mutated TP53. *Methods*. CLL patients harboring TP53 mutations detected by FASAY (53 mutations/46 samples) or gDNA sequencing (3 mutations/3 samples) were selected together with 12 samples previously assessed as wt-TP53 by FASAY. In all cases, the 17p

deletion was examined by I-FISH. All samples were analysed using two research assays based on an Affymetrix Microarray platform- (i) AmpliChip p53 Test (Roche) - a resequencing microarray designed for detection of single base substitutions and single base deletions in the coding and splice site regions of exons 2 - 11. (ii) CLL custom resequencing microarray (Affymetrix) containing probes for TP53 exons 2-11 and splice sites and for 9 other cancer-related genes; in particular, probes for 1nt substitutions in the whole coding region and a probe for a common 2-nt deletion in TP53 (del 2N 209) were included. For discordant cases, the particular exon was analyzed by Sanger sequencing. *Results*. In all 12 samples previously assessed as wt-TP53 by FASAY, no mutation was found by either microarray assay. Across all three methods, 64 mutations were detected in 49 samples - 49 (76,6%) substitutions (i.e. 41 (63,1%) missense, 5 (7,7%) splice-site, 3 (4,6%) nonsense mutations), and 15 (23,4%) ins/del mutations (including 3 one nucleotide deletions). However, only 35 (54%) mutations out of 64 were recognized by all methodologies - 32 substitutions and only 3 ins/>1bp del mutations. Both microarray assays reached the same number of recognized mutations. Irrespective of the FASAY results, each microarray detected 47 mutations out of 64 (73,4%), 41 of which overlapped. Vice versa, 10 additional mutations identified by microarrays were not detected by FASAY (2 missense, 2 nonsense, 4 splice-site mutations, two 2-nt deletions), 4 of which were not possible to be detected by gDNA sequencing. *Summary/Conclusions*. Microarray resequencing methods are less time-consuming than classical sequencing. AmpliChip p53 Test is more user-friendly, while the CLL custom resequencing microarray enables parallel mutational analysis of multiple genes. All tested platforms may fail to detect mutations present in low percentage of cells (approx. 15-25%). Ins/>1bp del mutations and one nucleotide deletions may not be detected by all methods, suggesting that no single methodology currently used for TP53 mutational screening is able to detect all present mutations. Therefore, further studies should be done in order to design the most suitable approach for detection of the majority of TP53 mutations in CLL patients.

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THE RECEPTOR TYROSINE KINASE ROR1 IS EXPRESSED BY LYMPHOID AND MYELOID HEMATOLOGIC MALIGNANCIES

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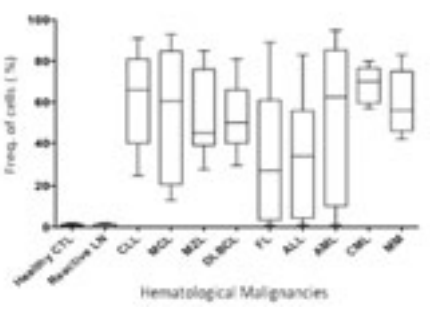
Background. ROR1 is a member of the receptor tyrosine kinase (RTK) family and related to muscle specific kinase (MUSK) and Trk neutrophin receptors. It is considered a potent survival kinase. ROR1 is of importance during embryogenesis and organogenesis and is not expressed on normal adult lymphoid and non-lymphoid tissues. Our published data showed an ectopic expression of ROR1 in all chronic lymphocytic leukemia (CLL) cases. A recent publication has described expression of ROR1 in hematologic malignancies of lymphoid origin. In the present study we have investigated the surface protein expression of ROR1 molecule in a series of patients with lymphoid and myeloid malignancies. *Aims*. To study the expression pattern of ROR1 in hematological malignancies of lymphoid and myeloid origins using a ROR1 monoclonal antibody recognizing the extracellular domain of ROR1.



Methods. 137 cases belonging to different hematological malignancies including 35 cases of CLL, 8 cases of mantle cell lymphomas (MCL), 7 cases of marginal zone lymphoma (MZL), 11 cases of diffuse large B-cell lymphoma (DLBCL), 30 cases of follicular lymphoma (FL), 16 cases of acute lymphoblatic leukemia (B-ALL), 12 cases of acute myelogenous leukemia (AML), 9 cases of chronic myelogenous leukemia (CML) and 9 cases of multiple myeloma (MM) were analysed by flow cytometry. Ten aged-matched healthy donors PBMC and 10 reactive lymph nodes were used as controls. *Results*. Our results showed a statistically significant variation in the expression of ROR1 in various hematological malignancies as compared to controls (no expression of ROR1 in PBMC of healthy donors or *reactive* lymph nodes). In flow cytometry, 35/35 of CLL cases were positive for ROR1 surface expression range of 25-91%, 8/8 MCL (13-93%), 7/7 MZL (28-76%), 11/11 DLBCL (30-81%), 20/30 FL (12-89%), 12/16 ALL (11-83%), 9/12 AML (32-95%), 9/9 CML (57-80%) and 9/9 MM (42-83%). *Conclusions*. ROR1 is widely expressed at the protein level in most types of lymphoid and myeloid malignancies but not on normal blood cells. These results suggest that ROR1 may be a candidate molecule for targeted therapy in various types of hematological malignancies

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REARRANGEMENT OF λ IMMUNOGLOBULIN LIGHT CHAIN IN CLL IS COUPLED WITH MIRNA-650 EXPRESSION

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Background. MicroRNAs constitute 3-5% of predicted genes in human genomes, and are located in the introns (~60%) or exons (~10%) of protein-coding genes, or outside of known genes (~30%). Although their important role in the regulation of protein-coding mRNAs was described, very little is known about the actual regulation of their expression. Interestingly, the locus for immunoglobulin λ light chain (IgL) in humans includes an annotated miR gene miR-650 (encoded by 4 members of V2 subgenes for IgL) (*Das, 2009*). This could be of particular importance because it is known that the biology of CLL is driven by processes that depend on immunoglobulin structure and microRNA expression (*Calin, 2005; Mraz, 2009*). Moreover, microRNAs are physiologically involved in regulation of IgG production and V(D)J recombination (*Vigorito, 2007; Koralov, 2008*). *Aims*. The aim of this study was to characterize the expression and function of miR-650 in CLL. *Methods*. We have defined the immunoglobulin λ light chain rearrangement in a cohort of 40 CLL patients using BIOMED-2 protocol and the expression of surface immunoglobulins was verified by flow cytometry. The qRT-PCR (TaqMan, ABI) was used to study the expression of miR-650 in this cohort. To assess the function of miR-650 cell lines (MEC-1, NALM-6) and CLL cells were electroporated with a RNA mimicing miR-650 (Dharmacon). This was followed by microarray expression profiling and western blot analysis 24/48 hours post transfection. *Results*. The analysis of IgL rearrangement by PCR showed that 51% patients (21/40) had a productive rearrangement in immunoglobulin λ light chain. Interestingly, the Real-Time PCR for miR-650 reported ~10 fold higher expression of miR-650 in CLL cells utilizing specifically V2-8, V2-5, V2-14, V2-23 subgenes for IgL (n=10) compared to samples utilizing different V lambda family (n=11) or expressing kappa Ig (n=19). These IgL subgenes of V2 family include homologues for miR-650. The coupled expression of miR-650 with IgL is remarkable because miR-650 was first identified in colorectal cells and it was believed to be regulated independently of immunoglobulin gene. Interestingly, the higher expression of miR-650 in our cohort was associated with statistically significant longer OS (161 vs. 173 months) and TFS (28 vs. 60 months). To elucidate the role of miR-650 in CLL we have transfected miR-650 into B-cells and assessed protein expressions of targets previously identified in solid tumors ING4 (Inhibitor of Growth 4) and CDK1 (cyclin dependent kinase 1) (Zhang, 2010; Chien, 2010) and also putative targets (EBF3, Bcl2, MDM2, MDMX, cyclin D1, pRb, Dicer). We were not able to demonstrate the regulation of any of these targets by miR-650 in B-cells. The microarray analysis of transfected cells revealed that the electroporation with miR-650 led to a down-regulation of dozens of potential target mRNAs, and the validation of the putative targets is currently in progress. *Conclusions*.

We have shown that the expression of miR-650 is coupled with the regulation and usage of variable subgenes for lambda immunoglobulin light chain and this has potential relevance for B cell biology.

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CIRCULATING REGULATORY T-CELLS IN *CLINICAL* MONOCLONAL B-CELL LYMPHOCYTOSIS

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Background. Monoclonal B-cell lymphocytosis (MBL) is a disease entity characterized by lower than 5000/μL circulating clonal B-cells in absence of other features of lymphoproliferative disorders. Regulatory T-cells (Tregs) constitute a small subset of cells involved in antitumour immunity and are generally increased in patients with chronic lymphocytic leukemia (CLL), for the diagnosis of which more than 5000/μL clonal B-cells in peripheral blood are required. *Aims*. To evaluate the number of circulating Tregs in patients diagnosed with *clinical* MBL and CLL and to compare it to clinical-biological features of the diseases. Methods. Tregs number have been detected, by means of multicolor flow cytometry (CD45/CD4/CD25/CD127), in the peripheral blood of 56 patients with *clinical* MBL (30 M/26 F; mean age 66.5 ± 9.7 years; range 44 - 86 years), 74 patients with previously untreated CLL (36 M/38 F; mean age 68.7 ± 12.5; range 35 - 90 years) and 40 healthy subjects (20 M/F 20; mean age 55.8 ± 14.3 years; range 30 - 81 years). *Results*. MBL patients showed a lower absolute number of Tregs (37.6/μL ± 38; range 4-154/μL), compared to CLL patients (70.7/μL ± 112; range 10-820/μL; p=0.0004), but higher than controls (27.3/μL ± 10.9; range 5-49/μL) without statistical significance. Noteworthy, there was not a higher number of CD4+/μL cells within the CLL subset compared to MBL (p=0.16). No significant correlation was found between Tregs number and CD38+B-cell/μL or ZAP-70. Moreover, the absolute cell number of Tregs directly correlated both with more advanced Rai/Binet clinical stages (p=0.02) and peripheral blood B-cell lymphocytosis (p<0.0001). Of note, the absolute number of Tregs was found lower within MBL patients than within CLL patients staged as 0/A Rai/Binet (p=0.02). *Conclusions*. This study showed that Treg cells are abnormally increased in *clinical MBL* patients compared to normal subjects, despite at a lower degree than in CLL patients. A significant direct relationship was also found with the tumor burden expressed by B-lymphocytosis. In light of these data, MBL seems to be a preliminar phase preceding the onset of CLL and the progressive increase of Tregs number might contribute to the evolution of MBL to overt CLL.

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NOD/SCID IL2RGAMMA(-/-) XENOGRAFT MODEL FOR CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. The transplantation of CLL into immunodeficient mice represents an attractive model for the studies of disease biology and drug resistance. Recently, Bertilaccio et al. (2010) developed a xenograft model by engrafting the CLL cell line MEC-1 into Rag2(-/-)gamma(c)(-/-) mice. These mice lack a functional receptor for interleukins and do not have functional T, B and NK cells, in contrast to NOD/SCID mice that retain NK cells. Several animal models utilizing NOD/SCID mice were also tested for engraftment of primary CLL (Shimoni,1997; Durig,2007). Unfortunately, the efficiency of the CLL engraftment in mouse models is not yet optimal. *Aims*. The aim of the study was to establish a CLL xenotransplant model using NOD/SCID IL2Rgamma(-/-) mouse (NSG). We hypothesized that the absence of functional T, B, and NK cells might enable sufficient engraftment of CLL cells. *Methods*. We used 31 non-irradiated NSG mice at age two months. The first group of mice was injected i.p. (n=5) and s.c. (n=5) with 5x10e6 MEC1 cells and sacrificed after 4 weeks. In the second group (n=14) mice were injected i.p. with Histopaque-separated 25x10e6 CLL cells. Cells from 3 patients were transplanted each in two mice and animals were sacrificed after 4 weeks. Cells from other 4 CLL samples were injected each in two mice and analyzed 4 and 6 weeks after the transplantation to assess the evolution in number of engrafted cells. Seven control mice were injected with PBS only. Samples of PB, spleen, liver, and bone marrow were analyzed by flow-cytometry and immunohistology for expression of human CD45/CD3/CD5/CD19/CD20/CD23. *Results*. The s.c. and i.p. injection of MEC1 cell line led to successful engraftment in all animals. The transplantation of MEC1 led to a significant weight loss, hepatomegaly, and massive splenomegaly in all subjects. Severe anemia, thrombocytopenia, and leukocytosis with the presence of immature human B-cells in the peripheral blood occurred in the transplanted animals. Immunohistological analysis revealed a massive focal infiltration of liver, spleen, and bone marrow by immature polymorphic human cells (CD19+, CD20+). Moreover, rapidly growing subcutaneous tumors occurred after s.c. injection of MEC1. In mice transplanted with CLL samples (n=7) flow-cytometry confirmed the engraftment with human CLL cells in all subjects after 4 weeks. The highest relative engraftment of CLL B-cells was observed in the spleen with an exception of 1 CLL sample where B-cells were present only in peripheral blood. The immunohistological analysis revealed a mild infiltration by CD20 positive cells in the spleen, liver, and bone marrow for 2 samples and massive infiltration by CD20+CD23- (B-cells) and CD3+5+ (T-cells) cells in spleen for 4 CLL samples. The detected B and T cells had high proliferative activity (80% of cells Ki67+). This corresponds with the observation of increased number of human cells for 3/4 of sequentially tested samples (in one case only T-cell counts increased). *Conclusions*. The use of NOD/SCID IL2Rgamma(-/-) mouse strain led to 100% engraftment efficiency for MEC1 and CLL cells making it a promising tool for CLL xenotransplant studies.

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IGHV UNMUTATED CLL B CELLS ARE MORE PRONE TO SPONTANEOUS APOPTOSIS AND DEPEND FROM THE ANTI-APOPTOTIC EFFECT OF ENVIRONMENTAL SIGNALS

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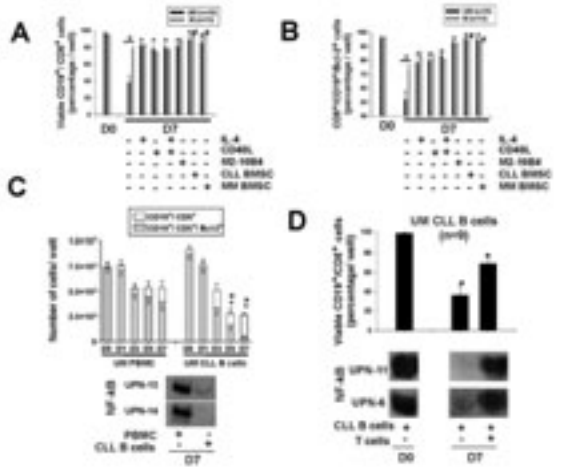
Background. Chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease. Patients with unmutated (UM) tumor immunoglobulin heavy chain variable regions (IGHV) have a worse prognosis than patients with mutated (M) IGHV. The tumor microenvironment [cytokines (i.e. IL-4 and CD40L) bone marrow stromal cells (BMSC) and nurse-like cells (NLCs)] are important survival factors for CLL B cells. *Aims*. Aim of this study is to assess whether and to what extent UM and M CLL cells differently depend on the local microenvironment for their survival. *Method.* M and UM CLL cells negatively selected by magnetic beads isolation were cultured in the presence or in the absence of IL-4, CD40L, murine stromal cells (M2-10B4), NLCs, CLL-derived BMSC and autologous, negatively selected T lymphocytes. Apoptosis an necrosis were evaluated by annexin V and propidium iodide (PI) staining. The intracellular expression of Bcl2 and the activity of NF-kB were evaluated by flowcytometry and by EMSA, respectively. Quantitative analysis of RelA and RelB NF-kB subunits was performed with the TransAM Flexi NF-kB Family assay kit (Active Motif, USA). CK and chemokines (CC) were measured using a multiplex suspension array system (Bio-Rad Laboratories S.r.l.) in culture supernatants. *Results*. Leukemic cells purified from the peripheral blood (PB) of UM patients (UM CLL B cells) showed a significantly higher apoptotic rate in 7-day cultures as compared to M CLL B cells. In both M and UM CLL B cells, high basal levels of Bcl-2 expression and NF-kB activity were detected. On day 7, Bcl-

2 and both RelA and RelB NF-kB subunits were significantly lower in UM than in M CLL B cells. CD40L, IL-4 and stromal cells significantly improved UM CLL B cells viability with a Bcl-2 dependent (Figure 1A-B), but NF-kB independent, mechanism. Interestingly, cell viability, Bcl-2 expression and NF-kB activity were preserved when cells were cultured as UM unfractionated PB mononuclear cells (UM PBMC) as compared to purified UM CLL B cells (Fig. 1C).



This observation suggested the presence of a pro-survival element in the PB of these patients. NLCs were not responsible of this prosurvival effect since, unexpectedly, NLC were defectively generated from the PBMC of UM patients, whereas they were abundantly generated by the PBMC of M patients. In spite of the lack of generation of NLC, leukemic cells viability was very similar in the non adherent fraction of M and UM PBMC. Conversely, autologous T cells played an essential role in supporting UM CLL B cells survival. Indeed,a significant NF-kB-mediated prosurvival effect was observed when purified UM CLL B cells were cultured in the presence of autologous purified T cells (Figure 1D). This prosurvival effect of circulating T cells was exerted both in cell-to-cell contact and in trans-well condition and was associated to increased secretions of TNF-α, PDGF-BB and IL-8. *Conclusions*. Despite their more aggressive features, UM CLL B cells are more susceptible to spontaneous apoptosis and their viability strictly depend from the presence of environmental prosurvival signals. This vulnerability of UM CLL B cells might be exploited as a selective target of therapeutic interventions.

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GENE EXPRESSION PROFILE OF AURKA AND AURKB IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): CORRELATION WITH CLASSICAL CYTOGENETIC (GTG) AND HEMATOLOGICAL PARAMETERS

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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. However, the majority of studies involving AURKA/B are addressed to cancer immunotherapy and no study has comprehensively examined the role of these genes in leukemogenesis. AIMS In the present investigation, we have evaluated AURKA and AURKB gene expression in peripheral blood (PB) cells of CLL patients, by real time quantitative PCR and correlate the findings with hematological parameters established for CLL and classical cytogenetics METHODS Sixty two CLL patients (23 female and 39 male) and 10 age-matched hematologically healthy donors were selected for our study. The majority of the patients were classified as Binet A (63%). The comparative analysis of AURK/B expression of leukemic and normal samples was calculated as a relative quantification to the GAPDH gene. Moreover, AURKA/B gene expression from leukemic samples was calculated as relative quantification to normal CD19+ cells from healthy donors and expressed as 2-ΔΔCt. The 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 (AURKA/B) were validated by FISH analysis with a specific set of probes. RESULTS According to median value of AURKA/B expression, patients were divided into two groups (≥ 3.4, considered as AURKA+ and > 2.3, considered as AURKB+) and their clinical and biological characteristics were correlated. Higher AURKA/B expression were observed in CLL samples compared with PB normal samples (AURKA [mean value of ΔCt ± SD]: 0.093±0.003 vs 0.071±0.001, p=0.02; AURKB: 0.166±0.01 vs 0.09±0.002, p=0.02). Moreover, AURKA/B+ patients presented a significantly high leukocyte count compared with AURKA/B- patients (AURKA [WBC countx103 ± SD]: 37.8±5.5 vs 68.8±5.8, p=0.0003; AURKB: 40±5.5 vs 66.6±6.2, p=0.0023, respectively). However, no significant differences were found regarding to Binet classification, gender or platelets count. In addition, Pearson correlation showed that there is a significant association between high expression of AURKA/B and complex karyotype (relative risk: 2.4 [95%CI: 1.46-3.93], p < 0.001). 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%). CONCLUSION In this investigation we demonstrated a significant correlation among high expression levels of AURKA/B genes in CLL with chromosomal abnormalities and other hematological parameters. Overexpression of aurora kinase genes have been extensively studied in solid tumors. In CLL this observation 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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PERSISTENT POLYCLONAL BINUCLEATED B-CELL LYMPHOCYTOSIS (PPBL): IDENTIFICATION OF A SPECIFIC CYTOGENETIC PROFILE AND A LONG TERM FOLLOW UP OF 151 PATIENTS

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Background. PPBL is a rare disease characterized by a chronic, stable, persistent and polyclonal B-cell lymphocytosis, the presence of binucleated lymphocytes in the peripheral blood and a polyclonal increase in serum immunoglobulin-M. *Aims*. We report here the cytogenetic characteristics and the long-term follow-up of 151 patients with PPBL. Moreover, a pangenomic study of 10 patients using the most resolutive cytogenetic method based on SNP array was performed in order to study more extensively cytogenetic abnormalities in PPBL. *Methods*. PPBL was diagnosed in 151 patients. Conventional cytogenetic analysis (CCA, n=139), fluorescent in situ hybridization (FISH, n=129) and SNP array were performed at diagnosis and during the course of PPBL. We used Affymetrix® Cytogenetics Whole-Genome 2.7M Arrays® in 10 typical PPBL patients. The DNA was extracted from peripheral CD19+ B-cells and CD19- cells purified using Miltenyi® technology (AutoMACS Pro Separator®). *Results*. PPBL was diagnosed in 26 male and 125 female patients, with a median age of 40 years (18.9-66.2). At diagnosis, supernumerary isochromosome 3q (+i(3)(q10)) was detected in 82/139 patients (59%) using CCA and FISH. PCC was detected as a sole abnormality in 7/139 patients (5%). No abnormality was identified in 50/139 patients (36%). Chromosomic instability was observed in 77/139 patients (55%) at diagnosis and persisted during PPBL follow-up. SNP arrays were performed in 10 patients (3 male, 7 female). CD19+ B-cells were separated by immunomagnetic cell sorting. We performed DNA arrays on CD19+ and CD19- cells in 7 patients, CD19+ cells in 2 patients and CD19- cells in 1 patient. Recurrent gene copy number (GCN) gains were detected on the long arm of chromosome 3 (3q) and identified only in CD19+ cells. The size of GCN gains was variable between patients, from thirty kilobases to the whole 3q. GCN gains did not involve all the CD19+ B-cells (mosaicism phenomenon). Non recurrent GCN aberrations involving the whole genome confirmed the presence of genetic

instability in all patients. Out of the genes amplified on 3q of CD19+ cells, we identified with a high frequency (7/9 patients) partial or complete amplification of one particular gene. After a median follow-up of 34 months (1-347), six cases of IgM MGUS were observed. Three patients developped solid cancers (2 pulmonary cancers and 1 cervical carinoma). Three patients developped diffuse large B-cell lymphoma (DLBCL), two patients a splenic marginal zone lymphoma (SMZL). *Conclusions*. Isochromosome 3q has been implicated in the progression of cervical carcinomas. In addition to MGUS cases, we report here 5 cases of lymphomas developped during the course of PPBL. The clinical follow-up with genetic instability and the presence of recurrent cytogenetic abnormalities on 3q led us to consider PPBL as a premalignant state and require a carefully long-term follow-up of PPBL patients.

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MUTATIONAL STATUS AND GENE REPERTOIRE OF IGHV-D-J REARRANGEMENTS IN SERBIAN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background. The mutational status of immunoglobulin heavy variable region (IGHV) genes is the most stable molecular prognostic marker in chronic lymphocytic leukemia (CLL). It defines two CLL subtypes, mutated (M-CLL) and unmutated (U-CLL), with significantly different clinical course. M-CLL cases usually present a non-progressive disease and have considerably longer survival than unmutated cases, who tend to have a progressive disease. Biased IGHV gene usage in CLL vs. normal B lymphocytes as well as in mutated vs. unmutated CLL cells, and the expression of almost identical, "stereotyped"heavy complementarity-determining region 3 (VH CDR3) sequences among some patients implies the role of specific antigens in the pathogenesis of the disease. Furthermore, there are population differences in mutational status and IGHV gene usage which may reflect different genetic background and/or effect of environmental factors. *Aims*. The aim of this study was to analyze the mutational status and IGHV gene repertoire in Serbian CLL patients. It included 85 CLL patients, 57.4 % with progressive and 42.6 % with non-progressive disease. *Methods*. IGHV-IGHD-IGHJ rearrangements were amplified by multiplex RT-PCR using 5' primers specific for leader sequences of IGHV gene subgroups, in conjunction with 3´ primers complementary to IGHJ genes. Clonal RT-PCR products were sequenced and analyzed using the ImMunoGeneTics database. *Results*. Overall 88 alleles have been analyzed, since 3 patients (3.5%) expressed biallelic rearrangements, both of the same mutational status (mutated). We found that 56.8% of rearrangements carried mutated IGHV genes, whereas 43.2% carried unmutated IGHV genes. Among M-CLL patients, 44.7% presented a progressive disease, in contrast to 73.3 % among U-CLL patients. Comparison of PFS means revealed a significantly longer PFS in mutated cases ($p<0.01$). The most frequent IGHV subgroup was IGHV3 (55.7%) followed by IGHV1 (27.3%), IGHV4 (12.5%), IGHV5 (2.3%), IGHV2 (1.1%) and IGHV6 (1.1%). No IGHV7 subgroup members were identified. IGHV3 subgroup genes were found predominantly in mutated rearrangements (69.4% vs. 30.6%), in contrast to IGHV1 subgroup genes which were predominantly unmutated (70.8% vs. 29.2%). A total of 29 IGHV genes were identified, 7 of them accounting for 56.9% of all rearrangements. The most frequent were IGHV3-23 (14.8%), IGHV1-69 (10.2%), IGHV3-33 (8%), IGHV1-2 (6.8%), IGHV1-18 (5.7%), IGHV3-30 (5.7%) and IGHV3-48 (5.7%). GHD genes showed the following distribution: IGHD3 - 38.4%, IGHD2 - 22.1%, IGHD6 - 12.8%, IGHD1 - 10.5%, IGHD4 - 8.1%, IGHD5 - 7% and IGHD7 - 1.2%. The most frequent IGHJ gene was IGHJ4 (48.9%), followed by IGHJ6 (28.4%), IGHJ3 (11.4%) and IGHJ5 (11.4%). No IGHJ1 and IGHJ2 genes were identified. IGHJ4 was used preferentially in mutated (72.1%) and IGHJ6 in unmutated rearrangements (80%). There was a significantly lower median VH CDR3 length in mutated (15 a.a.) vs. unmutated (21 a.a.) cases ($p=<0.001$). *Conclusions*. Our study showed strong correlation between IGHV gene mutational status and clinical course of CLL. Progressive disease was observed predominantly in patients expressing unmutated rearrangements, while non-progressive disease predominated among M-CLL patients ($p=0.026$). Frequencies of IGHV subgroups and their mutational status resembled those observed in other Mediterranean countries, with exception of IGHV4 subgroup genes which were underrepresented in our cohort.

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THE INTERPLAY BETWEEN PKC α AND PKC β II IN CLL

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Background. PKCα and PKCβII belong to a classical subfamily of serine/threonine protein kinases that are involved in regulation of proliferation, differentiation, cell migration and apoptosis in normal cells. These two enzymes are structurally similar and are activated by phospholipids, diacylglycerol (DAG) and Ca2+ binding. In a number of cancers including breast, prostate, brain and gastric cancer, PKCα has been implicated as a tumour promoter. However, in other cancers such as colon, thyroid, pituitary and pancreatic cancer, it serves a tumour suppressive role. Thus it is important to delineate the particular function of PKCα in specific cancers in order to better direct therapies. PKCβII plays an important role in regulating signals generated by the BCR, and has been shown to be overexpressed in CLL compared to normal mature B cells, correlating with a worse overall prognosis. In the current study, we explore the relationship between these two isoforms in CLL. *Aims*. Although the expression profile of PKCβII has been associated with a more aggressive CLL phenotype, its function in the generation and propagation of CLL remains largely unclear. Here we characterise the role of PKCα and PKCβII in CLL by investigating the signalling mechanisms associated with their expression. *Methods.* Murine CLL-like cells were generated from stem/progenitor cells of wild type ICR mice by retroviral introduction of kinase inactive PKCα construct, and subsequently maintained in an *in vitro* B-cell supportive system or injected into RAG-/- mice for *in vivo* studies. For translational significance, human CLL cells were isolated from peripheral blood of diagnosed patients and subsequently co-cultured in a microenvironment mimicking that of circulating and proliferative compartments. *Results*. Our studies indicate that PKCα acts as a tumour suppressor in CLL, and its subversion leads to an upregulation and sustained overexpression of the PKCβII isoform. Importantly in human CLL, we show a decrease in PKCα transcript and protein levels in more than half of CLL cases studied, complimented by an increase in PKCβII transcript and protein levels. In addition, the increase in PKCβII expression positively correlates to increases in tumour-associated ERK and mTor signalling. Finally we identify cyclin D1 to be overexpressed in our mouse model of CLL and in human CLL, which, alongside the implicated signalling pathways leads to cells that have a survival and proliferation advantage over normal B cells. *Summary*. Although structurally similar, PKCα and PKCβII serve very different and significant roles in CLL. The downregulation of tumour suppressor PKCα results in an upregulation of PKCβII coupled with increased oncogenic signals, contributing to the malignant phenotype of CLL.

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CD38 RS6449182 POLYMORPHISM IS ASSOCIATED WITH OVERALL SURVIVAL IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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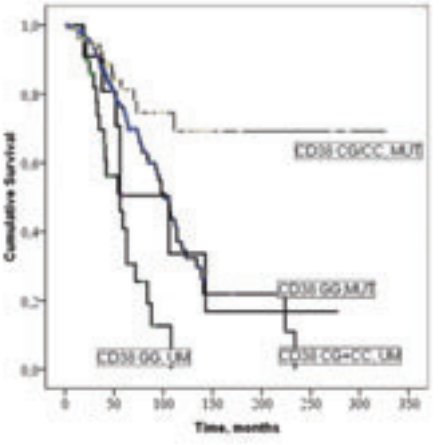
Background. CD38 antigen is well-established independent marker of poor prognosis in B-CLL. CD38 molecule is thought to be also one of the key pathogenetic elements in the disease development, which is involved in delivering of growth and survival signals in leukemic cells. The single nucleotide polymorphism 184C>G in the regulatory region of CD38 gene (CD38 rs6449182 SNP) has recently shown to affect CD38 expression. The association of variant G allele with some poor prognosis markers, advanced clinical stage and elevated risk of Richter transformation was demonstrated, the association between CD38 polymorphism and CLL risk has been questioned. *Aims*. Aim of our study was to evaluate if CD38 rs6449182 polymorphism influence the risk of CLL development and whether it provide prognostic information on the clinical outcome of the disease. *Methods*. PCR-RFLP analysis was used for CD38 rs6449182 genotyping: genomic DNA was amplified in PCR using primers designed by *Aydin et al*., 2008, with following enzymatic digestion of PCR products. The digested products were resolved on 3% agarose gel and analyzed. Results: SNP was studied in a total of 304 CLL patients and in the group of 217 control individuals. The median follow-up from diagnosis in CLL patients was 4.1 years (range 0.1-27

years). The majority of patients were at Binet A or B stage at diagnosis (45% and 40% correspondently), 70% cases were IgVH unmutated. We found that the frequency of the variant homozygous CD38 rs6449182 GG genotype was significantly higher in CLL group in comparison with control (14.8% vs 6.9%, P=0.012).



The distribution of heterozygous CD38 rs6449182 CG genotype did not differ significantly between groups. The variant G allele frequency was found in CLL group and controls as 0.30 and 0.26 (P=0.03). The highest CD38 rs6449182 homozygous GG genotype frequency was observed in group of 29 patients who developed Richter syndrome (RS) during follow-up period (34.5%). It differed significantly when compared both with the CLL cohort without RS (12.7%), and with treated CLL patients without RS during follow-up at least 2 years (11.8%), P<0.05. The distribution of heterozygous CG genotypes in these groups was comparable. The overall survival (OS) of patients with GG genotype was significantly shorter in comparison with those with at least one wild C-allele: for the entire CLL cohort (median 56 vs 111 month, P=0.0001), for Binet A/B stage patients (median 59 vs 123 month, P=0.0001), for CLL group with unmutated IgVH status (median 56 vs 101, P=0.0001), and for patiens with mutated IgVH genes (median 106 vs did not reach , P=0.019) (Figure1). We failed to found some significant correlation between the CD38 rs6449182 genotypes and other relevant prognostic factors such as Binet stage, IGHV mutation status, or CD38 expression. *Conclusions*. Our results suggest that CD38 rs6449182 SNP may be a factor predisposing to CLL. The variant homozygous CD38 rs6449182 GG genotype identify poor risk CLL group concerning OS and RS transformation.

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IMPAIRED ANTIGEN PRESENTATION AND POTENT PHAGOCYTIC ACTIVITY IDENTIFYING TUMOR-TOLERANT HUMAN MONOCYTES: DEMONSTRATION IN ISOLATED CELLS FROM CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Monocyte exposure to tumor induces a transient state in which these cells are refractory to further exposition to cancer. This phenomenon, termed "tumor tolerance", is characterized by a decreased production of proinflammatory cytokines in response to tumors. *Aims*. Identify tumor tolerance phenomena in monocytes from Chronic Lymphocytic Leukemia patients and their relationship with prognostics markers. Methods: Samples of peripheral blood from CLL patients were procesed for monocyte isolation and phagocytic activity was measured. Tumor lymphocytes were cocultured with control monocytes and induced tolerance status was measured. Results: We have established a human model of tumor tolerance and have observed a marked downregulation of MHCII molecules as well as the MHCII master regulator, CIITA. These cells combine an impaired capability for antigen presentation with potent phagocytic activity. We also show that circulating monocytes, isolated from patients who suffer from mature B-cells neoplasm, share all the determinants that characterize cells locked in a tumor tolerant state. In addition, lymphocytes from these patients and supernatants from their culture induced a tumor tolerance state in controls monocytes. *Conclusions*. Monocytes from CLL patients are locked in a tumor tolerant state with a lower capability of antigen presentation and therefore an impaired immune response.

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ANALYSIS OF POTENTIALLY BICLONAL CLL PATIENTS WITH TWO OR THREE PRODUCTIVE IGH REARRANGEMENTS

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Background. Majority of chronic lymphocytic leukemia (CLL) patients develop a monoclonal disease and thus can be characterized by single immunoglobulin heavy chain gene (IGH) rearrangement. Interestingly, double productive IGH rearrangements can be sporadically detected with an incidence about 2-4% and can indicate simultaneous biclonal/biphenotypic disease. Malignant subclone diversification due to BCR receptor editing or monoclonality with a lack of IGH allelic exclusion has been previously described as other possible reasons of this phenomenon. Here we present the detailed analysis of patients expressing two/three functional IGH rearrangements who were examined for IGHV mutational status. Aims: We performed a detailed characterization of selected CLL cases with double/triple productive rearrangements and followed their clonal evolution during course of the disease. *Methods*. B-lymphocytes were separated from peripheral blood using Ficoll-Paque PLUS (GE Healthcare) and RosetteSep Kits (StemCell). IGHV status was assessed according to the ERIC recommendations and Ig light chain (IGL) utilization was examined using cDNA as an input material. At DNA level, clonal IGH and IGL rearrangements were confirmed by BIOMED-2 protocol and also unproductive rearrangements were analyzed. Immunophenotypization was performed on separated CD19+ cells using panels of monoclonal antibodies including those against CD19/IgLambda/IgKappa, CD19/CD20/CD23, CD19/CD43/FMC7. Other prognostic markers, such as chromosomal alterations and TP53 mutational status, were available in all cases. *Results*. Double/triple productive IGH rearrangements were in total detected in 35 patients comprising 3,36 % of our cohort. Ig light chains (IGL) utilization was assessed and according to the number of double productive IGL rearrangements, 18 patients were considered as biclonal CLL. Interestingly, in nine patients, alterations in the proportion of IGH rearrangements were observed during the disease course. In three patients, selection of other clone was accompanied by unfavorable de novo TP53 mutation detection. Out of these 35 patients expressing multiple in-frame IGH rearrangements, 12 of them (34%) were further analyzed in three subsequent samples per patient in average. At DNA level, all productive rearrangements were confirmed; out-frame IGL rearrangements were found in nine cases (75%). Immunophenotypization revealed two separated populations in five cases (42%) differing mainly in Kappa and Lambda IGL that corresponded with PCR results and were considered as true biclonal cases. In one patient, biclonality was presumed while two in-frame IGL Lambda were present. Based on bioinformatics analysis, BCR editing was excluded in all 12 patients because no pair of IGH rearrangements shared D-J junction. *Summary/conclusions*. Biclonal CLL is a very infrequent phenomenon representing approximately 50% of cases with double productive IGH rearrangements. We performed particular characterization of 12 potentially biclonal patients. Six of them were biclonal at least in one time-point during their course of CLL according to immunophenotype and/or PCR. In several cases, proportion of particular populations changed in time. BCR editing was excluded in all these patients. We conclude that subsequent monitoring of CLL patients and repeated IGH rearrangement assessment is necessary for understanding of malignant disease evolution. Further analysis of these patients is in progress.

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GENOMIC ABNORMALITIES DETECTED BY MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) MAY PRECEDE THE CLINICAL ONSET OF CHRONIC LYMPHOCYTIC LEUKEMIA BY SEVERAL YEARS

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Background. Chronic lymphocytic leukaemia (CLL), the most common leukaemia in adults in Western countries (Linch, 1992), is a biologically heterogeneous disease with a variable clinical course and many aspects of the pathogenesis are awaiting final clarification. B lymphocyte monoclonal rearrangements may last for longer than 7 years before the disease becomes clinically detectable (Frezzato 2005, Landgren 2009) but the triggering events leading to the disease progression are largely unknown. No data are available on the time of the first onset of the cytogenetic abnormalities detected by Fluorescence In Situ Hybridization (FISH) in more than 50% of cases at diagnosis (Dohner, 2000). *Aims.* To identify genetic abnormalities in healthy subjects, enrolled in a prospective population based investigation, who subsequently developed CLL. *Methods.* Six patients with a diagnosis of CLL, according to standard morphologic and immunophenotypic criteria (Cheson 1996) have been identified in a cohort of 14396 healthy subjects who had been enrolled during years 1993 - 1996 in an ongoing prospective clinical survey also providing DNA samples preservation. FISH analysis has been performed in all these cases as part of the diagnostic work-up for CLL. We performed Multiplex Ligation-dependent Probe Amplification (MLPA) analysis (an emerging new tool apt to detect some genomic abnormalities with high sensitivity and specificity) (Buijs, 2006; Al Zaabi, 2010) in all these subjects as well, after an informed consent was obtained. If a chromosomal abnormality was detected (by FISH and/or MLPA) we performed MLPA on DNA preserved samples collected at the enrolment in the clinical survey, 54 to 89 months before diagnosis. Interphase FISH with SEC63 (6q21), C-MYC (8q24), ATM (11q22), GLI (12q13), DLEU1 (13q14 and p53 (17p13) probes was performed on peripheral blood samples according to the ISCN criteria (Brothman, 2009). DNA was analysed with MLPA P040 test kit (MRC Holland), including set probes for 11q23 (ATM), 12p12.3-12p13, 12q14-12q24.3, 13q14.2 (RB1), 13q14.3 (KCNRG-ATP7B), 17p13.1 (p53) chromosomal regions, according to the manufacturer's protocol. *Results*. FISH analysis at diagnosis showed a del:13q14 in 3 subjects (nuclear positive interphases: 90% in 2 cases; 10% in 1 case). MLPA result was consistent with FISH only in the two patients with 90% deletion. In one of them MLPA revealed the same deletion to be present 54 months before diagnosis (see Table for details). *Conclusions*. We firstly report a CLL-associated genetic deletion detected a long time before the clinical diagnosis. The deletion includes the DLEU1 locus, proposed as the most likely candidate CLL-associated tumour suppressor gene (*Wolf, 2001; Ouillette, 2008*). We have been able to evaluate only a few cases but our report could prompt future investigations on the role of different genetic abnormalities and their meaning in the pathogenesis of CLL

Sex Age	FISH at diagnosis % interphases	MLPA at CLL diagnosis RCN	MLPA at enrolment RCN	Enrolment to CLL diagnosis Months
F 65	del 13q14 90%	del 13q14 (KCNRG-DLEU7) RCN: 0,63	del 13q14 (KCNRG-DLEU7) RCN: 0,73	54
M 56	del 13q14 10%	Neg	Neg	83
F 62	del 13q14 90%	del 13q14 (KCNRG-DLEU2) RCN: 0,55	inadequate material	89

RCN: relative copy number

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CHARACTERIZATION OF CLL CASES BY I-FISH, IGHV STATUS AND CD38 EXPRESSION: A SINGLE CENTER STUDY

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Background. Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with a variable clinical course. *Aims*. Here we report the characterization of a cohort of 90 CLL patients from central Sardinia using an integrated diagnostic laboratory work-flow including immunophenothyping, cytogenetics and IGHV mutation status. *Methods*. Ninety CLL patients (M 54, F 36, age 41-90) were diagnosed between 2007-2010 by standard criteria . FISH analysis on interphase nuclei with probes for the detection of trisomy 12, 11q22.3 (ATM), 13q14 (D13S25 and D13S319) and 17p13 (TP53) was perfomed in peripheral blood cells. In a subgroup of 30 CLLs, peripheral blood was cultured in the presence of the immunostimulatory CpG-oligonucleotide DSP30/Interleukin-2 (IL2) and RNA was analyzed for IGHV mutation status following the Biomed-2 protocol. Sequence data were analyzed using the International Immunogenetics database (IMGT, http://imgt.cines.fr). The IGHV status was assigned as mutated with a cut-off of 2%. Expression of CD38 was analyzed on FACScan flow cytometry using direct conjugate antibodies (anti-CD38-FITC/anti-CD19-PE) and positivity was assigned with a cut-off of 20%. *Results*. All patients included in the study could be successfully analyzed by interphase FISH. Trisomy 12 was detected by FISH in 15,5% (14/90) patients, 13q14 deletion in 40/90 (44%), TP53 deletion in 12% (11/90), while the 11q deletion was observed in 7 patients (7,7%). Correlation between CD38 expression and FISH aberrations showed del13q14 in 40% of the CD38-negative CLLs while TP53 was present in the 74% of the CD38-positive subgroup. The status of somatic mutations in the IGHV gene was available in 45 cases. Twentysix (58%) displayed an unmutated IGHV status, while the remaining 19 cases (42%) had a mutated IGHV gene. The IGHV gene family usage within the mutated subset was VH4>VH3>VH2> VH1>VH7, whereas IGHV3 was the most frequently used in the unmutated group, being expressed in 13 patients. IGHV1-69 genes were present in 3 CLL patients overall (2 unmutated). Correlation between cytogenetic categories and mutation status showed that the poor prognosis marker TP53 deletion was present in 3/26 (11,5%) of unmutated CLLs and in 2/19 (10%) of mutated cases. CD38 expression was detected in the 43% and 28% of the mutated and unmutated cases respectively, and showed a tendency to correlate with cytogenetic abnormalities. According to age, TP53 and 13q14 deletions (21% and 60%, respectively) were more frequently detected in CLL cases diagnosed before age 55 when compared to older patients. When clustering analysis was performed, only 8 out of 45 sequences were assigned to a specific subset according to Murray et al, while 13 sequences, all unmutated, clustered based on the pattern they shared following the TEIRESIAS algorithm. This cluster included stereotype rearrengements in the IGHV3-21, IGHV3-11 and IGHV1-69 with the longest HCDR3 sequences. *Summary*. We found that cytogenetic adverse prognostic markers were more frequently detected among younger CLL patients and the ratio of unmutated/mutated patients is higher than in other population. We have a low incidence of IGHV3-21 and IGHV1-69 in Sardinian CLLs suggesting that the frequency of specific IGHV CLL may be related to geographic, ethnic, or environmental background.

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GENE EXPRESSION PROFILE IN PATIENTS WITH CLL TREATED WITH CLADRIBINE AND CYCLOPHOSPHAMIDE REGIMEN

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Background. An accumulation of CD5/CD19/CD23 positive cells in bone marrow and peripheral blood due to their resistance to apoptosis is a key event in chronic lymphocytic leukemia (CLL). Therefore, apoptotic gene expression could be essential to understanding the background of the disease. The microarray technique is one of the most innovative and powerful tools, providing a wealth of information on gene expression which can be helpful in identification of diagnostic and prognostic factors and than can lead to a selection of the most appropriate therapy. Cladribine and cyclophosphamide (CC) is a safe and very effective drug combination used in the first line therapy for CLL patients. *Aims.* The aim of this study was to investigate the gene expression profile in CLL cells with regard to factors involved in the apoptosis cascade in patients treated with CC protocol. *Methods*. The study involved 8 CLL patients who were previously untreated. All the patients were diagnosed and followed at the Department of Hematology, Medical University of Lodz, Poland, according to the IWCLL 2008 criteria. The study received the approval of The Ethical Committee of Medical University of Lodz (Dnr. RNN/196/07/KE). Informed consent was obtained from all the patients. Fresh blood samples were collected from all the enrolled patients before and after 2 weeks of the first cycle of CC treatment

(cladribine 0.12mg/kg - days 1-3, cyclophosphamide 600mg/m2 - days 1-3; q 4 wks × 6 courses). Mononuclear cells (MNCs) were separated according to the standard protocol and used for further studies. The gene expression profiling was measured by means of microarray method (TaqMan Low Density Array, Human Apoptosis Panel). This microarray method was based on real-time PCR technique. Ninety six transcripts were placed on the array, 3 of which were endogenous controls. Measurements were conducted in duplicates. The relative expression of each gene was quantified by the comparative cycle treshold (Ct) method (ΔΔCt), using 18S as an endogenous control. Fold change (RQ) for each gene was evaluated. RQ is the differential expression after treatment to gene expression before treatment. For significant fold change the value of 2 or greater than 2 was chosen. *Results.* Data analysis highlighted 8 out of 93 examined apoptotic genes whose expression was significantly important. 5 genes (BIRC 1, BIRC5, BIRC8, CARD6, HRK) form a cluster by means of average linkage method. The most significant differences in gene expression before, against and after treatment are demonstrated by antiapoptotic genes such as: BIRC1, BIRC5, BIRC8 whose expression decreased. However, the expression of 5 proapoptotic genes such as: PUMA, CARD6, HRK, APAF1 and TNFRSF10B (TRAIL-R2) significantly increased. *Conclusions*. Our results show that the treatment with CC regimen leads to overexpression of genes involved in the intrinsic pathway of apoptosis. Nevertheless, further studies into the clinical usefulness of this observation for the development of new therapeutical strategies are vital.

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USEFUL OF MONOCLONAL AND POLYCLONAL SERUM FREE LIGHT CHAINS AS A PREDICTIVE BIOMARKER OF PROGRESSIVE DISEASE IN A SERIE OF B-CLL PATIENTS: EPIGEN STUDY

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Background. The B-CLl, is the most common type of leukaemia in the West countries. Several immunophenotypic, genetic and molecular markers allow their inclusion in different prognostic groups, recently serum free light chain (FLC) quantitation have shown abnormalities in 39-54% of CLL patients and this marker is associated with shorter time to treatment (TTT) and inferior overall survival (OS). *Objectives*. To determine FLC abnormalities in a prospective cohort of CLL patients (EPIGEN project) and explore the effects of both monoclonal and polyclonal FLC elevations in the outcome of CLL patients. *Material and Methods*. A prospective, descriptive, and analytical and cross section of incidental cases of B-CLL in Aragon, Navarra, Basque Country and Cantabria during the period: 1/09/2007- 31/08/2008. The study involved 13 hospitals that have collected the clinical characteristics at diagnosis and biological samples for DNA, RNA and serum extraction stored in the Biobank. The study has the approval of the Committee on clinical trials and investigation of Aragon (CEICA) and patients have signed IC. We designed a database for the study where the variables are collected: demographic, staging (Rai / Binet), lymphocyte count, immunophenotyping (IP) (CD38, Zap70), genetic testing (CG) and molecular (Ig VH genes mutation), esearch serum samples using the Freelite FLC assay (The Binding Site, Ltd., Birmingham, UK). Elevated FLC was defined as either kappa or lambda above the reference range (k>19.4 mg/L, l>26.3 mg/L). Monoclonal FLC elevation was defined as elevated FLC with an abnormal FLC ratio. Polyclonal FLC elevation was defined as elevated FLC with a normal FLC ratio (0.26-1.65). Follow-up to progression, development of primary tumors and response to treatment were analyzed. RESULTS: We collected data from a total of 96 new patients. The analysis includes 61 cases: men (H) 31 (50.8%) females (M) 31 (50.8%). Average age: 70.8 years (41-91) Males: 63.3 years and Females: 71.6 years. 52.4% were older than 70 years. Staging at diagnosis (Binet and Rai): A0 68.8%, 13.1% AI, AII 3.2%, 1.6% BI, BII 8.1%, BIII 3.2%, 3.2% CIV. 13.1% starts with splenomegaly. 29.5% had no lymph node involvement or extranodal affectation. CG: del13q (18.0%), trisomy 12 (18.0%), del11p (6.5%), del17p (1.6%), normal (45.9%). IP: CD38 positive (22.9%), Zap70 positive (16.3%). Ig VH mutated (21.3%). Abnormalities in FLC were present in 48% of the CLL patients. In 35.0% elevated kappa or lambda was observed, 20.0% had a monoclonal FLC and 15.0% had a polyclonal FLC elevation. Monoclonal FLC elevation was associated with high risk features of CD38+, CD49D+, ZAP70+, IGHV unmutated and having high risk FISH (del 17p13; del 11q22). *Conclusions*. The more common genetic aberrations were: del13q and trisomy 12. Normal kariotype apparently is related to early stages, absence of spleen enlargement and B symptoms, typical morphology and predominance of IgVH gene mutation and CD38 and Zap70 negatives. The analysis of FLC at diagnosis could be a predictive biomarker in CLL.

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MATURATION OF THE MIR15A/MIR-16-1 FAMILY IS IMPAIRED IN CHRONIC LYMPHOCYTIC LEUKEMIA

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A role as tumor suppressor has been proposed for the miR15a/miR-16-1 family of miRNA genes localized in a critical region in 13q14 that is deleted in more than 50% of CLL patients. These two miRNA genes regulate cell cycle progression and apoptosis in cell lines and in vivo, and knockout of the syntenic region in mice results in the development of a CLL-like phenotype. We measured by qRT-PCR mir15a and miR-16 in CLL samples (n=38) and normal B-cells (n=14) and observed reduced levels in CLL independent of the 13q deletion status. We then assessed the levels of the primary precursors of the mature miRNAs (pri-miRNAs 15a, 16-1 and 16-2). No difference could be identified between patients with a deletion of 13q and patients with a retention of both copies of 13q14. However, CLL cells had increased levels of pri-miRNAs when compared to CD19-sorted normal B-cells. Moreover, in CLL cells levels of miR-16 and miR15a were inversely correlated to their pri-miRNA transcripts, while a miRNA used as a control (miR-155) had a positive correlation with its primary transcript as expected. In addition, also the levels of precursor miRNA molecules of the 13q14 miRNA genes were low in CLL patients. This strongly suggested a defect of miRNA maturation at the Drosha processing step that produces the pre-miRNA from the pri-miRNA molecule. Therefore, we grouped patients according to the observed levels of pri-miRNA and pre-miRNA: CLL patients with a ratio of these two processing intermediates (pri-/pre) above the average levels observed in non-malignant CD19-B-cells were included in the *high ratio*group (58% of our cohort CLL patients), while the rest was grouped in the *normal ratio* group (42%). Patients with high pri-/pre ratio had significantly lower mature miRNA levels compared to patients with normal ratio. In contrast, the respective ratio of the precursor molecules of miR-155 that were used as control did not differ in the two groups. These findings suggest that there is a processing defect that reduces maturation of the miR15a/miR-16 in a subset of CLL patients. In order to test the actual processing activity in CLL cells, we used a luciferase based in-vivo Drosha processing assay and could show a significant reduction of pri-16-1 processing in patients belonging to the "high pri-/pre ratio"group. These findings underline the role of miR15a/miR-16 in the pathomechanism of CLL and show a complementary route of inactivation that supplements genomic loss of the critical region in 13q14 and its transcriptional downregulation in CLL.

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MEASUREMENT OF SERUM FREE LIGHT CHAIN (SFLC) IS A SIMPLE, COST EFFECTIVE AND POWERFUL TEST TO EVALUATE ADVERSE FORM OF CLL AT DIAGNOSIS AND TO FOLLOW THE RESPONSE TO TREATMENT

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Background. Previously published data have emphasized the utility of SFLC assessment in newly diagnosed CLL and have concluded that abnormal SFLC ratio was related to adverse prognostic factors and a shorter time to first treatment. *Aims.* The aims of our study were to confirm the utility of SFLC at CLL diagnosis in a prospective study, to compare with known prognostic factors, and to evaluate the interest of the

test in treated patients' follow-up. *Methods.* We investigated SFLC level and ratio in 63 patients newly diagnosed with CLL in our centre between August 2007 and December 2010. Blood lymphocytes immunophenotype systematically included CD for RMH scoring and surface light chain expression analysis. Prognostic factors such as Binet stage, lymphocyte doubling time, CD38 and ZAP-70 expression, FISH analysis for P53, ATM, or RB deletion and chromosome 12 number, serum thymidine kinase and serum soluble CD23 were systematically evaluated. Serum B2-microglobulin and immunoglobulin (G, M and A) levels were also measured. In the same period of time, SFLC were assessed before and after chemotherapy in 52 treated patients, allowing 121 control measurements (one to seven per patient), and were compared to clinical and haematological response and minimal residual disease (MRD) measured by 5-colour flow cytometry, according to international recommendations. *Results.* Thirty patients (48%) had an abnormal SFLC level and/or ratio at diagnosis. The ratio kappa/lambda was increased in 19/21 cases (90%). No relation was found between the level of SFLC and white blood cell and/or blood B lymphocytes number, neither with the level of light chain expression on B lymphocytes as determined by immunophenotype. The type of free light chain was constantly the same than the one expressed at the B lymphocyte surface. Abnormal SFCL was associated with adverse prognostic factors such as advanced Binet stage at diagnosis (B and C, $p < 0.001$), short lymphocyte doubling time (LDT) ($P<0.0001$), ZAP-70 positivity ($p < 0.05$), adverse cytogenetics (P53 deletion, ATM deletion or trisomy 12, $p <0.01$). No relation was found with the presence of a serum monoclonal immunoglobulin peak, which was detected in 3 patients only. However a decrease in normal immunoglobulin serum level was evidenced in 22 of the 30 patients with abnormal SFLC (73%), with a significant correlation ($p = 0.001$). Analysis of treatment-free survival (TFS) duration between diagnosis and first line treatment showed a relation to SFLC. Multivariate analysis confirmed that short LDT, ZAP-70 expression, and P53 or ATM deletion were independent risk factors in Binet stage A CLL, and that abnormal SFLC was a high independent risk factor. Abnormal SFLC CLL had shorter TFS (HR = 27, 95% CI = 4.15-175.5), confirming that the disease was more aggressive in this group. When analysed during patients' follow-up after treatment, abnormal SFLC were associated with a positive MRD ($p< 0.0001$), with a specificity of 86% and a positive predictive value of 96%. *Conclusions.* SFLC assessment is a simple, immediate and cost-effective test to appreciate the aggressiveness of CLL at diagnosis and to follow the response to treatment.

1182

CLONAL EVOLUTION IN CLL PATIENTS. ACQUISITION OF NEW CYTOGENETIC ABERRATIONS IS RELATED TO THE EXPRESSION OF CD38 AT DIAGNOSIS.

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Introduction. The acquisition of new genetic alterations in the development of CLL is a known phenomenon, but published studies show conflicting results. For some authors, this phenomenon is not related to the expression of CD38 while others find a relationship either with unmutated cases or the expression of ZAP-70. *Patients and Methods.* In a series of 200 patients diagnosed with CLL, we included those who had been studied by FISH both at diagnosis and later on during their clinical course. We analyzed clinical and biological prognostic factors (age, stage, B2M, ZAP70, CD38, del13q, +12, and del17p del11q) in the diagnosis and follow-up. We also studied variables such as the number of treatment lines, vital status, lymphocyte doubling time, newly acquired cytogenetic abnormalities and secondary neoplasms. *Results.* We evaluated 60 patients with a mean age of 64 years (range 35-79). In the group that did not show clonal evolution (29/60), 21 had neither cytogenetic abnormalities at diagnosis nor at follow-up, and the remaining 8 showed the same abnormalities throughout their evolution [del13q (4 / 29), del11q (1 / 29), +12 (2 / 29), t (2; 5) (1 / 29)]. 31 patients showed a clonal evolution. In patients with normal FISH at the time of diagnosis, the most frequent acquired abnormality was isolated del13q (11/31), followed by +12 (1 / 31), del17p (1 / 31) and t (11, 14) (1 / 31). With regard to changes involving del11q, 4 patients with normal FISH acquired both del11q and del13q, 1 patient with del11q acquired del13q. Del17p acquisition was associated with gaining of either trisomy 12 or del13q in two patients with a normal initial study; del17p was observed in two patients one with previous trisomy 12 and another with del13q; finally two patients with del13q plus del11q acquired deletion of 17p. Two patients with initial trisomy 12, one of whom had an associated del11q, acquired del13q. When performing univariate analysis, variables related to the diagnosis of clonal evolution were: advanced clinical stage, splenomegaly, B2-M> 3 mg /dl and expression of CD38. Also significant was the number of treatment lines. ZAP-70 was not significant. In addition, the group of clonal evolution showed a trend to the appearance of second malignancies ($p = 0.05$). In multivariate analysis only CD38 expression at diagnosis was significantly associated with decreased survival. Finally, when examined separately, the subgroup of patients with high risk cytogenetics at diagnosis, were more likely to acquire new cytogenetic abnormalities and show a trend to be treated earlier ($p = 0.09$). *Conclusions.* In our series, clonal evolution is associated with the expression of CD38 at diagnosis. In addition it is related with a greater number of treatment lines and lower survival. Ultimately these results reflect a more aggressive disease that might require a specific therapeutic approach.

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RITUXIMAB-BASED CHEMOTHERAPY FOR AUTOIMMUNE CYTOPENIA OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Chronic lymphocytic leukemia (CLL) is characterized by an acquired immune defect that can cause autoimmune complications, including anemia (AIHA) and thrombocytopenia (ITP). AIHA occurs in 10% of advanced stage CLL patients; ITP occurs in 2 - 3% in early stage disease and may be a presenting manifestation. And there are limited effective treatment options for steroid refractory autoimmune cytopenias. Rituximab, an active agent against B cell malignancies, has also been noted to be active in certain autoimmune hematologic disorders. The purpose of this study was to evaluate the safety and efficacy of Rituximab-based chemotherapy in this complication of CLL. *Patients and Methods*. This prospective study examines the outcome in CLL patients with autoimmune phenomena (AIHA, Evans' syndrome). Five patients were treated at our institution. Two patients who had been previously treated with alkylating agents (CHOP/CVP), one of them had received Fludarabine, two patients were newly diagnosed and had not receive any treatment. Treatment regimen (RCD): Rituximab was given at a dose of 375? m2 intravenously (i.v.) on day 1 (D-1). Dexamethasone 40 mg i.v. D-1. Cyclophosphamide at a dose of 1000 mg (total dose) i.v D-1. The treatment was repeated every 2 weeks of a total of six cycles. *Results*. Median age was 65 years (range 44 -74) and there were five male patients. All of them had Binet stage C disease, and ECOG Performans status was 1. Response in autoimmune cytopenias was evaluated by frequent blood counts and Coombs test. 4 of five patients achieved a remission of their cytopenia. One of them is still received the treatment. Median pretreatment hemoglobin was 6.7 g/dl and post-treatment hemoglobin was 12.3 g/dl. The mean pretreatment platelet was 147 G/L and post treatment was 213 G/L. Three patients converted to Coombs negative after RCD. Median duration of response was 14 months. The patients were evaluative for toxicity: grade ¾ toxicity neutropenia was noted in one patient and needed supportive care by haemopoitic growth factors. One patient died of progressive disease (CLL) 06 months after the response to RCD therapy. *Conclusions*. Autoimmune phenomena, largely related to blood cells, are based in the immune dysregulation of CLL. Our results indicate that a rituximab-based combination regimen (RCD) is highly effective in treating this complication of CLL, and show a safety profile.

1184

ASSOCIATION OF HEMATOLOGICAL NEOPLASIAS AND MERKEL CELL CARCINOMA

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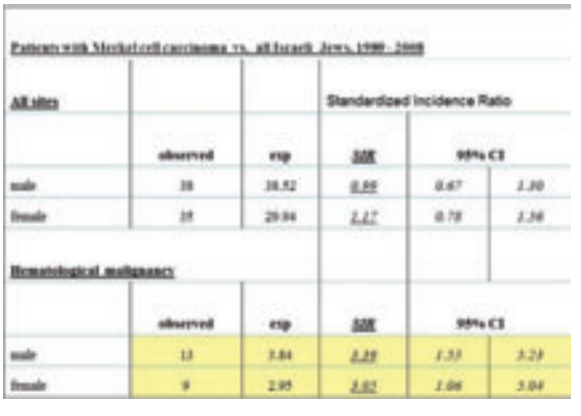
Background. Merkel cell carcinoma (MCC) is a rare and aggressive neuro-epithelial skin tumor which has been reported to occur in association with other primary neoplasms, including haematological malignancies. Immune suppression and dysregulation are well established risk factors for the development of MCC and may provide the explanation for the reported association of this tumor with neoplasms of the immune sys-

tem, predominantly lymphoid malignancies. *Aims*. The purpose of this study was to analyze data from the Israel Cancer Registry (ICR) relating to the epidemiological characteristics and the incidence of second cancers in patients with MCC, with special emphasis on haematologic malignancies and especially chronic lymphocytic leukaemia (CLL) /small lymphocytic lymphoma (SLL) *Methods*. Examination of the ICR records revealed 305 cases of MCC diagnosed in Israel during the period 1982 and 2009. Data were collected on age, gender and ethnic origin, dates of diagnosis of MCC and other tumors, as well as causes and dates of death when applicable. The incidence of second neoplasms in MCC was compared to those recorded in 4446 age-ethnic and period matched controls diagnosed with primary neoplasms in the Jewish population.Age specific standardized incidence ratio(SIR) was calculated with a 95% confidential interval (CI). *Results*. Incidence of MCC increased during the time periods reported. 97 % of MCC cases occurred in Jewish Israelis, while the remaining 3 % included Arabs and non- Arabs non- Jews. Median age at diagnosis in the Jewish patients was 73.3 years and 56 years in the Arab population respectively .One hundred and four patients (34%) had a second neoplasm, 73 evident before, and 31 after the diagnosis of MCC. The SIR for non- haematologic malignancies did not show a higher proportion of second tumors among MCC patients.Thirty of the 104 second cancers (28.8%) were haematological malignancies and of these 23 were detected before and 7 after the diagnosis of MCC. The SIR for second haematologic malignancy was 3.39 for males (95 % CI: range; 1.55-5.23) and 3.05 for females (95 % CI: range; 1.06-5.04).The most frequent haematologic neoplasias recorded were SLL/CLL (46.6%) and lymphoma (30%).There were also two cases of myeloproliferative diseases and single patients with multiple myeloma, hairy cell leukaemia, myelodysplastic syndrome (CMML) and mycosis fungoides. *Conclusions*. There is a high incidence of second haematologic neoplasms in Israeli Jews with MCC and these are mostly CLL and lymphomas. These results are in accordance with some reports from other countries showing a high prevalence of MCC especially in patients with CLL and lymphoma. This rare but significant association should be taken into consideration when evaluating patients with B-cell lymphoproliferative disorders and a co-existent skin tumor.



Patients with Merkel cell carcinoma vs. all Israeli Jews, 1989 - 2008					
All sites			Standardized Incidence Ratio		
	observed	exp	SIR	95% CI	
male	38	38.52	0.99	0.67	1.30
female	35	29.04	1.17	0.78	1.56
Hematological malignancy					
	observed	exp	SIR	95% CI	
male	13	3.84	3.39	1.55	5.23
female	9	2.95	3.05	1.06	5.04

Patients with Merkel cell carcinoma vs. all Israeli Jews, 1989 - 208

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CLINICAL EXPERIENCEOF BENDAMUSTINE-RITUXIMAB TREATMENT FOR RELAPSED INDOLENT NHL AND CLL

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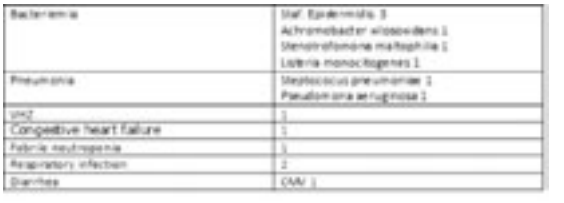
Background. Promising results have been reported in studies evaluating the combination of Bendamustine and Rituximab (B-R) in patients with relapsed/refractory indolent or mantle cell lymphomas. *Patients and methods*: we have analysed the role of the combination B-R in 8 patients since January 2009. They received Rituximab 375mg/m2 (day 1) plus Bendamustine 90-100 mg/m2 (days 1+2) every 28 days for a maximum of 6 cycles. The median patient age was 69.9 years (56-86 range). Most patients were in advanced stages (III-IV). Histologies were distributed: MALT NHL 10 %, grade 2 follicular NHL 10%; mantle cell NHL 30%, CLL/lymphocytic lymphoma 50%. Patients were heavily treated with a median of 3 prior regimens, including anthracycline containing chemotherapy (n= 4) and purine analog chemotherapy (n= 7). All patients had received previously rituximab. *Results*. Of the 10 patients, 2 are going in the treatment and they have not yet evaluated, 1 patient died at 3 cycles to infection, and 1 patient was lost follow-up. A median number of 4.5 cycles was given (2-6 range). At the time of analysis 2011 February, the median observation time was 10 months (2-22). Overall response 100% rate for patients treated with B-R. CR rate was 42.9% and 57.1% PR. Two patients in PR progressed at 9 and 10 months after they completed therapy. 1 patient in PR progressed, after 3 cycles. Hematologic toxicities were observed for neutropenia grade 3+4. Patients were admission in hospital in 22% cycles of bendamustine (Table 1). The B-R regimen was good tolerated by the patients, as evidenced by a lower rate of alopecia, number of infectious complications and estomatitis. We observed drug-associated erythematous skin reaction (rash) in one patient. There is not association between prior purine analog chemotherapy. *Conclusions*: the combination of Bendamustine +Rituximab have a excelent tolerability profile, and CR rate in heavily treated patients with relapsed/refractory indolent or mantle cell lymphomas. Further follow-up will determine whether the high RC/RCu rate corresponds to prolonged PFS. Additional updates on response will be available at the time of presentation.



Bacteriemia	Staf. Epidermidis 3 Achromobacter xilosoxidans 1 Stenotrofomona maltophilia 1 Listeria monocitogenes 1
Pneumonia	Streptococcus pneumoniae 1 Pseudomona aeruginosa 1
UTI	1
Congestive heart failure	1
Febrile neutropenia	1
Respiratory infection	2
Diarrhea	CMV 1

Table 1. Causes of admission in patients.

1186

MONOCLONAL ANTI-CD52 ANTIBODY TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS THAT ARE REFRACTORY TO FLUDARABINE-CONTAINING CHEMOTHERAPY REGIMENS

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Anti-CD52 monoclonal antibody (alemtuzumab) is a new treatment option in chronic lymphocytic leukemia (CLL). *Aims*. To study the efficiency and toxicity of alemtuzumab in a monoregimen in recurrence and refractory CLL. *Materials and methods*. Twelve CLL patients (11 male and 1 female) were included in the study. The age of the patients ranged from 36 to 66 years (median: 54). Nine patients were in stage B, and 3 in stage C according to the Binet classification. The ECOG performance status was ≤2 balls. The median time from diagnosis until alemtuzumab treatment was 16 months (range, 1-28). Nine patients received FC, FCM, and RFC treatment, 3 of them were refractory to the FC program, and 1 to FCM. Five CLL patients were in relapse, 3 of these after FC (6 courses), one after FCM (4 courses), and one after RFC (6 courses). The remaining three patients were treatment-naïve. In all tested patients ZAP-70 protein expression before treatment was revealed by using flow cytometry on more than 20% lymphocytes and expression membrane antigen CD38 on more than 30% lymphocytes. The average β2-microglobuline level in blood serum was 5.92±0.8 mg/l (3.91-8.7 mg/l). Cytomegalovirus (CMV) screening was done by serology and the PCR-method before alemtuzumab therapy. CMV monitoring was undertaken every 2 weeks and if a fever of unknown origin occurred. Alemtuzumab therapy was performed in a monoregimen by subcutaneous injection with dose escalation (3 mg, 10 mg, 30 mg) by 30 mg 3 times a week. The treatment duration was 14 weeks in 1 patient, 12 weeks in 9 patients, and 8 weeks in 2 patients. *Results*. As a first line therapy alemtuzumab induced complete remission in 2 patients after 6 and 8 weeks of treatment, which progressed to molecular remission; one patient developed partial remission. Among the patients resistant to earlier treatment and with relapse, overall response was achieved in 8 (89%) patients, complete remission developed in 5 (56%) patients, and partial remission in 3 (33%). One (11%) patient had no remission on therapy.

It is necessary to note that in 3 (60%) patients with complete clinical and morphological remission, molecular remission developed too. The results of the study have shown that a response on alemtuzumab therapy depends on the duration of this therapy and the spread of the tumor process. *Conclusions*. Monoclonal anti-CD52 antibodies are high effective among CLL patients that are refractory to fludarabine-containing chemotherapy regimens, and with the relapses of the disease, but toxicity was considerable.

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THE INTERNATIONAL ON-LINE REGISTRY OF RARE, CUTANEOUS AND CNS LYMPHOMAS OF CHILDHOOD

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Background. Certain types of lymphomas (follicular lymphoma, small cell lymphoma, marginal zone lymphoma, mantle zone lymphoma etc.) are rare in childhood. Also, CNS lymphomas and skin lymphomas are also relatively rare in childhood. Because of this rare occurrence the knowledge of clinico-pathologic correlations and prognostic factors is low worldwide. *Aims*. The project objective is to study clinico-pathologic correlations and analyse prognostic factors in rare lymphomas, CNS lymphomas and skin lymphomas in children (i. e. in the fragile population), further standadisation of therapy protocols, which will mean a benefits in the health care in Oncology and Pediatrics. Owing to the fact of rarity/low incidence of such lymphomas even in specialised centres there is a necessity of not only interinstitutional, but also international cooperation. *Methods*. Starting international database/registry in Brno (under auspices of I-BFM) is being designed as a virtual site which will collect all diagnostic and clinical information by means of filling the on-line accessible (but secured by codes) forms, placed on the web site of the database/registry. A part of the database/registry will be freely accessible www atlas of microscopic/histologic images (on-line virtual microscope). Data will be acquired inter-institutionally and internationally by means of filling of secured web forms (initial data will be put along with sending a case by cooperating institution, follow-up data will be added prospectively. Number of cases will depend on amount of these rare diagnoses in cooperating institutions. After collection of statistically significant amount of cases from one type of lymphoma, data will be analysed. Clinico-pathologic correlations and therapy responses will be analysed by standardised statistic methods. Prognostic markers will be analysed mostly by means of detection of expression of those potential markers by immunohistochemical antibodies which will work on lent formalin fixed paraffin embedded tissue material (paraffin tissue blocks), then after semiquantitative evaluation of presence/absence of expression will be statistically analysed with aim to find possible prognostic correlations. Information about cases will be supported by web atlas. The atlas will contain free online accessible high resolution diagnostic images of histology of every case with short English description (high resolution virtual misroscope) which can help to histopathologists in making the right diagnoses. *Results*. After collecting significant amount of cases from cooperating institutions there will be statistic analysis of clinico-pathologic correlations and analysis of prognostic factors. Standardisation of therapy protocols will be performed. *Summary/conclusions*. The project will enable a study of prognostic factors, clinico-pathological correlations and standardisation of therapy protocols in rare lymphomas of childhood, in CNS lymphomas of childhood and in skin lymphomas of childhood (i. e. in fragile population) by means of interinstitutional/international database/registry shared on secured web site (*www.rarelymphomas.eu*).

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EFFICACY, TOLLERABILITY, 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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Backgroun. The treatment decision of elderly patients has to be made individually considering, not only the 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. The oral formulation offers equivalent efficacy and an improved tolerability profile compared to the IV formulation. IV fludarabine requires several administrations that will expose patients to the risk of IV injection complications and the cost of trip to the 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 2010, 10 elderly untreated patients (mean age 75, range 68-86) with treatment requiring B-cell lymphatic leukaemia (according to ESMO guidelines working group) and 10 elderly indolent stage ≥3 lymphoma non Hodgkin untreated patients (mean age 74, range 59-80) received therapy with low dose of oral fludarabine (25mg/mq/die) and cyclophosphamide (150mg/mq/die) (FC) both from days 1 to3 in once a day administration. 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 a week) and allopurinole (300 mg once a day from days 0 to 4). Performance status was WHO ? 2 in all patients. Comorbidities, which included diabetes, hypertension and chronic heart disease, were present in 12 patients. The mean of administered cycles was 4 with range 2-6. No patients reduced dose and number of cycle 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.17 of 20 (8 RC and 9 RP) obtained a response with an overall response 85%. All responder patients are alive and maintained response after mean follow-up of 20 months (range 2-44). We used Genzyme sponsored Excel program to compare direct hospital cost of oral to 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 for more aggressive treatments. 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 progress, 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 an environment friendly, with fewer complications and minor use of hospital resources.

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TREATMENT OF B-CLL AND T CELL NON-HODGKIN LYMPHOMAS PATIENTS WITH ALEMTUZUMAB; THE EXPERIENCE OF A SINGLE ROMANIAN CENTER

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Background. Treatment with Alemtuzumab has been useful not only to consolidate remission in patients with B-CLL treated with Fludarabine or/and Rituximab as frontline,but also as salvage therapy for patients refractory or resistant, especially those with del 17p. In T cell lymphomas Alemtuzumab is combined with intensive chemotherapy inducing a rate of response around 70% (*Gallaminic&al- Blood, 2007*), but complications due to serious infections are frequent. *Objectives*: to evaluate a lot of patients diagnosed with B-CLL and Tcell lymphoma type treated with Alemtuzumab between May 2006- May 2010 in Fundeni Clinic Of Hematology(indications for treatment, adverse reactions, complications and treatment response). *Material and Methods*: a single center retrospective clinical and epidemiological study of 44 patients diagnosed with B-CLL(37 patients) and T cell lymphomas(7 patients- 1 angioimmunoblastic, 1 anaplastic , 5 PTCL- NO). *Results.* Alemtuzumab was administered subcutaneously, in standard dose, as first line therapy(2 patients), as consolidation for previously treated responsive patients(9 patients) or for refractory/ progressive disease(33 patients).15 patients were checked for del 17p (3 patients del17p+). CMV reactivation was confirmed in 8 patients(2 deaths with encephalitis). 19 patients died; main causes of death were: progressive disease (10 patients), sepsis(6 patients) and hemorrhagic events (5 patients). *Conclusions*. Alemtuzumab is efficient and well-tolerated for treating early stages of B-CLL and for consolidation therapy; heavily pretreated patients gets a lower and transient rate of response and high rate of morbidities and deaths. In

Tcell lymphomas the efficacy was lower in our study than in the literature studies, mainly because Alemtuzumab was used as salvage therapy, in multiple treated patients.

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INCIDENCE OF ADVERSE EFFECTS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA TREATED WITH FC VERSUS FCR

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Background. CLL is the most common form of adult leukemia, accounting for 25%. CLL is a slowly unfolding from year to decades. Treatment of FC was and still is widely used in the treatment of first line in patients with CLL, with good results. However, introduction of Rituximab treatment in CLL patients has resulted in better outcomes and especially lower adverse reactions. *Material and Methods*. Between January 2008 - January 2011 we evaluated 62 patients with CLL in different stages of disease, of which 46 have followed treatment of FC and rituximab was introduced at 14, Of these, 24 were stage A, 6 stage B and 32 stage C, 32 were men, 30 women. All six cycles were performed either FC or FCR. *Results*. Blood complications occurred in 35 patients, 26 with FC (56.5%), 9 FCR (64.2%). Of these, neutropenia appeared in 20 patients treated with FC (43.5%) and 6 with FCR (42.8%), anemia in 15 patients treated with FC (32.6%) and 4 with FCR (28.6%), thrombocytopenia in 11 patients with FC (23.91%) and 3 with FCR (21.4%). Infectious complications occurred in 10 patients treated with FC (21.7%) and 2 with FCR (14.3%), most viral infections (herpes, EBV). Gastrointestinal side effects such as nausea and vomiting occurred in 2 patients, one from each group, given preventive treatment used antiemetic therapy. Anorexia and stomatitis occurred in the first group in 3 patients (6.52%) and 1 patient in the FCR treated group (7.14%). Asthenia and fatigue, due in part to anemia were reported in 40% of patients with FC and 45% of those with FCR treatment. Alopecia with marked psychological impact was seen in 13 patients (28.26%) of the first group and six in the second (42.8%). *Conclusions*. The data reported in our study are comparable with the literature, not statistically significant differences between the two groups.

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CLINICO-BIOLOGICAL FEATURES FEMALES AND MALES WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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B-cell chronic lymphocytic leukemia (B-CLL), the most common leukemia in the Western world is characterized by quite variable clinical course, which have been correlated with some clinical and biological markers. Among proposed prognostic factors the significance of gender is unclear, although in published trials females survive longer. A group of 286 unselected patients with B-cell chronic lymphocytic leukemia were studied for clinical and biological features. Sample analysis included CD38 and ZAP-70 stainings, fluorescence in situ hybridization (FISH) for chromosomes 11, 12, 13 and 17, level of bFGF, VEGF and TNF. There were168 men and 118 woman. The sex ratio was 1.4:1. The median age at presentation was similar, for men was 64.0 (range 34.0-83.0) and woman 64.5 (range 36.0-86.0) p=0.8207. Patients were staged at diagnosis according to the Rai classification. The distribution of patients with Rai stage eg. low-risk (stage 0), intermediate-risk (stage 1 and 2) or high-risk (stage 3 and 4) was: 28.8%, 50.8% 20.4% for female and 22.6%, 54.2%, 23.2% for males respectively. There was significant lower median rate of hemoglobin and higher rate of thrombocytes and percentage of CD3+ cells in females than males with B-CLL (12.63±1.96 vs 13.17±2.44, p=0.0045; 193.44±68.33 vs 170.36±70.05, p=0.0007; 16.02±13.41 vs 13.15±12.13, p=0.0369). Overall, the median survival of females was 56.91 months compare to 50.49 months for males (p=0.1076). Treatment-free survival was better for females than males (median time to treatment 17.55 months versus 9.27 months, p=0.0208). All group of studied patients received different kind of chemotherapy as front line treatment (chlorambucil alone, chlorambucil and prednisone, fludarabine containing regiments, COP and CHOP regiments); 63.6% females and 75.4% males had required treatment. There were significance differences in how females and males were treated (p=0.0255): 31.3% females and 28.4% males received chlorambucil alone or chlorambucil and prednisone, 8.6% females and 16.6% males were treated with COP or CHOP, and 23,7% females and 30.4% males received fludarabine containing regiments. 48.6% females and 46.0% males achieved complete response after front line treatment; in 22.2% females and16.8% males partial response and in 12.5% females and 15.1% males progression disease was observed. There was no a significant correlation between percentage of CD38 and ZAP-70 cells, percentage cells with genetic abnormalities, level of bFGF, VEGF, TNF and gender of patients with B-CLL.

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PROGNOSIS OF RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AFTER COMBINED FLUDARABINE, CYCLOPHOSPHAMIDE AND RITUXIMAB (FCR) TREATMENT

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Backround. FCR regimen, as the first-line therapy in CLL patients, results in high response rates, improved progression free survival (PFS) and overall survival (OS). However, more than 30% of patients relapse or have disease progression in 3-4 years after. An appropriate therapy for these patients still has not been determined. *Aims*. The aim of our study was to analyze further course of the disease in relapsed CLL patients (pts) after FCR treatment. *Methods*. 39 CLL patients relapsed after FCR, followed in our center from January 1995 to January 2011, were included in this retrospective analysis. We identified 2 main subgroups - relapsed patients after FCR regimen given as the first-line therapy (23pts, Group A) and patients who had been given FCR as the second-line (or more) therapy (16pts, Group B). *Results*. Groups' characteristics were as follows: Group A, a median age at the start of FCR was 62 years (49-74; median FCR cycles: 3). Rai stage before therapy: Rai I n=4 (17%), Rai II n=6 (26%), Rai III n=5 (22%), and Rai IV n=8 (35%). Regarding new prognostic factors, unmutated IgVH gene was detected in 91%, mutated in 9% of patients. A certain evolution in cytogenetic aberrations was observed in pts pre- and post FCR treatment: normal karyotype (4 vs. 6pts), del(13q) (5 vs. 3pts), del(11q) (7 vs. 5pts), trisomy 12 (2 vs. 2pts.) and del(17p) (1 vs. 3pts). In terms of response to therapy, 14pts (61%) achieved complete remission (CR), 7 (30%) partial remission (PR) and 2pts (9%) had progressive disease (PD). The same analysis was performed in the Group B. A median age was 64.5 years (38-78; median FCR cycles: 3). Median number of previous treatments was 1 (range 1-5), 7pts were treated with fludarabine. Rai stage before the start of FCR: Rai I n=2 (3%), Rai II n=4 (25%), Rai III n=5 (31%), and Rai IV n=5 (31%). Unmutated IgVH gene was detected in 88%, mutated in 12% of patients. Cytogenetic abnormalities: normal karyotype (7 vs. 5pts), deletion of 13q (5 vs. 4), deletion of 11q (2 vs. 2), trisomy of 12 (2 vs. 0) and deletion of 17p (0 vs. 2). Nine patients (56%) achieved CR, 3pts (19%) PR, 1pt (6%) had stable disease and 3pts (19%) PD. Median PFS1 (after FCR treatment) was similar in both groups: 11 (A) vs. 12 months (B), p=0.59, PFS2 (after a subsequent therapy, irrespective of the type) was 10.4 (A) vs. 3 months (B), p0.03. Median OS calculated from the time of diagnosis was 85.8 (A) vs. 100.9 months (B), p0.38 (Figure). *Conclusions*. Compared with other treatment options, FCR is superior in CLL patients regardless being administered as a first-line therapy or a treatment of relapsed CLL, however increased number of poor prognosis cytogenetic aberrations is observed after FCR regimen in relapsed patients. Prognosis of CLL patients relapsed after FCR appears to be very poor irrespective of the type of subsequent therapy.

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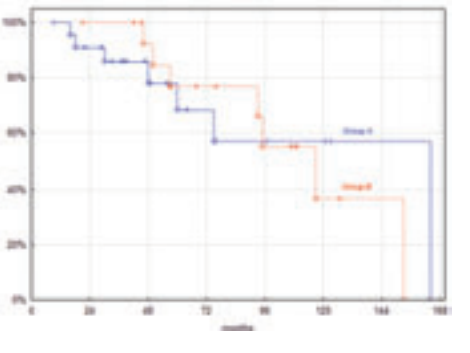

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RITUXIMAB THERAPY IN PATIENTS WITH EXTRA NODAL MANIFESTATIONS OF CHRONIC LYMPHOCYTIC LEUKAEMIA

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Chronic lymphocytic leukaemia (CLL) is a common adult leukaemia, occurring in patients over 50 years of age with median age at diagnosis of around 65 years. While it remains an incurable disease, its indolent nature results in a varied prognosis with a median survival of greater than 10 years reported in early stage disease. Treatment is typically initiated when patients become symptomatic including alkylating agents (chlorambucil or cyclophosphamide) but combination chemotherapy with vincristine, prednislone or fludarabine may be required in some patients. Rituximab, a chimeric anti-CD20 monoclonal antibody, has shown significant activity in a variety of B-cell lymphoproliferative disorders. The CD20 antigen is also found on B-cells in CLL and rituximab therapy has shown clinical benefit in these patients. Extra nodal infiltration by small lymphocytes in patients with CLL is an uncommon manifestation. There is limited data on specific therapeutic strategies for treating these patients. The following case reports highlight the usefulness of rituximab in CLL patients with extranodal infiltration, with minimal additional use of chemotherapy. We present seven patients with various extranodal manifestations including 3 patients with cutaneous lesions with one of them showing amyloid deposits; 2 patients with renal involvement including minimal change glomerulonephritis(GN) and mesangial GN; 2 patients with pulmonary nodal infiltration. All these patients received a short course of chlorambucil therapy followed by rituximab therapy with significant improvement. The therapy consisted of weekly infusions at 375 mg/m2 followed by monthly infusions for 4 months. Some of the patients have required maintenance rituximab therapy. Follow up period range: 2-8 years (median 4 years). 5 patients are alive and well and 2 patients died of unrelated causes. The surviving patients have not required any additional therapy to treat CLL or extranodal manifestations. Hence, rituximab can be used as a single agent therapy in these patients.

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CLONAL DIVERSITY IN CONCOMMITANT MULTIPE MYELOMA AND CHRONIC LYMPHOCYTIC LEUKEMIA CASES DEMONSTATED BY IMMUNOPHENOTYPIC, FISH AND WHOLE GENOME SNP ANALYSIS

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The occurrence of multiple myeloma (MM) and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) in the same patient is rare. We present three such cases and document clonal diversity by flow cytometry, FISH and molecular analyses. Informed consent was obtained for all studies. Case 1 is a 54 year old female diagnosed originally with CLL who presented with bone pain. On FISH analysis two distinct cell types were noted - small cells having +12 and plasma cells (PCs) showing 3-4 copies of FGFR, D12Z1, CCND1, ATM, TP53 and MYB. Marrow showed κ restricted plasma cells with aberrant CD117 and CD19, CD5, CD23 + λ restricted B-cells. Whole genome SNP analysis (Infinium System) confirmed dual clonal disease. Case 2 is a 68 year old male diagnosed with CLL 2 years previously. Marrow exam showed panmyelosis with increased PCs showing λ restriction. Lymph node showed lambda restricted CD19, CD5, CD23 + B-cells. CLL FISH probes were negative. IEF showed three M components - IgM λ, IgG kappa and IgG lambda. Case 3 is a 27 year old female diagnosed with MM. Flow cytometric analysis performed on marrow showed kappa restricted PCs with aberrant CD117expression. FISH analysis two morphologically distinct cell types: cells with smaller nuclei had an extra copy of 1q and loss of 13q14; cells with larger nuclei appeared tetraploid with 4 copies of 1p and 6 copies of FGFR3, CCND1, TP53 and BCR and 5-6 copies of IGH. Genome SNP analysis is pending. The results in all cases support distinct clonal evolution of these two B-cell neoplasms.

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PILOT STUDY OF THE COMBINATION OF RITUXIMAB, IFOSFAMIDE AND FLUDARABINE (R-IFLU) IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Fludarabine given with cyclophosphamide and rituximab is considered the cornerstone of chronic lymphocytic leukemia (CLL) treatment. While several purine-analogs have been evaluated in combinatory trial, no other member of the oxazaphosphorines family has been investigated in this setting. Ifosfamide has shown significant activity in non-Hodgkin lymphomas, but has been not yet evaluated in CLL. *Aims*. We conducted a pilot phase II study of ifosfamide given in combination with fludarabine and rituximab (R-IFLU) in relapsed/refractory CLL patients. *Methods*. Thirteen patients with relapsed/refractory CLL were enrolled. Therapy consisted of ifosfamide (750 mg/m2) and fludarabine (25 mg/m2) for three consecutive days (D1-3), and rituximab (375 mg/m2) on D3. Treatment was administered every 21 days up to 6 cycles. Response was assessed using the NCI-WG 1996 criteria. Maintenance with monthly rituximab (375 mg/m2) infusions was administered in responders for a total of 4 months. *Results*. Median patient age was 63 years (range 51-71). The median WBC count was 61,800/mmc (3,160-122,000), and 9 patients were in advanced Binet stage (5 B and 4 C). Six (46%) patients presented with bulky lymph nodes (>5 cm). Five patients (38%) showed an unfavorable cytogenetic profile, including 4 patients with del(11q) and 1 patient carrying del(17p). The median number of previous lines of therapy was 2 (1-4); all the patients were previously exposed to chlorambucil (69% being refractory) and 29% to fludarabine (54% being refractory). A median of 4 cycles of therapy was administered (2-6); the overall response rate (ORR) was 69%, including 23% of CRs. After a median follow-up of 4 years all the patients progressed, with a median progression free survival of 20.5 months (95% CI 8.1-40.1). Median overall survival was 47.6 months (95% CI 33.8-86.6). Hematologic toxicities were frequent: grade 3-4 neutropenia, thrombocytopenia and anemia were reported in 69%, 15% and 8% of the patients, respectively. Infusion related toxicities occurred in 3 patients and were mild in all the cases. Infections occurred in 8 patients (62%) during therapy, a grade 3-4 event was reported in one patient. *Conclusions*. This pilot study shows that combination of ifosfamide, fludarabine and rituximab is feasible and effective in relapsed/refractory CLL. Further studies are needed to evaluate the potential role of ifosfamide as alternative oxazaphosphorine drug in those patient only partially responding to cyclophosphamide-containing regimen.

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CORRELATION OF BCL2 GENE EXPRESSION AND BCL2/BAX RATIO WITH CLINICAL AND IMMUNOPHENOTYPIC CHARACTERISTICS OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. B-cell chronic lymphocytic leukemia (B-CLL) is a lymphoproliferative disorder characterized by accumulation of clonal B-lymphocytes, mainly due to aberrant apoptosis. The Bcl2 gene regulates apoptosis and is known to be overexpressed in B-CLL. The activity of this protein is opposed by Bax, a homologous protein that accelerates the rate of cell death. B-lymphocyte Bcl-2 and Bax protein levels were found to be significantly altered in B-CLL. The mutational status of immunoglobulin heavy chain variable region (IGHV) divides B-CLL into two prognostic groups, depending on the presence or absence of somatic hypermutation, where unmutated IGHV genes are associated with considerably worse prognosis than mutated IGHV. *Aims*. We analyzed clinical characteristics, immunophenotypic profile, IGHV mutational status, Bcl2 and Bax gene expression in 53 patients with B-CLL. We evaluated the immunophenotypic caracteristics in correlation with Bcl2 gene expression and Bcl2/Bax gene expression ratio in patients with mutated and unmutated IGHV. *Methods*. Bone marrow (BM) and/or peripheral blood (PB) derived cell flow cytometric analyses were performed for following antigens (Ag): CD19, CD20, CD22, CD23, CD25, CD10, SmIg, kappa, lambda, CD79b, CD38, FMC7, CD3, CD2, and CD5. Cut-off for Ag expression was accepted positive > 20%. Pathohistology and immunohistochemical testing were performed on BM (bone marrow) biopsy samples. We used following monoclonal antibodies: CD5, CD20, CD23, CD10 and CD79b. Multiple RT-PCR and sequencing analysis were used to determine IGHV mutational status. RQ-PCR was used to determine Bcl2 and Bax gene expression. The correlation between clinical parameters, surface antigen expression Bcl2 and Bcl2/Bax ratio was estimated by Pearson correlation coefficient. All patients signed informed consent according to Declaration of Helsinki.

Results. High expression level of CD23 surface antigen correlates with shorter PFS (p=0.04), higher level of bone marrow infiltration (p=0.035), shorter lymphocyte doubling time (p=0.004) and advanced Rai clinical stadium (p=0.014). The expression of CD38 surface antigen correlates with shorter PFS (p=0.008). High level of Bcl2 gene expression and Bcl2/Bax ratio correlates with shorter lymphocyte doubling time and shorter PFS, but only in the group of patients with mutated IGHV (p=0.01; p=0.004). High level of Bcl2 expression correlates with the following immunophenotypic profile: low expression level of FMC7 (p=0.02) and high expression level of CD23 (p=0.032) in all patients. High Bcl2 expression correlates with lower FMC7 expression level in the group of patients with unmutated IGHV (p=0.003). High Bcl2 expression correlates with higher expression level of CD23 in the group of patients with mutated IGHV (p=0.042). There is no correlation of immunophenotypic profile with Bax expression, nor with Bcl2/Bax ratio in this study. *Conclusions*. Our analyze showed that B-CLL with higher CD23 surface antigen expression have more aggressive course with shorter progression free survival. Higher Bcl2 gene expression and Bcl2/Bax ratio correlate with the shorter progression free survival in B-CLL patients with mutated IGHV. We suggest that B-CLL with high level of Bcl2 gene expression is characterized by distinct immunophenotypic profile with increased CD23 and reduced FMC7 surface antigen expression.

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CLL REGISTRY IN ROMANIA - A PRELIMINARY REPORT OF THE ROMANIAN INITIATIVE GROUP IN DIAGNOSIS OF CLL (RGIDCLL)

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Background. Chronic lymphocytic leukemia(CLL), the most frequent chronic lymphoproliferative disorder, mainly in elderly people seems to have real incidence higher than in reports. The diagnosis based especially on flowcytometry, and molecular and genetics changes should define the prognosis subgroups of CLL. *Aims*. The present study done by the Romanian Group of Initiative in Diagnosis of CLL proposes to introduce flowcytometry in current screening of patients with lymphocytosis suspected for CLL in Romania. At the same time, to assess the real incidence of CLL in Romania based on the same panel and protocol concerning the recommended ESMO guidelines. *Methods*. We have analyzed 214 patients suspected with CLL in last year in few centers in Romania. Clinical assessement was done as the guidelines recommended in all patients. The samples for immunophenotyping were collected from peripheral blood and sent from the centers to the central laboratory. The diagnosis was made by flowcytometry on a BD FACS Flow Calibur, with classical association of markers CD19,CD20,CD5, CD23,CD79b. In these patients we have analyzed other surface markers, like CD43,CD38, and intracellular markers ZAP-70. P53 mutation was done by FISH in a central laboratory, too. Statistical analysis with SPSS software was used to find correlations between clinical, immunophenotypical and genetic parameters, and therapy, too. *Results*. The patients with CLL were stratified by Rai clinical stage as follows: 17.39% stage 0, 16.30% stage I, 39.13% stage II, 10.87% stage III, and 16.30% stage IV. Positive diagnosis for CLL was found in 146 patients, represented 68% from the suspected cases. Negative cases represent other lymphoproliferative disorders and reactive lymphocytosis. 11 cases(5%) were diagnosed as monoclonal B-cell lymphocytosis. A presumptive incidence was calculated from the regions which were involved in subjects' recruitment. We found an incidence of 4.0559 : 100,000 people related to 3,476,357 people, higher than the incidence reported in US(3.35-3.69). p53 mutation was found positive in 8% of cases, in a higher percentage than usual(5-7%). Related to the diagnosis panel, we found a significant correlation between CD5 and CD79b(r:0.62), and between intensity of CD79b and CD38(r:0.60), which could be explained by an atypical immunophenotype associated with progression risk. A correlation between CD38 and ZAP-70 expression was found and with lower expression of CD23(r:0.666), which correspond to an atypical immunophenotype with progression risk. *Conclusions*. This is the first study which uses the same protocols in diagnosis of CLL cases from multiple centers, and different regions. The positive ratio of diagnosis is considered very well, and could be a useful way to identify earlier phase new CLL cases in Romania. The incidence of CLL seems to be higher than in other regions of world, and with a higher number of high risk (p53 positive) cases. It could be a high risk of this region related to the nuclear incidents from 80's involved in this pattern or not? We consider that this preliminary data are very useful to assess the real incidence and ethiologic factors of CLL occurrence in Romania.

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RITUXIMAB IN COMBINATION WITH HIGH DOSE METHYLPREDNIZOLONE FOR THE TREATMENT OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND P53 DELETION

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Chronic lymphocytic leukemia (CLL) patients with del (17p) or del p53 do not respond to conventional treatment and have shorter survival. Studies have demonstrated that high dose methylprednisolone (HDMP) either alone or in combination with Rituximab is effective in such patients *Purpose*. We evaluated the efficacy and complications of the combination of high dose methylprednisolone(HDMP) with the anti-CD20 monoclonal antibody, rituximab (R-HDMP) in heavily pre-treated CLL patients with del p53. *Patients and Methods*. We retrospectively studied 9 patients with CLL, (4 men and 5 women), who had del p53. The median age was 72 years (range 59-82 years). Two patients had Rai stage I, one patient Rai stage III and the remainder 6 patients were Rai stage IV. Four patients had bulky disease. All 9 patients had previously received other chemotherapy regimens (2-7 prior therapies). HDMP was given intravenously at a dose of 1 gm daily for 5 days in combination with rituximab 375mg/m2 on day 1 of a 28 day cycle for 2-6 cycles. They also received trimethoprim-sufamethoxazole, valacyclovir and fluconazole as prophylaxis during therapy and for two months after its completion *Results*. Eight out of nine patients developed side effects (hyperglycemia: 3 patients, edema of low extremities: 3 patients, increase of infections: 4 patients, osteoporotic fracture:1 patient and myocarditis: 1 patient). Four out of 9 patients (44.45%) achieved partial response (PR), 2/9 patients (22.23%) had steady disease (SD), and 3/9 patients (33.33%) had progressive disease (PD). With a median follow up of 5 months all patients with PR had progression of the disease and one of them died, while all patients with SD and PD died. *Conclusions*. Although the number of patients is small, the results indicate that the combination of high dose methylprednisolone(HDMP) with rituximab (HDMP-R) is an active regimen in a poor prognostic patient group like heavily pretreated CLL patients with del p53. Further evaluation in controlled trials is needed.

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LYMPHOCYTE CELL POPULATION DATA PROVIDED BY UNICEL DXH 800 IMPROVE IDENTIFICATION AND CLASSIFICATION OF LYMPHOPROLIFERATIVE DISORDERS

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Background. Lymphoproliferative disorders (LD) and B cell chronic lymphocytic leukemia (B-CLL), are heterogeneous disease whose diagnosis is based on automated WBC count, microscopy and immunophenotyping . Our previous experience with VCS technology of Beckman Coulter showed important correlation between VCS Cell Population Data (CPD) and other data coming from microscopy, flow-cytometry, cytogenetics and molecular biology.2. Aims: Since all these test are still part of the routine in clinical practice for B-CLL and LD diagnosis and classification, we decided to further explore UniCel DxH800 CPD could provide the same useful information to laboratory and clinical hematology.3. Methods: 16 patients samples (7 of them, untreated CLL) and 50 healthy donors were analyzed in this study with UniCel DxH800 that

performs leukocytes differential with the Flow Cytometric Digital Morphology (FCDM) technology, based on the measurements of Volume (V), Conductivity (C) and 5-angle Scatter light laser (UMALS, MALS, LMALS, LALS, AL2) on cells in their native state. Mean (M) and standard deviation (SD) of FCDM measurements are collected in 56 CPD. Diagnosis of CLL and LD are based the WHO classification criteria.4. Results. Reference interval for lymphocyte CPD were calculated and compared with pathological samples' ones. We confirmed our previous data showing the statistically difference ($p<0.05$) of mean volume LY (MV-LY) in CLL samples versus normal samples (83 a.u. vs 89 a.u.); SD-V-LY was 18.8 in CLL and 14 in normals. We discovered that axial-light-loss (M-AL2-LY) is also lower (41 vs 68). These CPD were able to describe the morphological findings of both clonal lymphocyte populations with low homogeneous volume and CLL with heterogeneous features. Even in some leukemic lymphomas we found correlation between MV-LY (95), AL2-LY (74) and morphological abnormalities of lymphoma cells. As an example we found a 3-years old ALL sample with lymphocytosis and without leukocytosis in which the value of SD-LY-AL2 (14) vs normal (10), induced us to follow-up in the diagnosis. Scatter light CPDs seem to be also useful in differentiating abnormal cells and therefore they need to be investigated.5. Conclusion. In this study we presented some considerations on the useful information to laboratory and clinical hematology provided by UniCel DxH800 CPD. Different patterns of scatterplot with different CPD values can be the basis for the validating process both in large laboratories and in clinical hematology lab. The first ones need screening tools while the second ones need classification tools useful also in the follow-up of the patients' therapy and prognosis. These preliminary observations are now under investigation in a multicentric evaluation.

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BORTEZOMIB ENHANCES THE SENSITIVITY OF IMATINIB TO K562/G01 CELLS

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Objectives. To explore the effect of proteasome inhibitor Bortezomib (BOR) on the sensitivity of imatinib to K562/G01 cell (imatinib-resistant chronic myeloid leukemia cell line) and the mechanism. Methods MTT assay was used to observe the effect of growth inhibitory of cells. flow cytometry was uesed to detect cell cycle; Real time-PCR was performed to detect expression of COX-2 and MDR-1 mRNA. Results Combined with 10、20nmol/L BOR could significantly enhance the sensitivity of drug, the reverse fold respectively was 1.83 and 2.72 . G2 / M phase cell cycle arrest could be seen by flow cytometry with BOR. K562/G01 cell over-expression of COX-2 and MDR-1, BOR could down-regulate COX-2 and MDR-1 expression. Conclusion BOR can enhance the sensitivity of imatinib to K562/G01 cell,the mechanism may be related to cell cycle G2 / M phase arrest and down-regulating the expression of COX-2 and MDR-1.

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STUDY OF THE ASSOCIATION OF CYP2D6*4 POLYMORPHISM WITH THE RISK OF CHRONIC MYELOID LEUKEMIA

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Background. PCYP2D6 is a member of cytochrome P450 enzymes family which are involved in the detoxifcation of a wide range of xenobiotics and drugs. Several genetic polymorphisms had been shown to affect its activity which may results in increased susceptibility to malignant disorders. Aim of the Study: This study aimed to determine whether any association exists between genetic polymorphism in CYP2D6*4 and risk of chronic myeloid leukemia (CML). Subjects and Methods: Our study groups consisted of 50 CML patients and 40 unrelated healthy volunteers as a control group. *Results*. The frequencies of EM genotype (wild type) were 64% and 95% in CML and control groups, respectively. The frequencies of polymorphic IM genotype (heterozygous variant) were found to be 28% in CML patients and 5% in controls. The PM genotype (homozygous variant) was 8% in CML and not observed in control group. There was a statistical signifcant correlation between the CYP2D6*4 gene polymorphism and chronic myeloid leukemia patients with the frequencies of IM and PM CYP2D6*4 were higher in CML cases compared to controls (28% versus 5%, 8% versus 0%, $p=0.004$), on contrary, the frequency of EM was higher in controls cases compared to CML patients (95% versus 64%). As an estimate for the relative risk, the odds ratio for CYP2D6*4 polymorphism was 10.688 with a 95% confdence interval of 86.597-1.319. Conclusion: These data indicate a higher risk for CML in individuals carrying the IM and PM CYP2D6*4 and reflect the major role of environmental factors in CML pathogenesis. The present study establish signifcant association of CYP2D6*4 polymorphism with CML.

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EXPRESSION OF PHOSPHORYLATED STAT5 IN CHRONIC MYELOID LEUKEMIA: RELATION TO DISEASE STAGESA

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Background. Chronic myeloid leukemia (CML) is characterized by the presence of the Ph chromosome (BCR/ABL chimeric gene) in hematopoietic stem cells. Clinically, it is manifested in three distinct phases: chronic, accelerated, and blastic. Signal transducer and activator of transcription (STAT) proteins are known to be regulated by cytokine receptors and are critical for driving transcription necessary for growth, survival, and differentiation of hematopoietic cells. BCR-ABL expression results in constitutive activation of STAT5 and essentially bypasses cytokine or growth factor-dependent activation of STAT5. Aims: The aim of our work was to investigate the state of STAT5 phosphorylation in relation to CML disease stages as a possible indicator of BCR/ABL tyrosine kinase activity. Methods: The study was conducted on 39 CML patients including 17 male and 22 female with an age range of 19-79, a mean of 39.88±15 and a median of 41.5 years. Patients were diagnosed as CML and stages defined according to the WHO classification of myeloid neoplasms. Twenty two patients were in chronic phase (group I) and 17 were in accelerated phase or blastic crisis (group II). Patients were divided into 3 risk groups according to Hasford score: Low risk group: score 780, Intermediate risk group: score 781-1480 and High risk group: score > 1480. After informed consent, analysis of phosphorylated STAT5 (pSTAT5) was done using Flow Cytometry. Results were expressed as percentage positivity and florescent ration in CD34 positive and CD34 negative cells. pSTAT5 expression was studied in relation to various hematological and clinical parameters. Results: pSTAT5 was expressed in all cases tested. The level was statistically significantly higher in advanced phases than in the chronic phase ($p=0.006$). CD34 positive cells% was 1.74 ± 1.61% and 21.3 ± 20.4% in group I and II respectively ($p=<0.001$). All CD34 positive cells were pSTAT5 positive. CD34 negative cells were pSTAT5 negative (<10%) in 8/22 (36.4%) and 5/17 (29.4%) patients in group I and group II respectively. pSTAT5% expression was significantly higher in group II as compared to group I (56.4 ± 27.6% vs. 33.9 ± 21% respectively; $p=.006$); however the florescent ratio was comparable in both groups. CD34-ve/pSTAT5+ve % was higher in Group II than in Group I but the difference did not achieve statistical significance. pSTAT5% expression showed significant positive correlation with both peripheral blood and bone marrow blast percentage ($r= 0.39$ and 0.37; $p= 0.017$ and 0.02 respectively). The distribution of the 3 Hasford risk groups among the two patients groups revealed 8/21 (38.1%) low risk, 8/21 (38.1%) intermediate risk and 5/21 (23.8%) high risk in Group I as compared to 4/16 (25%), 8/16 (50%) and 4/16 (25%) in group II; the difference is statistically insignificant. No correlation was encountered between pSTAT5 expression on one side and age, Hasford score or duration of chronic phase on the other side. Summary/Conclusions: The level of expression of pSTAT5 is higher in advanced phases of CML reflecting a higher tyrosine kinase activity of the BCR/ABL chimeric protein. It may serve as an indicator of the BCR/ABL expression level.

1203

TREATMENT WITH IMATINIB INHIBITS NF-KB AND AP-1 ACTIVATION AND INTRACELLULAR CALCIUM LEVELS INSP3 OR ATP-INDUCED IN CML PATIENTS

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Background. Chronic myeloid leukemia (CML) is a hematopoietic stem cell disorder caused by the oncogenic activity of the Bcr-Abl protein, a deregulated tyrosine kinase. The iperactivity of tyrosine kinase (TK) of this fusion protein activates multiple signal transduction pathways, which leads to malignant transformation. Our previous results demonstrated that peripheral blood mononuclear cells derived from CML patients displayed decreased intracellular calcium [Ca2+]i fluxes both after InsP3 and ATP administration (*Ciarcia et al. J.Cell.Physiol.,2010*). On the other hand several transcription factors are activated in response to physiopathological increases and intracellular calcium levels. It is widely known that BCR-ABL can inhibit apoptosis also by activating at cytoplasmic level the PI3K/AKT pathway and furthermore, the important anti-apoptotic pathway passing through the transcription factor NF-kB/Rel, appears activated in CML patients. *Aims*. In present study we evaluated the levels of transcription factors NF-kB and AP-1 as well as the intracellular calcium measurement in peripheral blood leukocyte of control or CML patients before and after treatment for three months with imatinib 400 mg in order to determine whether treatment with imatinib was able to change these parameters. Methods. NF-kB, AP-1 and intracellular calcium levels were measured on lymphocytes isolated from blood of n. 8 healthy volunteers and n. 8 CML patients in first of diagnosis and after three months treatment with imatinib. Intracellular Ca2+ concentrations were measured by using the radiometric fluorescent indicator dye FURA-2/AM, the membrane-permeant form of FURA-2/AM as previously described and opportunely modified by Pagnini U et.al (Anticancer research, 2000). To detect and quantify NF-kB and AP-1 activation in our samples, we used ELISA-based Trans-Am transcription factor kits (Active Motif, Carlsbad USA). Results. Our results showed that InsP3 induced an increase in maximum levels of [Ca2+]i by depletion of the intracellular calcium stores with a significant higher effect in CML untreated than treated patients for three months with imatinib increasing from 121,9±8.1 to 373,5±29.7nM in CML untreated and from 120.3±7.4 to 252.2±18.7nM (-53.1 %) in treated patients. ATP increased intracellular Calcium concentration by Ca2+ influx with a significant higher effect in CML untreated from 115.2 ± 6.4 to 251.8±22.3 nM than treated patients from 121.4 ± 7.6 to 197.3 ± 14.2nM (-52.9 %). The results of NF-kB and AP-1 in CML samples, demonstrated that respect to the constitutive expression of NF-kB and AP-1 in primary CML lymphomonocytes, Imatinib treatment was able to significantly reduce NF-kB (-92.7%) and AP-1 (-84.6%) activation. Conclusions. Our results suggest that the inhibitory activity of imatinib on both intracellular calcium levels and NF and AP-1 activation could be used as a prognostic factors to address the follow-up in patients with CML treated with imatinib.

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1204

COMBINATION OF RESVERATROL AND NILOTINIB INHIBITS CELL PROLIFERATION AND INDUCE APOPTOSIS SYNERGISTICALLY IN CHRONIC MYELOID LEUKEMIA CELLS

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Background. Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a naturally occurring phytoalexin, which presents especially in red grapes. It is a potential anticancer agent which inhibits tumor initiation and progression. This inhibitory effect of resveratrol causes cell death in various cancer cells. Nilotinib is a recently developed and an effective tyrosine kinase inhibitor used for the treatment of chronic myeloid leukemia. Nilotinib was methodically and rationally designed to create a better topological fit in the ABL kinase domain of BCR-ABL resulting in enhanced BCR-ABL inhibition. It is an aminopyrimidine derivative of imatinib, structurally changed to eliminate two energetically unfavorable hydrogen bonds with the replacement of the N-methylpiperazine ring of imatinib by a trifluoromethyl-substituted phenyl group. Nilotinib does not inhibit only BCR-ABL kinase activity, but also inhibits c-KIT and platelet derived growth factor . It only binds the inactive conformation of ABL. Aims. The natural product resveratrol has anti-cancer effects on cancer cells. Furthermore, it was demonstrated that resveratrol do not have side-effects on cells. This makes resveratrol powerful therapeutic agent in cancer. In this study, we aimed to determine the possible synergistic antiproliferative and apoptotic effects of nilotinib and resveratrol in K562 chronic myeloid leukemia cells. Methods. Human K562 cells were grown in RPMI1640 medium containing 10% FBS and 1% penicillin-streptomycin. Cytotoxicity analyses of resveratrol, nilotinib and combination of both were conducted by XTT cell proliferation assay. Then, possible synergistic apoptotic effects of both agents were carried out via measuring loss of mitochondrial membrane potential and changes in caspase-3 enzyme activity. Results. IC50 values (the drug concentration which inhibits cell proliferation by 50% as compared to untreated control group) of nilotinib and resveratrol, were found as 42 nM and 85 µM, respectively. There were 10 and 15% decreases in cell proliferation in response to 0.1 and 1 nM nilotinib, respectively, while the same concentrations of nilotinib in combination with of 85 µM of resveratrol resulted in 43 and 55% decreases in cell proliferation, respectively. 0,5 and 5 nM nilotinib or 85 µM resveratrol increased caspase-3 enzyme activity 14 and 25% or 44% respectively. But, combination of the same doses of nilotinib in combination with 85 µM resveratrol increased caspase-3 enzyme activity to 280 and 404%, respectively. Furthermore, in K562 cells exposed to combination of 0,5 and 5 nM nilotinib or 85 µM resveratrol, there were 1,26 and 1,29 or 96,60-fold loss of mitochondrial membrane potential comparing to untreated controls while combination of resveratrol with the same doses of nilotinib resulted in 106,95 and 154,47-fold increases in loss of mitochondrial membrane potential, respectively. *Summary/Conclusions*. The results of this study demonstrated that resveratrol may increase therapeutic efficiency and activity of nilotinib in chronic myeloid leukemia cells.

1205

HIGH RESOLUTION MELTING CURVE ANALYSIS FOR THE DETECTION OF SNPS IN CYP3A4 OF SESOTHO CML PATIENTS BEING TREATED WITH IMATINIB

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Background. Tyrosine kinase inhibitors (TKIs) have become the treatment of choice for chronic myeloid leukemia (CML). However, up to 5% of patients may experience intolerance to TKIs as a result of adverse drug reactions (ADRs). TKIs are primarily metabolized by Cytochrome P450 enzyme CYP3A4. Although it appears that the metabolism of TKIs, specifically imatinib, is not affected by the inhibition of CYP3A4, the role of this enzyme in ADRs to TKIs is uncertain. Allelic variants based on single nucleotide polymorphisms (SNPs) in CYP3A4 have been found to be associated with altered catalytic activity in exons 5, 7, 10, 11 and 12 that may potentially contribute to inter-individual differences in drug metabolism. Since SNP profiles differ between populations, it is important to screen all 13 exons of CYP3A4. Unfortunately, sequencing all the exons of CYP3A4 is time consuming, laborious and expensive. However, high resolution melting curve (HRM) analysis has successfully been used to screen for SNPs in different genes reducing the need for unnecessary sequencing. *Aims*. To determine whether HRM analysis can be used to screen for SNPs in CYP3A4 of Sesotho CML patients. *Methods*. Blood samples were obtained from 38 Sesotho CML patients being treated with imatinib after obtaining informed consent. DNA was extracted from samples after Trizol stabilization. HRM primers were designed for exons 5, 7, 10, 11 and 12 using the online primer design program Primer3Plus. HRM analysis was performed using MeltDoctor HRM reagent on the ABI 7500 Fast. Samples were sequenced using the BigDye Terminator v3.1 cycle sequencing kit. *Results*. Of the 38 patients screened by HRM analysis, only 3 were found not to have any SNPs. The range in Tm for variant samples overlapped with that of the reference sample making it difficult to distinguish between them based on the use of the Tm. In comparison, the use of difference plots identified a total of 3 variants in exon 5, 20 in exon 7, 25 in exon 10, 2 in exon 11 and 13 in exon 12 with SNPs. Of the 27 SNPs identified, 21 have not been previously described. *Conclusions*. HRM analysis was successfully used to detect the presence of single and multiple SNPs in exons 5, 7, 10, 11 and 12 of CYP3A4 prior to sequencing. A total of 21 SNPs, not previously described, were identified in Sesotho patients. The use of HRM analysis in this study allowed the exclusion of 112 exon sequencing reactions which is a major cost saving factor in SNP screening. The effect of these SNPs on CYP3A4 gene expression now needs to be tested.

1206

CYTOGENETIC CHANGES IN PHILADELPHIA CHROMOSOME NEGATIVE CELLS OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA DURING IMATINIB TREATMENT

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Background. Since treatment of chronic myeloid leukemia (CML) with imatinib mesylate (IM) has been improved there is an increasing number of reports on chromosomal aberrations in Philadelphia chromosome negative cells (Ph-) in patients with CML. Here we describe 5 cases of the emergence of clonal evolution in Ph- cells in patients treated with IM. Methods. The group of 76 CML patients treated with imatinib and achieving cytogenetic response were examined for the presence of chromosomal abnormalities in Ph negative cells. The methods of conventional cytogenetics (GTG) and fluorescence in situ hybridization (FISH) were used. *Results*. Cytogenetic abnormalities in Ph negative cells were observed in 5 patients in whom the first diagnosis of CML showed a sole t(9;22) (6,6% of investigated group). Such a clonal evolution of Ph- cells was observed between 12 and 36 months after starting of IM treatment (median: 27 months), after obtaining a cytogenetic response. Trisomy 8 was found in 4 cases (80%), and del(7)(q22) was present in 1 case (20%). The appearance of aberrations in the Ph- clone occurred during complete cytogenetic response (CCyR) in one patient. In the remaining 4 cases, progression in Ph- clone was revealed during a partial cytogenetic response (PCyR) in 3 patients or minor cytogenetic response (MCyR) in 1 patient with del(7). Ph- cells with aberration on time of first detection accounted from 10% to 52% of examined bone marrow. During follow-up trisomy 8 was present in 3-30% of bone marrow cells; the patient with del(7q) was not re-examined yet. None of the patients showed hematological progression after appearance of trisomy 8 in Ph- cells. One CCyR patient who developed trisomy 8 at 24 months of IM treatment, remained in complete remission with +8 clone within the next 12 months. The next patient developed +8 at the same time losing CCyR (PCyR in 38 month of treatment) and then maintained both PCyR and +8 clone up to 51 months follow-up. One patient continued in PCyR from 12 up to 56 months of therapy, when CCyR without +8 was established. The last patient maintained PCyR up to 29 months of observation with the continuous presence of +8 cells. The patients with trisomy 8 were examined retrospectively by FISH for the presence of trisomy 8 before treatment and no +8 cells have been identified. *Conclusions*. Our observations indicate that aberrations in Ph- cells appear *de novo* during of imatinib treatment in patients with cytogenetic response. The most common abnormality in this group is trisomy 8. Regardless of the variable course of the disease, the emergence of trisomy 8 is not a serious threat of imatinib failure. During time of observation no clear clinical consequences could be identified. However, the presence of other aberrations such as del(7q) may be an early symptom of acute hyperplasia.

1207

THE HMOX1 GTN POLYMORPHISM IS ASSOCIATED WITH CHRONIC MYELOID LEUKEMIA

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Background. Chronic myeloid leukemia (CML) is a complex disease with strong inter-individual susceptibility. Human genetic variation is based on the presence of polymorphisms along the DNA sequence. Polymorphisms located at the promoter or the coding region of the gene could change the level of the encoded protein or the protein activity, respectively. To avoid the damage caused by oxidative stress or diverse environmental hazards such as, xenobiotics, the cell has developed several anti-oxidant and detoxificant mechanisms. Genetic alterations in components of these protection mechanisms have been associated with cancer susceptibility. *Aims*.The aim of this study was to determine the association among CML susceptibility and polymorphism located in a battery of anti-oxidant and detoxificant. Methods.-We collected blood sample from 250 individuals with CML and 250 healthy subjects. Cases and controls were matched by age, gender and ethnicity. Single nucleotide polymorphism (SNPs) at the genes GSTP (A1404G) and NQO1 (T609C), were genotype by TaqMan assays, meanwhile short tandem repeat at HMOX1 promoter region (GTn) was determined by Gene Scan analysis. Significant differences in allelic and genotype frequency distribution and odd ratios (OR) was determined by Chi-squared and Fisher's exact test. After genotyping, all polymorphisms tested were in Hardy-Weinberg equilibrium in both, case and control population. *Results*. We found no significant differences in allelic and genotypic distribution of GSTP and NQO1 SNPs between CML and healthy individuals. However, short allele (S) from HMOX1 (GTn) polymorphisms showed a higher frequency in CML individuals compared to healthy subjects (0.18 vs 0.13). Likewise, the SS genotype were associated with CML susceptibility (OR=1.497 CI[1.23-4.78], p=0.008). *Conclusions*. Our data suggested that the short allele from HMOX1 could be a susceptibility factor against CML.

1208

KINETIC PROFILE OF BCR-ABL PROTEIN IN AN IMATINIB-EXPOSED CML CELL LINE

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Introduction. Imatinib (STI571; Gleevec) is a specific inhibitor that targets the tyrosin kinase activity of the BCR-ABL fusion protein in CML cells (t9;22). The biological response to imatinib exposure is not well understood. Using a surrogate K562 (t9;22) CML model, we examined the imatinib effect on the BCR-ABL fusion protein, Bcr-Abl fusion mRNA, cell viability, and cell differentiation. *Methods*. The K562 cells were continuously exposed to 5 µM of imatinib; imatinib was replenished at 24, 48, and 72 hours. Cells were harvested at 24, 48, and 72 hours and underwent protein, mRNA, viability, and differentiation analysis. BCR-ABL fusion protein in the cell lysate was quantitatively measured at each time-point using the BD® Cytometric Bead Array Research Use Only BCR-ABL Protein* (BD Biosciences, San Jose, CA, USA). Bcr-Abl mRNA transcripts were measured using Q-PCR assay (Ipsogen Corp, Stamford, CT). Cell viability was determined by trypan blue staining and apoptosis was determined by flow cytometry through Annexin V/7-AAD double staining. Erythroid differentiation was scored by benzidine staining and Glycophorin A as hemoglobin synthesis and erythroid lineage markers by flow cytometry, respectively. *Results*. Exposure to imatinib for 24, 48, or 72 hours reduced the BCR-ABL protein concentration by 50%, 70%, and 90%, respectively (Figure 1). At the same series of time points, the Bcr-Abl mRNA transcript level was reduced by 20%, 70%, and 90%, respectively. The fusion protein showed significantly faster reduction than fusion mRNA at 24 hours. The trypan blue and Annexin V/7-AAD stainings showed that the continuous drug exposure induced cell apoptosis in a time-dependent manner.

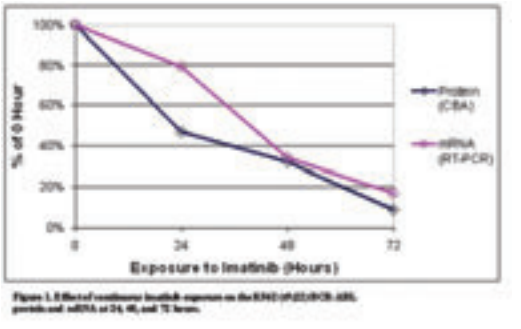

Figure 1. Effect of continuous imatinib exposure on the K562.

Appearance of benzidine-positive cells and increased Glycophorin A expression were observed after 48 hours of imatinib exposure indicating erythroid lineage differentiation. *Conclusions*. Using the K562 (t9;22) in vivo CML model, the present work suggests that the BCR-ABL fusion protein has a quicker response to imatinib than fusion mRNA at 24 hours. Both morphologic and phenotypic analyses indicate that imatinib exposure reduces cell viability, possibly through apoptosis, and induces erythroid lineage differentiation with hemoglobin and Glycophorin A expression. With the availability of the BCR-ABL Protein Kit, researchers can conduct more systematic studies on the BCR-ABL protein and its drug interaction.

1209

CHARACTERISTICS OF NILOTINIB AND IMATINIB RESISTANT CHRONIC MYELOID LEUKEMIA CELLS

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Background. Imatinib has improved the treatment of BCR-ABL posi-

tive leukemia such as chronic myeloid leukemia (CML) and Philadelphia chromosome (Ph) positive acute lymphoblastic leukemia (ALL), however, imatinib resistance is often reported in patients with advanced-stage disease by the mutations in kinase domain (KD) of BCR-ABL. Nilotinib is also a potent and selective inhibitor of BCR-ABL tyrosine kinase. In the phase 3 Evaluating Nilotinib Efficacy and Safety in Clinical Trials-Newly Diagnosed Patients (ENESTnd) clinical trial, nilotinib was demonstrated superior efficacy to imatinib with higher and faster molecular responses against CML patients. *Aims*. Nilotinib is the very high rates of responses were achieved during the first 12 months on treatment, however, there are fully not known about nilotinib resistance. *Methods*. We established the imatinib (K562 IM-R) and nilotinib (K562 AMN-R) resistant cell line and examined the mechanism of drug resistance by cell proliferation assay, microarray assay and immunoblot assay. *Results*. We first evaluated the gene expression profiles and intracellular signaling of CML cell line K562 exposed to imatinib or nilotinib. When their gene expression profiles were compared, there was an increase of 934 genes in imatinib and 1234 genes in nilotinib and 854 genes overlapped. In contrast, there was a decrease of 582 genes in imatinib and 816 genes in nilotinib and 515 genes overlapped. In apoptosis related gene, Myb and Myc gene was decreased in nilotinib treatment. We next examined the drug sensitivity of imatinib and nilotinib resistant cell line. Cell proliferation of K562 IM-R and K562 AMN-R did not decrease after imatinib (10 μM) or nilotinib (2 μM) treatment compared with parental cell line, K562. Imatinib resistance occurs through a variety of mechanisms, including BCR-ABL kinase domain mutation, and amplification or overexpression of BCR-ABL. The BCR-ABL expression was not enhanced in these cell lines compared with K562 by FISH and immunoblot analysis. The BCR-ABL kinase domain mutation was not found in K562 IM-R and K562 AMN-R cells by direct sequence analysis. We next examined the intracellular signaling by using these cell lines. One of the Src family kinase, Lyn was activated in resistant cells. The protein expression of Lyn was also enhanced. Ponatinib (AP24534), an oral multiple tyrosine kinase inhibitor, is a potent pan-BCR-ABL inhibitor with activity against imatinib resistant cells. We next examined the efficacy of ponatinib against imatinib and nilotinib resistant cell lines. We found the cell proliferation was decreased after ponatinib treatment. We also found the phosphorylation of BCR-ABL, Lyn and Crk-L was reduced and Poly (ADP-ribose) polymerase (PARP) was activated after ponatinib treatment. *Summary*. These results suggest that the expression and protein activation signatures identified in this study provide insight into the mechanism of resistance to nilotinib and imatinib and we demonstrate ponatinib has anti-leukemia effect by reducing ABL and Lyn kinase activity.

1210

COMPARISSON BETWEEN TWO QUANTITATIVE REAL TIME PCR FOR BCR-ABL DETECTION IN CML: EAC METHOD AND GENEXPERT

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Background. Recently, some efforts have been made in Europe in order to standardize BCR-ABL quantification as well as to certify laboratories which are able to express the results in an international scale applying a conversion factor (CF). However, light variations in the techniques which are used can cause CF modifications. The GeneXpert is an automated closed system for the quantification process, performing a nested-PCR using ABL as control gene. AIMS: To determine whether BCR-ABL results obtained by GeneXpert are comparable with the ones given by the standard method with the CF. *Material and Methods.* BCR-ABL was determined by both methods in 58 samples from bone marrow and/or peripheral blood of patients with CML (25 in Carlos Haya Hospital; 33 in Dr. Negrin Hospital). For the analysis with the standard method, LightCycler platform was used according to Europe Against Cancer (EAC) conditions. Both laboratories showed the results in an international scale, applying each one its CF. GeneXpert analysis was performed according to manufacturer instructions. With both methods, the quantification is automated by every equipment software and the final result is expressed as BCR-ABL/ABL x 100. The obtained results were statistically analyzed by Passing Bablok method in order to establish the relation between these techniques and by Bland andAltman to estimate the concordance. RESULTS: GeneXpert results are slightly higher (0,14761 logs greater) than the ones obtained by the standard method, being significant the difference of the means between the logarithm of the standard one corrected with CF and the logarithm of the GeneXpert ($p<0,004$). The concordance indicates that both techniques are significantly correlated, being the correlation coefficient of 0,9507 (Pearson correlation factor of 0,9571). Besides, it doesn't exist a significant deviation of linearity ($P>0,10$) between both measurements. Finally, according to the standard technique and after applying the CF, 28 samples were in MMR (≤0,1%). Out of them, 22 matched up with the results given by the GeneXpert. The other 6 showed levels of >0,1%, but very close (range 0,11-0,23%). Just 2 samples in MMR by GeneXpert didn't show the same result with the standard method once CF was applied (0,11% and 0,12%). *Conclusions*. 1) The concordance level between both measurements is quite good. 2) The result obtained with the standard method can be predicted by GeneXpert, although slightly overestimated. 3) None of the measurements showed a relevant difference between standard variable scores and GeneXpert, so that we can consider GeneXpert as an alternative and useful method to determine molecular response.

1211

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS OF TRANSPORT PROTEINS SLC22A1, ABCB1 AND ABCG2 WITH RESPONSE TO IMATINIB AND IMATINIB PLASMA LEVEL IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background, Many cases of imatinib resistance leading to suboptimal response or failure of imatinib mesylate therapy can be explained by tyrosine kinase mutations but many cases are resistant without any mutation found. It can be caused by overexpression of BCR/ABL gene or polymorphic alleles of genes involved in transport and metabolism of imatinib. Several studies regarding role of relevant polymorphisms have been done. However, their results are often heterogeneous and role of many polymorphisms remain unclear. *Aims*. To study single nuclear polymorphisms (SNPs) of one influx (SLC22A1) and two efflux transporters (ABCB1 and ABCG2) and their association with response to imatinib and imatinib plasma level in our cohort of chronic myeloid leukemia (CML) patients. *Methods*. We retrospectively analyzed response to imatinib according to European LeukemiaNet recommendations of 58 patients in chronic phase of CML treated with imatinib (400 mg /day). Patients with treatment failure due to mutations in kinase domain of ABL were not included. SNPs were detected using allelic discrimination with labeled probes. Imatinib plasma level was detected by liquid chromatography from samples taken 24 hours (+/- 2 hours) after the last imatinib dose. Statistical analysis of response was done using chi-square test. Imatinib plasma level was evaluated using ANOVA. *Results*. No significant association of different response (optimal vs suboptimal or failure) of individuals carrying homozygotic allele T or C of ABCB1 C3435T (TT vs CT or CC, $p=0.437$; CC vs CT or TT, $p=0.460$) or heterozygotic genotype (CT vs CC or TT, $p=0.923$) was revealed. Response to imatinib therapy is not associated with ABCG2 C421A ($p=0.299$) and SLC22A1 G480C ($p = 0.496$) too. No association of imatinob plasma level was observed ($n = 51$; ABCB1 C3435T, $p = 0.890$; ABCG2 C421A, $p = 0.978$; SLC22A1 G480C, $p = 0.923$). *Conclusions*. Our study did not confirm any association of analysed SNPs with different response of CML patients to imatinib as observed in some other studies. Imatinib plasma level was not significantly influenced too. More pharmacogenetic studies will be needed to validate association of genetic polymorphisms of analyzed and other transport or metabolic genes with different clinical outcome.

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1212

INCREASED LEVELS OF MYELOID-DERIVED SUPPRESSOR CELLS AND PROGRAMMED DEATH RECEPTOR LIGAND IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background. The role of the immune system in cancer biology and therapy is currently in focus. Thyrosine kinase inhibitor (TKI) therapy intended to act directly on the tumor cells in chronic myeloid leukemia

(CML) has shown interesting effects also on the patients immune cells and the immune status of these patients is therefore of interest. Anti-tumor immune reactions in cancer patients may be hampered by immune escape mechanisms, including recruitment of suppressor cells like myeloid-derived suppressor cells (MDSC) or expression of immune inhibitory molecules such as programmed death receptor ligand 1 (PDL1) on tumor cells and programmed death receptor 1 (PD1) on T cells. Immune escape mechanisms in CML have so far not been extensively studied. *Aims*. The aim of the study was to investigate immune escape mechanisms in CML patients. *Methods*. Cryopreserved leukapheresis samples (n=18) from high (n=11) and low (n=7) sokal score risk group CML patients and buffy coats from healthy controls (HC, n=21) were investigated for the presence of MDSCs (CD11b+CD14-CD33+) and the immune inhibitory cell surface markers PD1 and PDL1 by flow cytometry. The level of the MDSC-associated molecule Arginase-1 in cells from CML patients (n=5) and controls (n=5) was assessed by real time PCR. T cell proliferation upon tumor cell encounter was determined by flow cytometry. *Results*. The level of MDSCs in leukapheresis samples from CML patients was significantly higher compared to the levels in healthy controls (medians 2,6% in CML and 0,8% in HC). When CML patients were divided into sokal high and low risk groups the high risk group had higher MDSC level (median 3,6%) compared to the low risk group (median 0,8%). The level of MDSC in sokal low risk group did not significantly differ from the level in HCs. Arginase-1 is a molecule linked to the immune inhibitory effects of MDSCs. The expression level of Arginase-1 was higher in CML patients compared to HCs as assessed by real time PCR. Further, the expression of PDL1 on CD11b+ myeloid cells was higher on cells from CML patients compared to controls. There was no difference in PDL1 expression levels between sokal low risk and sokal high risk groups. PDL1 is known to inhibit T cells by binding to PD1 on the T cell surface. In our study the expression level of PD1 on CML T cells was higher compared to the expression of PD1 on healthy T cells. However, blocking PDL1 could not increase proliferation of healthy T cells mixed with CML cells. *Conclusions.* CML exert multiple immune escape mechanisms including high levels of MDSCs, Arginase-1, PDL1 and PD1 expression. Interestingly, some mechanisms investigated seem to correlate with sokal score demonstrating the relevance of immune biomarkers in CML. Treatment with TKIs lowers tumor burden and affect immune cells. It remains to investigate if TKIs could affect immune escape mechanisms in CML leading to long lasting anti-tumor control.

1213

THE BCR-ABL TRANSCRIPT LEVELS IN CML PATIENTS AT PRESENTATION HAVE NO PROGNOSTIC SIGNIFICANCE FOR THE MOLECULAR RESPONSE TO IMATINIB

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Background. The chronic myeloid leukemia (CML) outlook has radically changed since treatment with Imatinib mesylate. Responce prediction has proven useful and might guide therapeutic decisions in the era of targeted therapy. As the majority of CML patients treated with Imatinib achieve a complete cytogenetic remission (CCyR), there is a need for a molecular analysis that measures the level of BCR-ABL transcripts in order to detect minimal residual disease. *Aims*. We have studied the significance of BCR-ABL transcript levels at presentation for the response to treatment with Imatinib. The BCR-ABL levels were measured during 18 months of treatment with Imatinib. *Methods*. We have monitored BCR-ABL transcript levels by RQ-PCR in 57 CML patients with CML-CP starting at presentation. The BCR-ABL transcript level at baseline was considered in order to evaluate a correlation with MMR achievement. Transcript quantification by RQ-PCR was measured every 3 months from baseline. As a national refference laboratory for CML molecular monitoring, a conversion factor (CF=0,7838) was used for International Scale alignment. *Results*. 55% of the patients that achieved CCyR at 6 months have a baseline transcript level range between 4-78%. 13% of patients achieved major molecular response (MMR) at 6 months, having baseline transcript levels between 21-61%. The remaining 32% of patients achieved MMR at 12 months and transcript level at baseline was between 4-75%. The type o transcripts was about 60% b3a2 and 40% b2a2 in these three groups. *Conclusions*. Achieving an early MMR seems to show no correlation with BCR-ABL transcript levels at diagnosis. The level of BCR-ABL transcripts at presentation should not be a prognostic factor for the response to Imatinib treatment. This study is in progress, in order to extend the group of patients.

1214

SLC22A1 POLYMORPHISMS ARE NOT ASSOCIATED WITH CYTOGENETIC AND MOLECULAR RESPONSE TO IMATINIB MESYLATE IN CHRONIC MYELOGENOUS LEUKEMIA PATIENTS

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Background. The roles of drug efflux and drug influx proteins have been investigated in the development of resistance to imatinib mesylate (IM). The activity of IM has recently been linked with the organic cation transporter 1 (OCT1), an influx transporter, which is codified by SLC22A1 gene. However, few studies have shown the effects of SLCA22A1 polymorphisms in cytogenetic and molecular response to IM in chronic myeloid leukemia (CML) patients. *Aims*. To investigate the relationship between SLC22A1 polymorphisms and response to IM in patients with chronic myeloid leukemia (CML). *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 first group (*responder*) comprised 70 patients who had a complete cytogenetic response within 18 months of treatment. The second group (*non responder*) 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 Leukemia Net. 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. The SLC22A1 (848C>T (rs4646277), 859C>G (rs4646278), 1222A>G (rs628031) and 480C>G (rs683369)) polymorphisms were detected by Real Time PCR. *Results*. Minor allele frequencies for SLC22A1 polymorphisms were similar between responders (859G: 8.7%; 1222G: 74.6% and 480G: 12.3%) and non responders (859G: 6.2%; 1222G: 67.7% and 480G: 12.5%; P>0.05). The 848C>T variant was not detected in this sample. The frequencies of SLC22A1 (859C>G, 1222A>G and 480C>G) haplotypes in both groups were also similar (P>0.05). Association between SLC22A1 genotypes or haplotypes and MMR was not found in CML patients independently of type of IM response. *Conclusions*. SLC22A1 859C>G, 1222A>G and 480C>G polymorphisms do not influence the cytogenetic and molecular response to IM in CML patients.

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1215

VARIANT PHILADELPHIA TRANSLOCATIONS - CYTOGENETIC EVOLUTION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background. The Philadelphia chromosome (Ph1) is the hallmark of chronic myeloid leukemia (CML). Whereas the majority of Ph-positive CML patients show the standard Ph translocation involving chromosomes 9 and 22, t(9;22)(q34;q11), the minority of cases presents a variant type of Ph translocation. Available data indicate that variant rearrangements do not confer any specific phenotypic or prognostic impact as compared to CML with a standard Ph chromosome. *Methods*. The study was conducted between January 2002 and January 2011. The cytogenetic studies were made on hematopoietic bone marrow using culture for 24-48 hours on a culture medium dedicated for hematopoietic cells, followed by standard cytogenetic exam, GTG banding and karyotyping. The hematologic and cytogenetic evaluations of patients were monitored during the treatment. *Results*. Out of 87 patients with chronic myeloid leukemia cytogenetic analyzed, 8 patients had Philadelphia (Ph) negative, 71 patients had Ph positive, of which 5 patients carried a variant Ph translocation. Four patients had complex translocations involving 9q34, 22q11, and a third chromosome: t(9;10;22)(q34;q24;q11), t(6;9;22)(p21.3;q34;q11), t(7;9;22)(p22;q34;q11) and (9;11;22)(q34;p11;q11). One patient had a complex variant Philadelphia (Ph) translocations, t(8;19;22), with no obvious involvement of chromosome

9. Two patients, had after treatment the unusual secondary changes t(1;4)(q32.1;q13.2) and t(14;20)(q24.2;13.1), which were transient. *Conclusions*. The present findings strongly suggest that variant Ph translocations of CML occur as primary cytogenetic changes similar to the classical Ph1 translocations. Some complex chromosomal rearrangements are associated with rather poor prognosis and respond poorly to antileukemic treatment.

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THE EFFECT OF IMATINIB TREATMENT OF CHRONIC MYELOID LEUKEMIA (CML) ACCODING TO CLINICAL AND EPIDEMIOLOGICAL DATA OF REGIONAL MONITORING

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Background. Since 2005-2007 Imatimib (IM) has been the current standard of real-life out-patient therapy of patients with CML in the Russian Federation and is financed by the federal program. *Aims*. To evaluate the clinical effectiveness of IM in unselected population in the real-life out-patient therapy and epidemiological rates of CML on the basis of the regional monitoring from 2000 to 2010. *Methods*. We analyzed the data of the regional register of CML-patients. Clinical effectiveness criteria: 1) frequency of no 'failure' after 18 month according to the ELN, 2009, 2) cumulative frequency of complete cytogenetic response (CCgR) at the moment of the last research after ≥ 12 months of IM treatment. The analysis of IM effectiveness has been carried out in the three groups of patients: 1st - the early chronic phase (ECP) - ≤6 months from the establishment of CML till the beginning of IM treatment, 2nd - the late chronic phase (LCP)-> 6 months from the establishment of CML till the beginning of IM treatment (7 to 127, median 23) and 3d - the progression phase (PrP) which combined the patients with the accelerated phase and the blast crisis. Epidemiological rates: incidence, prevalence, mortality were calculated by the standard methods of the variation statistics to the number of the acquisitive population (adults >14 years) with an assessment of average multiyear rates, multiyear dynamics and trends. Statistical validity was determined by the method Chi-square. *Results*. Since 2000 CML, Ph-positive was established in 248 patients, the age (13-82 years, a median 52, including 2 children cases), sex (M-112, F-135), 161 CML patient have received IM treatment: 76 in ECP, 75 in LCP and 10 PrP. The frequency of no 'failure' after 18 months and the cumulative frequency of CCgR at the moment of the last research after ≥12 mouths of IM treatment are presented in the table 1. Nobody in PrP has not reached CCgR. The IM treatment has been stopped in 42 (25.5 %) to 161 patients. The average multiyear incidence rate was 0,77±0,080/0000: max 1,040/0000 in 2004, min- 0,640/0000 in 2008, without trend (T=-0,04%). The quantity of deaths of CML patients was 197. The average multiyear mortality rate was 0,6±0,120/0000: max 1,1 0/0000 (in 2000), min 0,170/0000 (in 2010), - with a marked trend to decreasing for 10 years of mortality (T =-6,6%). The average multiyear prevalence rate was 3,6 + 0,420/0000: max 4,90/0000 in 2010, min 2,70/0000 in 2002, - with a marked trend to growth of CML prevalence for 10 years (T=+7,8%), especially for the last 5 years. *Conclusions*. IM in real-life out-patient therapy of CML patients has shown high clinical effectiveness without changes of average multiyear incidence rate with decreasing average multiyear mortality rate and increasing average multiyear prevalence rate.

	ECP (n=76)		LCP (n=75)		p
	n	%	n	%	
IM treatment ≥18 months	37	48.7	68	90.6	
No "failure" after 18 months	24	64.9	32	47.1	0.05
IM treatment ≥12 months	45	60.5	71	94.7	0.05
CCgR at the last investigation	39	86.7	44	62	

1217

LONG-TERM RESULTS OF IMATINIB THERAPY AND SURVIVAL IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA RESISTANT OR INTOLERANT TO INTERFERON- ALPHA THERAPY

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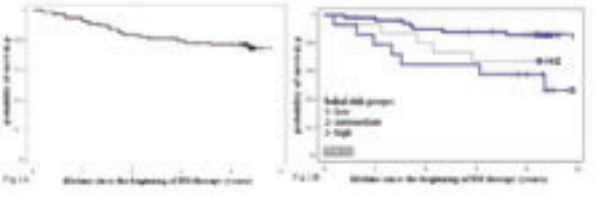
Background. In Russian Federation a selective BCR/ABL tyrosine kinase inhibitor (TKI) imatinib (IM) was introduced for the first time in 2000y for chronic phase (CP) CML patients(pts) after interferon α (IFN-α) failure. The 2nd and more potent generation of TKI (TKI-2) became available since 2005-2007y. At present time we summarize the long-term therapy results. *Objectives*. To evaluate the efficacy of 1st and 2nd generation TKI therapy: frequency and stability of complete cytogenetic response (CCyR), frequency of major molecular response(MMR) and complete molecular response (CMR), incidence of second tumors and overall survival (OS). *Patients and treatmen*t. The analysis included 79pts. Criteria for inclusion into an open non-randomized study were: CP CML; diagnosis confirmation by standard cytogenetic study; IFN-α therapy discontinuation and initiation of IM therapy from Jul.2000 till Sep.2001. Median (Me) age 39 (15- 64) years, males:females =41:38. Distribution by Sokal groups: low-50 (63%) pts, intermediate + high - 15(19%) pts plus 14(18%) pts. Me duration of CML before IM therapy was 35.1 (3-157) months: less than 1 year in 12pts (15%), from 1 to 5 years in 50pts (63,5%) and more than 5 years in 17pts (21,5%). Me period of IFN-α pretreatment was 26 (0,5-156) months. Me duration of IM therapy (Jul2010) was 80 (2,4- 118) months. The initial dose of IM was 400mg/day. Me duration of TKI-2 therapy was 31 (2-51) months. 4 (5%) pts received more than two TKIs-2. Me duration of CML (from diagnosis to analysis- July 2010) was 120.5 (13-259) months. Statistical analysis was performed using the package v.SAS9.1.3. *Results*. 59 of 79pts (75%) alive on Jul2010 received TKI therapy: IM 34 (43%)pts /TKI-2 24 (30%)pts/ 1(1,3%)pt treated by hydroxyurea (resistant to all available TKI). For the entire observation period CCyR was obtained in 64%(51pts). The cumulative incidence of CCyR was 54% (43pts) with a Me to CCyR 9 months (5-53). Acquired cytogenetic resistance was observed in 16 of 43 (37%)pts (or 20% of the total group). CCyR was again achieved on IM for 27(34%) pts (still on IM) and for 8(10%) pts on TKI-2 therapy for the first time. 34(43%) pts with CCyR continue IM therapy (27 of them have stable CCyR). MMR in pts with CCyR on IM therapy was observed for 21 (26,7%) of 79 pts, CMR was achieved in 11 (14%) pts.



Discontinued IM therapy 45(57%)pts and 29 (35%)pts were switched to TKI-2 treatment. 20(25%) pts died. Death causes were: progression to advanced CML phases (16pts), brain hemorrhage (2pts), generalized infection (1pt), hepatocellular carcinoma (1pt). Importantly, during the 9-year IM therapy period there were only 2 (2,5%±1,8%) cases of secondary malignances (1 pt alive with CMR). Estimated 9-yr OS of all pts on TKIs is 75% (Figure 1A), progression-free survival is 78,5%. OS (108 months) by Sokal risk groups is 86% /67%/50% for low, intermediate and high risk group accordingly (p=0,0094)(Figure 1B). *Conclusions*. The results showed high survival rates and efficacy of IM and TKI-2 therapy in a special long-time observed group of patients in CP CML with IFN-α failures, with rare second tumors detection.

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CYTOKINE SYNTHESES BY T-CELL SUBSETS OF CML PATIENTS PRIOR TO AND DURING IMATINIB THERAPY: RELATION BETWEEN THE LEVEL OF RESPONSE TO THERAPY (OPTIMAL VERSUS NO OPTIMA) AND ENDOGENOUS T-CELL FUNCTION

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Background. The majority of imatinib (IM) treated chronic phase chronic myeloid leukemia patients achieved complete haematological and cytogenetic remission but relatively few of them achieve complete molecular remission. Although T-cells cytokines role in the long term

control of CML is well established, previous studies showed contradicting results regarding imatinib effect on the endogenous T-cell function by IM. The variability in the patients population included in these studies may contributed to this where in some of them cells were obtained from patients in CcyR or patients with resistant/intolerance to previous therapy with IFN-alpha. *Aims*. the purpose of this study was to determine the relation between the endogenous T-cell function prior to therapy and the degree of response to IM therapy in CP CML. In addition, modulation of the endogenous T-cell function during IM therapy was studied. *Methods*. Using flow cytometry, we studied Th1 and Th2 cytokine synthesis by PMA activated CD4 and CD8 T cell subsets of 22 patients with newly diagnosed CML in chronic phase prior to and during IM therapy compared to that of 5 patients with IM resistance and 10 healthy donors. Patients were grouped into optimal or no optimal response according to the disease status at last follow, disease progression or treatment failure. All patients and healthy donors signed and informed consent. *Results*. In 11 patients with optimal response, pre treatment cytokine studies showed lower Th1, but higher percentage of e Th2 and TNF-α producing CD4 and CD8 T cells compared to that of healthy donors. Significant higher levels of Th2 and TNF-α producing CD4 and CD8 T cells were found compared to those from patient with no optimal response and those known to have IM resistance. Lower level of Th2 and TNF-α producing T cells were found in the last 2 named groups of patients compared to that of healthy donors. Cytokine response with shift from Th2 profile to Th1 profile was detected early during IM therapy (6 weeks) in patients with optimal response coinciding with the CHR in addition to decline of TNF-α producing CD4 and CD8 T cells. This immunological response of T cell subset during IM therapy preceded both the cytogenic and molecular response which was maintained throughout the follow up period. In patients with no optimal response initially increase in the percentage of IFN-γ and TNF-α synthesizing CD4 and CD8 T cells at 6 weeks of therapy to levels exceeding that of healthy donors and those with optimal response followed by a rapid decline in the cytokine levels at later follow ups. *Conclusions*. CP CML patient with optimal response has Th2 cytokine and suppressed Th1 cytokine profile at diagnosis with restoration to normal levels during therapy. On the other hand patients with no optimal response and those known to have IM resistance showed suppression of Th1, Th2 cytokines and TNF-α. The role of pre treatment indigenous IL-4 and TNF-α in determining the response to IM therapy needs further investigation.

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IMPACT OF SWITCHING THERAPY FROM IMATINIB MESYLATE TO GENERIC COPY OF IMATINIB ON HEMATOLOGIC RESPONSE IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA: SINGLE CENTER STUDY

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Background. Imatinib mesylate is a tyrosine kinase inhibitor of BCR-ABL; it has revolutionized the treatment of chronic myeloid leukemia (CML). Imatinib is widely accepted as the standard of care for the first-line treatment of CML due to its high hematologic, cytogenetic, and molecular response rates and favorable long-term safety profile which has been documented in many studies. A copy version of imatinib, has become commercially available .The lower price has prompted some health-care authorities in Middle East to substitute the copy for the branded imatinib. *Aims*. The aim of this study was to evaluate hematologic responses of chronic phase CML patients who switched from imatinib mesylate to generic copy of imatinib. *Methods*. A prospective study conducted at the national center of hematology in Baghdad from January 2010 to November 2010.It included 126 patients with chronic phase CML who were switched from imatinib mesylate to a generic copy. At time of switching, all patients were in complete hematologic response and received generic copy of imatinib for at least 9 months. Informed consent was obtained. Physical examination, Complete blood count, and peripheral blood smear were performed in order to assess hematologic response. Evaluation of patients done at 3 month, and 6 months interval. *Results*. Eighty-four (66.6%)of the patients showed sustained durable hematologic response after switching that had no significant relationship with a patient's age or sex (P>0.05). while 42(33.3%)of patients lost their response to imatinib after switching to generic copy . Twenty-two out of 42 patients (52.3%) lost hematologic response after 3 months(4 patients had blast crisis, 18 accelerated phase).after 6 months ,twenty patient (47.6%) out of 42 had substantial hematological change(anemia, leucopenia ,and thrombocytopenia) in whom FISH study showed that they had cytogenetic relapse. *Conclusions*. The use of generic copy of imatinib instead of imatinib resulted in inferior outcome of a previously stable response. Loss of hematologic response in CML can lead to grave consequences of accelerated phase or blast crisis phase of the disease.

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THE RESPONSE TO TYROSINE KINASE INHIBITORS IS PREDICTABLE BY *IN VITRO* ANALYSIS OF BCR- ABL PHOSPHORYLATION

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Background and Aims. Although tyrosine kinase inhibitors (TKIs) targeting Bcr-Abl have dramatically improved the treatment of CML, a substantial proportion of the patients treated with imatinib have been reported to exhibit either primary or secondary resistance or intolerance. To overcome the resistance and intolerance to imatinib, second- and third-generation TKIs, such as nilotinib, dasatinib, bosutinib and INNO-406 have developed. In parallel with the availability of new therapeutic compounds, we need more information to select the best TKI. In this study, to establish a system with which we can predict the response of each patient to TKIs, we investigated the phosphorylation of Crkl, a major target of Bcr-Abl, after in vitro incubation with or without TKIs in peripheral blood (PB) samples from patients either newly diagnosed or resistant to imatinib. *Methods and Results*. Ten mL of PB samples were obtained from thirty-one patients with CML in the chlonic phase (CP) with informed consent at the beginning or before the initiation of imatinib, nilotinib or dasatinib. Half of each sample was used for examination of the Bcr-Abl sequence, and the other half was used for immunoblot analysis. From the preliminary experiments to establish the experimental procedures, incubation time was optimized to 5 hours. The concentrations of imatinib, nilotinib and dasatinib were fixed from pharmacokinetics to 5μM, 5μM, and 0.1μM, respectively. To quantify the in vitro responsiveness to TKIs, we measured the density of each blot using a densitometric method, and then defined "Residual Index (RI)"for each TKI by the numerical expression. Newly diagnosed patients (n=15) were separated into two groups in accordance with the most recent outcome, the imatinib-sensitive (n=13), who achieved an optimal response after the sample collection, and the imatinib-resistant (n=2), who did not. The median RI of the patients in the sensitive group was 4.2% and that in the resistant group was 43.2% (p<0.05). Among 8 patients imatinib resistant had undergone nilotinib-therapy, the median RIs of 4 achieved optimal responses and those of 4 failed were 3.5% and 31.2%(p<0.05), respectively. Also in the dasatinib treated patients, RIs of three achieved response were almost 0%, and that of one failed was 92%. Imatinib-responsiveness could predict with 100% of sensitivity and 77% of specificity when the RIs were separated at 10%. On the other hand, although the sample size was small, the immunoblot analysis was able to predict the clinical responsiveness to nilotinib or dasatinib with 100% sensitivity and specificity. We also examined the relation of RIs with Bcr-Abl point mutaions. Although, among 4 patients with mutations, RIs in 3 cases agreed with the reported IC50s of the mutations, the RIs in one patient were contradictory to the reported responsiveness to nilotinib or dasatinib but consistent with his clinical outcome. Conclusions. The immunoblot system described here has the capacity to detect TKI-resistant subclones, including CML cells with Bcr-Abl mutations, and when used together with the cellular IC50 values and Bcr-Abl sequence, this immunoblot system should help improve the treatment of patients with CML.

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SKIN REACTIONS DUE TO IMATINIB TREATMENT IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML)

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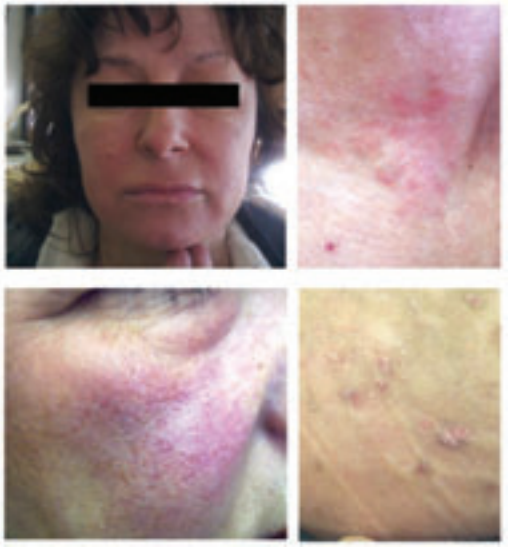
Background. Imatinib (IM) is current standard treatment for newly diagnosed patients (pts) with CML. Treatment with IM is generally well tolerated, and the risk for severe adverse events is low. Cutaneous reactions to imatinib are common and occur in 9.5% to 69% but in general are mild. Edema, periorbital edema, maculopapullar and erythematous eruptions are the most common side events. Imatinib can also induce severe skin eruptions (generalized rash, purpuric vasculitis, mycosis fun-

goides like reaction). Rare changes are hyperpigmentation, psoriasis and pithyriasiform eruptions. *Aims*. The aim of our study was to analyze cutaneous changes during IM treatment in large cohort of our CML pts. *Methods*. Between 2002 and 2010, 110 pts with CML were treated with IM in our institution. IM therapy was commenced in a dose of 400 and in some cases escalated to 800 mg/d.



Results. In our group, 76 patients (69%), 40 males and 36 females, mean age 39 years (28-78 yrs) had some skin changes. All pts were in chronic phase CML, 30 pts had low risk, 27 pts intermediate and 19 pts high risk Sokal score. Periorbital edema was the most common, occurring in 70 pts (64%); almost all had typical, mild form, pronounced in the morning. But 5 pts had also severe edema (CTC Gr. 2), without cessation of imatinib therapy. Edema developed in our group from two weeks to 20 months of IM treatment (median 12 months) and was treated by various means, e.g. low-salt diet, oral diuretics and in some cases topical 1% hydrocortisone. Six patients (5.4%) had other different cutaneous reactions. Two patients had acute maculopapullar rash (CTC Gr. 2) with pruritus, needed topical steroids bid during two weeks. One patient had worsening of vitiligo with extension in areas and pronounced edge hyperpigmentation, and second one worsening of psoriasis but responded to more frequent psoriatic treatment. Two previously healthy patients had a severe, recidivant psoriasis-like reaction with subcutaneous edema and pruritus from 3rd week of IM treatment proven by skin biopsy (CTC Gr. 2), responding to pause in IM treatment and to topical/systemic steroids. Unfortunately, in one patient due to recidivant skin changes IM treatment was discontinued for good. We have not found any correlation of any changes with Sokal score or some other pretreatment variable. *Conclusions*. Imatinib has many mild adverse events but the incidence and management of cutaneous side effects has been rarely reported. Most cutaneous eruptions caused by IM do not need discontinuation of treatment and are usually self limited. Administration of oral or topical corticosteroids can ameliorate some of imatinib induced cutaneous changes without need for treatment cessation.

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ECONOMIC BURDEN OF PROGRESSION IN PATIENTS WITH PHILADELPHIA CHROMOSOME-POSITIVE CHRONIC MYELOID LEUKEMIA (PH+ CML) IN CHRONIC PHASE

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Background. The ENESTndstudy showed that after 12 month of therapy, newly-diagnosed chronic phase (CP) Ph+ CML patients (pts) receiving nilotinib (300 mg BID) had a significant superior major molecular and complete cytogenetic response and improvement in the time to progression to accelerated phase (AP) or blast crisis (BC), compared with those receiving imatinib (400 mg QD) (P=0.01). *Aims*. To estimate the economic burden associated with early progression to AP in the US. Methods: A literature-based Markov model was developed to estimate the 5-year economic burden associated with early progression to AP despite first-line therapy. The model follows longitudinally cohorts of hypothetical pts who have progressed to AP after failure of first-line therapy in CP. Pts in the AP phase were assumed to initially receive therapy with a second generation TKI. These pts could discontinue this second generation TKI therapy due to intolerance or progression to BC. Patients who were off TKI (in the AP or BC) phase were assumed to receive primarily supportive care and/or transplant. The rate of transplantation in the latter patients was approximately 5% per year.

Resource	Avg Cost/Pt
TKI drug costs (including AE management)	$191,805
Inpatient care	$91,743
Transplants	$11,710
Physician and Nurse Visits	$9,964
Tests	$5,749
Palliative care	$3,323
Direct Costs	**$314,294**
Indirect Costs	**$176,179**
Total	**$490,473**

Prognosis was modeled using published data. Non-TKI-drug costs and productivity loss were assumed to increase as disease progressed. Quality of life varied by disease stage and treatment response. Resource use and costs were obtained from published estimates. Productivity was estimated using the human capital approach and considered the opportunity cost of CML-AP (i.e., productivity of progressed pts minus productivity of non-CML age/gender-matched individuals). Results: Pts who have progressed to AP experience high direct medical ($314,294) and indirect costs ($176,179) (TABLE) and poor prognosis (5-year overall survival=24%, average survival 3.74 years of which 2.66 years in AP and 1.09 years in BC, average quality adjusted life years = 2.11). 87% of costs were assumed to occur in AP and 13% in BC. Overall, the total direct and indirect costs of progression were $490,473/pt. *Conclusions*. In addition to poor survival prognosis, CML disease progression is associated with a heavy economic burden. Preventing progression is therefore an important therapy goal in CML- CP.

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EARLY CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS WITH IMATINIB FRONTLINE THERAPY : INCIDENCE AND CHARACTERISTICS OF PATIENTS ACHIEVING A COMPLETE MOLECULAR RESPONSE

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Background. Frontline therapy with imatinib (IM) have dramatically improved the survival of chronic phase chronic myeloid leukaemia (CML-CP) patients. While treatment with IM is effective, most of the patients have detectable residual disease documented by real-time quantitative polymerase chain reaction (RQ-PCR) and will have to continue IM at least indefinitely. Only a minority of them will achieve a complete molecular response (CMR). As sustained CMR can lead to cessation of IM without relapse in some cases, so it is the major goal of treatment and become the molecular endpoint in future trials with tyrosine kinase inhibitors. *Patients and methods.* We analyzed the data of previously untreated CML-CP patients who received IM as initial therapy in our department in order to identify the incidence and median time to CMR and characteristics of the patients who achieved a CMR under IM therapy. CMR was defined as CMR as undetectable BCR-ABL transcripts with sensitivity of at least 4.5 logs and undetectable BCR-ABL transcript on two consecutive assessments at least two months apart. Loss of CMR was defined as detectable BCR-ABL transcripts on two consecutive assessments. *Results*. Between September 2000 and December 2009, 161 ECP-LMC patients were initially treated with IM 400 mg (n=134-83%) or 600 mg daily (n=25-16%) or 800 mg (n=2-1%). The median age at diagnosis was 56.7 years [18.5-88.9] and 63% were male. The Sokal risk score was low in 28%, intermediate 42% high in 21%, unknown in 9%. Clonal evolution was documented in 12 patients (7%) at the time of

diagnosis. The median follow-up on imatinib therapy is 44.8 months [2.53-122.24]. One hundred and thirty patients (80.7%) achieved a CCR with a median time to CCR of 6.38 months, 116 (72.5%) a major molecular response (MMR) with a median time to MMR of 15.32 months. Fifty patients (31%) had stopped imatinib du to failure (n=22, 13%), intolerance (n=12, 7%), suboptimal response according to the molecular criteria (n=8, 5%) and participation to the French study STIM (n=9, 6%). The median time to imatinib cessation du to lack of efficacy was 27.09 months [2.53-110.93]. Fourty-six patients (28%) achieved a CMR. Median time to CMR was 35.3 months. The characteristics of those patients are detailled above (table). The median duration of CMR was 15.65 months [0.00-81.31]. *Conclusions*. Preliminary results suggest that patients who achieved a CMR had a faster MMR than the other . The identification of factors correlated with CMR in CML-CP treated with imatinib as frontline therapy is ongoing.

Table. Characteristics of the 46 patients who achieved a CMR as defined previously.

N= 46				
Median follow-up (month):	70.51 [33.07-122.24]			
Male:	57%			
Age (y):	median 56.3			
Sokal risk score:	low	intermediate	high	unknown (%)
	37	43	15	5
Clonal evolution (%):	15			
Splenomegaly(%)	yes	no	unknown	
	22	74	4	
Imatinib initial daily dose	400 mg	600 mg	800 mg	
	76%	22%	2%	
Median time to CCR (month)	5.97 [2.63-50.66]			
Median time to MMR (month)	10.82 [2.76-50.66]			
Imatinib plasma level (%):	< 1000 ng/ml	> 1000 ng/ml	Unknown	
	48	43	9	

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DASATINIB EVEN AT LOWER DOSES IS AN EFFECTIVE FOR CHRONIC MYELOID LEUKAEMIA TREATMENT IN PATIENTS RESISTANT OR INTOLERANT TO IMATINIB

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Background. Dasatinib a multitargeted tyrosine kinase inhibitor (TKI) is effective for the treatment of chronic myeloid leukaemia (CML) patients resistant or intolerant to imatinib. The recommended daily dose is 100 mg/day for patients in the chronic phase (CP) and 140 mg/day in the accelerated or blastic phases (AP, BP). However it is not always possible to keep the dose. The major reason is CTCAEv.3 grade 3/4 adverse events as well as persisting symptoms of prior treatment toxicity. *Aims*. To evaluate the efficacy of reduced dose of dasatinib on CML patients resistant or intolerant to imatinib managed in the context of every day clinical practice. *Methods*. We evaluated the outcome of 24/49 patients (48%) treated with reduced doses of dasatinib after the switch due to resistance and intolerance to imatinib. Group A: Daily dose of dasatinib was reduced in 14/24 patients due to haematological or nonhaematological adverse events (AEs) grade 3/4 on standard dose. Group B: patients with persisting symptoms of AEs prior imatinib toxicity (10/24). Two regimens were used: dasatinib 50 mg/day or alternative doses of dasatinib 50 mg and 100 mg/day. The median duration of reduced dosing treatment was 6 months (3-24) in group A, with total dasatinib duration 40 months (10-60), and 10 months (2-18) from total 28 months (14-39) in group B. Patients were followed by routine haematological and cytogenetic assessment and molecular monitoring. Resistant patients (n=15) were screened for baseline BCR-ABL mutations, in 6 patients mutations were identified. *Results*. Group A: After dasatinib dose reduction there was no worsening in response quality (i.e. cytogenetic and molecular) in 9/11 patients and quality of life improved with AE remission. The 10/11 (90%) patients survived for median 40 months (10-60), 9/11 (82%) without progression. In 5 patients 5 types of baseline mutations in BCR-ABL kinase domain were identified. Three of these completely disappeared during the treatment, in one patient the mutated clone declined from 100% to 23%. In one patient the mutated clone remained 100%. In two patients (with existing F311I and Y253H mutation) the further progression to AP was solved with nilotinib followed in one with allotransplantation, while the second one died from progression. One patient interrupted dasatinib treatment for pregnancy period (10 months). After dasatinib readministration she re-achieved CCgR and MMoIR. Group B: CCgR was achieved in 8/10 patients (80%) and MMoIR in 5 of them after 4-12 months. In other 3 pts even CMoIR was accomplished. In 2 pts CCgR has not been achieved yet, one of them had baseline E459K mutation, which disappeared after 9 months. No other mutation occurance was detected. All patients are alive with satisfactory quality of life with no progression. *Conclusions*. Our findings from clinical practice show the efficacy of reduced doses of dasatinib inducing or maintaining response in CML patients resistant or intolerant to imatinib. Further experience are warranted to confirm our findings.

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THERAPEUTIC RESULTS WITH IMATINIB IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA ACCORDING TO THE EUROPEAN LEUKEMIANET CRITERIA

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Background. Imatinib has changed the evolution of CML from a disease with an average survival of 3-5 years to a disease with a survival of 86% at 7 years. The implementation of the ELN criteria allows us to check the optimal response and to change the therapy in case of suboptimal response or therapeutic failure. The aims of this study are to evaluate the quality of the hematological, cytogenetic and molecular response, the overall survival and event free survival for 2 lots of patients treated with imatinib as a first and second line of therapy. *Methods*. This is an observational study, retrospective (01.01.2001-12.31.2006) and prospective (01.01.2007-01.07.2010). 170 patients with CML in chronic phase have been treated and monitored in 2 departments of haematology from Romania (Fundeni Clinical Institute and Emergency Clinical County Hospital Brasov). The collected data have been analyzed using the SPSS programs (Kaplan Mier method/log rank test Mandel Cox). *Results*. 56 patients (33%) treated with imatinib as a first line (median age of 41,5 years) and 114 patients (67%) treated with imatinib as a second line of therapy (median age of 50,4 years) have been included in this study. After 3 months of therapy, CHR was 91,07% in the first line lot and 84,2% in the second line lot. After 6 months, the rate of CHR was similar in both lots: 98,2% fot the first line and 95,8% for the second line, without significant diferences (p=0,508). The probability to lose the CHR until the 48th month is significantly higher for patients in the second line group comparing to the patients in the first line group (p=0,045). After 12 months, CCR was observed at 48,2% of first line patients and only at 17,5% of second line patients. The rate of MCR is 82,1% for the first line of therapy group and 68,7% for the second line of therapy group. CCR achived after 12 months was sustained at 48 months by 65% second line of therapy patients comparing to 96,3% first line therapy patients.(p=0,000019) After 18 months, 33,9% of first line therapy patients achived MMR, this type of response being sustained by 100% of the patients at 48 months. Event free survival after 48 months is superior in the first line group (80,3%) comparing to 52,6% in the second line lot (p=0,0152). The overal survival at 48 months is similar in both lots: 94,6% in the first line and 88,6% for the second line (p=0,46). *Conclusions*. Imatinib 400 mg/day induces high rates of CHR, MCR and MMR, these responses being sustained at 48 months by patients in the first line of therapy. The overal survival at 48 months is significantly improved in both of the lines of therapy lots.

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STUDY OF EFFECTIVENESS RECOMBINANT HUMAN ERYTHROPOIETIN IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH ANEMIA INDUCED IMATINIB THERAPY

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Background. Imatinib is a tyrosine kinases inhibitor (TKI) that revolutionaryly changed prognosis and survival in patients with chronic myeloid leukemia (CML). It allowed to achieve not only cytogenetic but so molecular response. TKI may cause hematological toxicity according to anemia, leukocytopenia and thrombocytopenia. *Aims*. To investigate the effectiveness of Recombinant Human Erythropoietin (rHuEPO) in CML patients who developed anemia as toxic effect of Imatinib on erythropoiesis. Methods. 90 patients with CML received Imatinib in

dosage 400-800 mg daily during 6-48 months. The patients were in chronic (n=82), accelerated (n=6) phases and blast crisis (n=2). The median age of patients was 62.4 years. 36 patients revealed anemia. The level of Hb in 18 patients was less than 10,0 g/dl and they werw administrated rHuEPO. If Hb concentration was <8,0 g/dl, the patients were performed red blood transfusions before rHuEPO treatment. rHuEPO was injected subcutaneously on 10.000IU 3 times a week (30.000IU a week). The target Hb level was 12,0 g/dl. *Results*. The anemia was revealed in 36 patients (40%) for the period of observation since 6 to 48 months. I degree (by WHO classification) was at 29 patients (32.2%), II degree - 5 (5.6%), III degree - 1 (1.1%) and IV degree - 1 patient (1.1%). 18 patients had level Hb less 10.0 g/dl and they needed correction of Hb concentration. CML patients with anemia were in chronic phase (complete cytogenetic response was in 11 patients and partial cytogenetic response - 4), acceleration phase (n=2) and blast crisis (n=1). Mean baseline Hb concentration was 9.24±1.08 g/dl (5.9-10.0 g/dl), RBC count was 2.89±0.47x1012/l (1.78-3.60 x1012/l) and Ht was 29.9±4.4% (19.9-38.0%). Two patients with III and IV degree of anemia (Hb 5.9 and 7.4 g/dl, respectively) before rHuEPO-therapy received red blood transfusions to correct the main symptoms of anemia and Hb concentration. Besides both patients was stopped treatment with Imatinib within two weeks. The period of rHuEPO-therapy was from 4 to 20 weeks (mean 9.7±4.6 weeks and median follow-up of 9 weeks). During the study period in whole group, the Hb concentration, RBC count and Ht increased from baseline to 12.41±2.08 g/dl (p<0.02), 3.80±0.64x1012/l (p<0.01) and 37.4±7.0% (p<0.02) before rHuEPO-therapy, respectively. An Hb increase >2 g/dl or achieving the target Hb level 12,0 g/dl was observed in 13 patients (72.2%), while a non-response was observed in 5 patients (27.8%). 5 patients (27.8%) needed the dose reduce (from 30.000IU to 20.000IU a week) because of too high response (Hb concentration increased <2,0 g/dl during 4 weeks). 12 positive response patients were observed above 18 months and needed in repeated administration of rHuEPO-therapy (3-4 times) because of anemia relapsed (Hb decreasing <10,0-11,0 g/dl). However we could to continue administration of Imatinib without break off. *Summary*. rHuEPO is effective at increasing Hb and prevention of stop therapy in patients developed anemia as toxic effect of TKI.

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THE GROWTH OF A PH-NEGATIVE, MONOSOMY 7 CLONE ARISEN UNDER SECOND-GENERATION TYROSINE-KINASE INHIBITOR THERAPY IS UNEXPECTEDLY SLOW DOWN BY BCR-ABL IN A CHRONIC MYELOID LEUKEMIA (CML) PATIENT

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Background. The appearance of clonal abnormalities in the Ph-negative metaphases during second-generation tyrosine-kinase inhibitors (2nd-TKI) therapy has been recognized in CML pts, but the frequency and clinical implications of this event have not been assessed. *Aims and Methods*. The present study describes the unusually indolent course of a -7, BCR-ABL negative acute myeloid leukemia developed in a Ph+ CML during treatment with a 2nd-TKI. *Results.* In June 2006, a 58-year-old man affected with CML switched from imatinb to dasatinib because of a less than partial cytogenetic response after 18 mos. Under dasatinib, he achieved a partial response and a near-major molecular response (BCR-ABL mRNA transcript: 0.5%-0.9% according to International Scale, IS). After 48 mos, he developed grade II cytopenia and monocytosis (2.66x10^9/L), with 10% myeloid blasts (CD34+ CD117+, CD33+, Cd13+, DR+) in peripheral blood. At this time, bone marrow biopsy documented 40%-50% of CD34+, CD117+, KP1/CD68+, PGM/CD68R+, MPO-, CD56- blasts. Bone marrow aspiration was consistent with acute leukemia, as 50% of atypical promonocytes and blasts were detected. On immunophenotyping 2 blast cell populations were observed: 26% CD34+, CD117-, CD33+, DR+ blasts and 40% CD34+, CD117+, CD33+, CD14+, DR+, Cd11c+ blasts. On RT-QPCR, BCR-ABL mRNA transcript was 0.5% (IS). Conventional karyotype was 46,XY, t(9;22)(q34;q11)[9]/45,XY, -7[11], and FISH on peripheral blood revealed monosomy 7 in 240/300 (80%) of the interphase nuclei (LSI D7S486 (7q31) SO/CEP7 SG probes, Abbott Mol.) and BCR-ABL rearrangement (LSI BCR/ABL ES dual color translocation probe, Abbott Mol.) in 8% of the interphase nuclei. MLL and c-MYC rearrangements were absent and RT-PCR excluded FLT3-ITD mutations. Interphase FISH revealed that the abnormal clone was undetectable at CML diagnosis, but was present in 6% of interphase nuclei at the time of switching to dasatinib and progressively increased over time in 48 mos. Dasatinib was discontinued and no other treatment was initiated. At this time, cytopenia improved to normal values, peripheral and bone marrow blast percentage slightly decreased, and the patient was in good clinical condition. At the latest evaluation (6 mos after documentation of overt leukemia), BCR-ABL transcript load was 3,4% (IS) and marrow karyotype was 46,XY, t(9;22)(q34;q11)[8]/45, XY, -7[11]/46,XY, del(7)(q31;q35)[1]. Interphase FISH showed that monosomy 7 was present in 199/300 (66%) nuclei and BCR-ABL rearrangement in 18% nuclei. *Conclusions*. Literature data suggest that monosomy 7 is an ominous sign when detected in Ph- cells under TKI therapy. Our case shows an unexpected discordance between the unfavorable cytogenetic and morphologic data and the indolent clinical course. Despite the unequivocal morphologic and immunophenotypic findings, the -7, Ph-negative leukemic clone expanded very slowly over 4 years under the selective pressure of the second TKI. This case is noteworthy, as it represents a therapeutic dilemma: the patient refuses both allotransplant from an unrelated donor and conventional chemotherapy, as without any treatment he feels well, with normal Hb level, and neutrophil and platelet counts. On the other hand, therapy with another TKI has no sense and new drugs effective in leukemic progression of Ph-negative myeloproliferative neoplasms have limited efficacy in Ph-positive CML.

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BCR/ABL MUTATION ANALYSIS IN CML - SIGNIFICANCE AND CONTROVERSIAL ISSUES

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Despite high efficiency of modern treatment of CML, the issue of disease resistance remains important. Among its main contributors are BCR/ABL gene mutations; however, their significance for occurrence of resistant clone is a subject of debate. This study comprises results of mutation analysis in a small group of patients with different stages of CML, including newly diagnosed. Aim of the study was to analyze efficacy of treatment with tyrosine kinase inhibitors (TKI) depending on presence and type of BCR/ABL mutations; to investigate significance of the method for understanding CML resistance; its importance for treatment selection. Methods. BCR/ABL mutation status was investigated in 19 patients with different stages of CML. In 16 of them it was tested due to disease progression or resistance; 3 patients were investigated at the initial diagnosis. BCR/ABL kinase domain mutations were investigated by method of direct sequencing. Results. The group of newly diagnosed patients included 2 chronic phase subjects and one in blast phase (BP). None of them was found positive for mutations. The second group included 15 patients tested for mutations at treatment change to nioltinib, the only 2nd line TKI registered in Ukraine. One patient was investigated due to decision to switch from interferon to imatinib. BCR/ABL mutations were detected in 6 of these patients (37,5%). In one case M351T mutation was detected. Treatment with nilotinib was started and appeared ineffective, despite reportedly high sensitivity of this mutation to the drug. In 3 months the patient developed BP and died. In two cases E255V and E255K mutations were revealed. Treatment was changed to nilotinib and both patients achieved complete hematological remission (CHR) with no cytogenetic response (CyR) detected. Two more patients had F359V mutation. The only therapeutic option for these subjects at that time was nilotinib. Their treatment results were surprisingly different: one patient achieved major cytogenetic response already at 6 months, complete CyR at 12 months; major molecular response and no BCR/ABL mutation at 18 months. The second patient with this mutation at 6 months achieved no response at all. The last patient of this subgroup with Y253H mutation was also resistant to nilotinib. In the second subgroup of 10 pretreated patients with no BCR/ABL mutations three responded to their treatment including one achieving complete molecular response. One patient lost CHR after 18 months of nilotinib. Another subject continued in CHR, yet no CyR was found at 6 months. The remaining patients in this group maintain their CHR; follow-up assessments of CyR were not yet performed. Conlusions: the data presented emphasizes controversy in the influence of BCR/ABL mutations on the efficacy of CML therapy taking into consideration different results of nilotinib treatment in 2 patients with F359V mutation; unsatisfactory treatment results of patient with M351T, considered as sensitive to TKIs; lack of treatment response in some of the patients without BCR/ABL mutations. Undoubtedly, T315I mutation is an indication for allogeneic stem cell transplantation; however significance of other kinase domain mutations for CML treatment selection needs further clarification.

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MEAN DAILY DOSE, MEASURED FROM 2~6 MONTHS OF IMATINIB THERAPY AS AN APPROPRIATE PREDICTOR FOR CYTOGENETIC AND MOLECULAR RESPONSE IN MONTH 6

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Background. Imatinib, tyrosine kinase inhibitor for BCR-ABL of CML has shown favorable results in clinic. Aims. This study was designed to observe the relationship among the response rate of imatinib (CyR; cytogenetic response, MR; molecular response), imatinib mean daily dose of month 1 treated, mean daily dose from month 2~6, imatinib plasma trough blood level of day 29 and day 168 (month 6) and Sokal score. Methods. In this study, 21 patients who receive imatinib 400mg QD as initial treatment after diagnosed as CP-CML were involved and cytogenetic and molecular assay are performed at baseline and every 3 month. Within 22-26 h after the previous dose of imatinib, blood sample were collected for measurement of imatinib plasma trough blood levels at day 29 and month 6. Results. When we compared the group achieving the complete CyR (CCyR) (n=14/21) and group not achieving CCyR (n=7/21), it has significant difference (p=0.002) in their mean daily dose from month 2~6 (means; 389.64? 25.1 and 362.86? 46.5, respectively). However, there is no significance in mean daily dose from month 1, imatinib plasma trough blood level of month 1 and 6 and Sokal score between these two groups. Group achieving 2-log reduction in MR in month 6 (n=14/21) has significant difference (p=0.000) in their mean daily dose from month 2~6 (395.42? 14.7 and 351.28? 45.7, respectively) compared with group not achieving 2-log reduction (n=7/21). Regarding CCyR, there is no significance in mean daily dose from month 1, imatinib plasma trough blood level of month 1 and 6 and Sokal score between these two groups. To observe the correlation with mean daily dose from month 1 and month 2~6, CyR and MR of month 6 and Sokal score, imatinib plasma trough level of month 1 and 6 respectively were grouped into 4 quartile. On the analysis based on quartile, imatinib plasma trough level of month 6 showed correlation with that of month 1 (p=0.024) and imatinib plasma trough level of month 1 had weak correlated with MR of month 6 (p=0.052). *Conclusions*. From statistical analysis, mean daily dose from month 2~6 showed correlation with CCyR and MR achieving 2-log reduction from baseline in month 6. This study on the process may show more relationship of the response rate of imatinib, imatinib mean daily dose of month 1 treated, mean daily dose from month 2~6, imatinib plasma trough blood level of day 29 and month 6 and Sokal score in the further follow-up.

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THE IMPACT OF THERAPY INTENSIFICATION AND OUTCOME OF 6 CML PATIENTS WITH THE SAME TYPE OF BCR-ABL MUTATION M244V

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Mutation in the kinase domain of BCR-ABL is not always the direct cause of imatinib resistance in CML patients. Also degree of mutation resistance to imatinib is very variable in different patients. The aim of this study was to evaluate the outcome and resistance overcoming by therapy intensification of 6 CML patients who on imatinib developed the same type of mutation M244V. In our clinical practice, three patients were treated with imatinib in the first line (group A) and other 3 were pretreated with hydroxyurea or hydroxyurea followed by interpheron-alpha for 32-67 months (group B). The transcript levels of wild type BCR-ABL and BCR-ABLM244V were quantified using standardized real-time qPCR (IS) and using Mutation Surveyor (Softgenetics) after direct sequencing (Polakova KM et al. Exp.Hem.2010;38:20), respectively. The group A responded to the first line imatinib optimally with CCgR 3-12 months from 400 mg initiation. One patient achieved MMR in the 3rd month however BCR-ABL transcript level has remained unchanged for 18 months at 0.01% with following rise over 2.5 logs. BCR-ABLM244V transcript (100%) was detected at the time of MMR lost. Daily dose of imatinib was increased to 800 mg three months after the rise of total BCR-ABL and mutation detection. This resulted in no further increase of total BCR-ABL and in BCR-ABLM244V lost, but novel mutation F359V appeared. Patient was switched to dasatinib 10 months after BCR-ABL increase and achieved CMR after 4 months. In other two patients, the BCR-ABLM244V was detected 10 and 8 months from imatinib start. However patients remained in CCgR for next 10 and 19 months, respectively. Patient who remained in CCgR for 10 months was switched to nilotinib 3 months after CCgR lost and responded with CMR after 6 months. The BCR-ABLM244V ratio fluctuated between 27-80% in the patient who remained in CCgR for 19 months after first mutation detection. Probably the presence of both clones with mutated and unmutated BCR-ABL resulted in partial sensitivity to imatinib and thus in longer CCgR duration. Patient was switched to dasatinib after CCgR lost with intermittent dose reductions. However, wild type BCR-ABL increased 5x and BCR-ABLM244V decreased 2x six months after dasatinib start. Patients from group B failed to achieve CCgR on imatinib and BCR-ABLM244V was detected 2, 12 and 70 months on imatinib; i.e. 37, 48 and 137 months from diagnosis. Imatinib dose was escalated in 2 patients, but their total BCR-ABLM244V transcript level did not decrease below 10%. Two patients were switched to dasatinib and one to nilotinib. In one patient mutation disappeared and MMR was achieved 6 months from nilotinib start (60 months from diagnosis). Other two patients on dasatinib showed slight decrease of BCR-ABL and BCR-ABLM244V 4 months from dasatinib initiation (103 and 142 months from diagnosis). In conclusion, our data outlined that type of BCR-ABL mutation itself is not predictive for sensitivity to therapy change alone. The stage of disease, treatment duration and other individual parameters influence disease outcome and response to therapy intensification.

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REAL-LIFE EXPERIENCE OF CML TREATMENT IN SAINT-PETERSBURG

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Background. Chronic myeloid leukemia (CML) is one of the first diseases, in which target therapy with high affinity to cornerstone of pathogenesis had become centerpiece of treatment. Implementation of tyrosine kinase inhibitors (TKI) made revolutionary improvements on CML course. At present, CML-associated mortality is less than other causes rate of death in CML patients. Wide use of imatinib was started in Russia in 2005. ELN CML (2006) recommendations gave rise to comprehensive treatment strategy. *Aims*. To assess the outcomes of CML patients' treatment in routine practice in population of Saint-Petersburg during 2005-2010 period. *Methods*. Patients charts, laboratory reports, population registry. *Results*. 182 CML patients living in Saint-Petersburg with diagnosis established in 2005-2010 were included into analysis. This group consisted of 88 males and 94 females. Median age was 57 years (17-86 years). Annual rate of newly diagnosed patients was 22-40 (median 30 patients per year). CML was diagnosed in chronic phase in 158 patients (86.8%), in acceleration phase in 22 (12.1%) patients and 2 (1.1%) patients were diagnosed in blastic phase. 118 patients received cytoreduction with hydroxyurea (median treatment time 1,9 months). 171 of 182 patients received imatinib as first-line treatment. Average time from diagnosis to start therapy with imatinib was 2.7 months. The remaining patients were treated with nilotinib (1), chemotherapy (6), hydroxyurea (4). Complete hematologic response (CHR) in three months was obtained in 151/171 patients (88.3%). The highest rate of CHR - 95.8% was observed in 6 months of treatment. In accordance with ELN criteria partial cytogenetic response (PCgR) after 6 months imatinib therapy was 67.8% (116 cases). 96 patients (56.1%) had complete cytogenetic response (CCgR) after one year of therapy with median time achievement of 11.8 months. The highest CCgR rate of 73.0% was registered in 36 months of therapy. Follow-up duration was 36.1 months in average (1-71 months). There were 21 deaths, overall survival was 88.5%. On time of analysis 151 patients were alive and regularly observed. 138 patients are still in chronic phase, 12 patients transformed into acceleration and 1 patient - in blastic phase. 110 patients are still continuing imatinib, 27 patients switched to second generation TKI (14-nilotinib, 11-dasatinib, 2-bosutinib), some patients were treated with hydroxyurea (9 patients), interferon (4 patients), 1 patient refused to

treatment. CHR was preserved in 129 (84.5%) patients. Cytogenetic monitoring was performed in 148 patients. At present: 90 patients (60.8%) had CCgR, 17 (11.5%) PCgR, 2 (1.4%) minor cytogenetic response, 10 (6.8%) minimal cytogenetic response and 29 (22.3%) had no cytogenetic response. Molecular response was assessed in 106 cases: 40 (37.7%) patients are in complete molecular response, 20 (18.9%) - major molecular response, 46 (43.4%) patients had no molecular response. *Conclusions*. Effectiveness of TKI in routine practice is similar to results obtained in most of clinical trials. Strict cytogenetic and molecular monitoring of minimal residual disease is of great value to accurate response assessment and timely switch to second generation of TKI.

1232

FREQUENCY OF BCR-ABL TYROSINE KINASE DOMAIN MUTATIONS IN CHRONIC PHASE-CHRONIC MYELOID LEUKEMIA PATIENTS WITH IMATINIB RESISTANCE ACCORDING TO DAILY MEAN DOSE OF IMATINIB

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Background. BCR-ABL kinase domain (KD) point mutation causes resistance to tyrosine kinase inhibitors (TKI) in CML patients through impaired binding of TKI to the target site. Such point mutations have been known as the clinically most relevant mechanism of TKI resistance. During TKI treatment, daily mean dose can be changed by several reasons including dose escalation, dose reduction, and temporary dose interruption. Among them, dose interruption by adverse events or patient's request is relatively common. However, there has not been much information about correlation between daily mean dose and frequency of BCR-ABL KD point mutations in imatinib resistant CML patients. *Aims*. We investigated effect of daily mean dose of imatinib on frequency of specific BCR-ABL KD mutations with imatinib resistant patients harboring BCR-ABL KD mutations. *Methods*. This study included 37 CP-CML patients registered in Seoul St. Mary's hospital since 2003. All of the patients started imatinib within 6 months of diagnosis without prior treatment for leukemia except for hydroxyurea, and were imatinib resistant with harboring any type of BCR-ABL KD mutations. Mutations in BCR-ABL KD were analyzed using direct sequencing when patients showed sign of imatinib resistance. Imatinib resistance was defined by one of the following criteria: lack of complete hematologic response (CHR) after 3 months of imatinib treatment, lack of cytogenetic response (CyR) after 6 months of imatinib treatment, lack of major cytogenetic response (MCyR) after 12 months of imatinib treatment, loss of hematologic response (HR) or CyR at any time during treatment, or disease progression from CP to AP occurring during treatment. Daily mean dose was defined as an average of drug amount taken actually from the imatinib start day up to a specific time point with the consideration of every medication of imatinib, dose modification and interruption. *Results*. We analyzed total 37 new CP-CML patients who showed BCR-ABL KD mutations after direct sequencing due to imatinib resistance. 8 patients started imatinib of 600 mg/day and 26 patients started 400 mg/day and 3 patients started 300 mg/day.

Table 1. Daily mean dose and mutation frequency.

	Group1 n=9	Group2 n=10	Group3 n=8	Group4 n=10	Overall n=37
Daily mean dose, Median(range) mg/day	293 (107-313)	380 (315-393)	400 (394-400)	441 (401-600)	393 (107-600)
Age, Median(range) yr	33 (20-62)	33 (20-61)	33 (18-60)	36 (19-71)	33 (18-71)
P-loop, n(%)	2(22.2)	2(20.0)	0(0)	4(40.0)	8(21.6)
T315I, n(%)	2(22.2)	4(40.0)	1(12.5)	2(20.0)	9(24.3)
Duration up to mutation detection, Median(range) mos					
P-loop	14.0 (11.2-16.8)	5.3 (4.1-6.4)	—	8.0 (3.2-11.6)	8.7 (3.2-16.8)
Any type	11.2 (4.2-80.6)	9.5 (2.1-57.3)	23.2 (4.1-37.8)	10.1 (2.9-44.1)	12.5 (2.1-80.6)

Daily mean dose up to the detection of the first mutation in each patient was calculated and patients were divided into 4 groups according to their daily mean dose; group1 with median 293 mg/day (107~313), group 2 with 380 mg/day (315~393), group 3 with 400 mg/day (394~400) and group 4 with 441 mg/day (401~600). Median age and Sokal risk score were not significantly different among four groups. We focused on P-loop mutations and T315I mutation. Frequency of them was not significantly different among four groups although frequency of P-loop mutation was higher in group 4. *Conclusions*. Lower daily mean dose was mainly caused by temporary interruption of imatinib treatment by adverse events while higher daily mean dose was caused by dose escalation due to non-optimal response. Daily mean dose did not make significant influence on selection of specific types of mutations in this study. However, investigation with large cohort can provide more solid correlation between daily mean dose and frequency of specific type of BCR-ABL KD mutation.

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QUANTIFICATION OF T315I MUTATION IN IMATINIB RESISTANT CHRONIC MYELOID LEUKEMIA

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Introduction. T315I mutation is located in the kinase domain of the ABL1 gene and represents one of the main mechanisms of resistance in Chronic Myeloid Leukemia (CML) patients, since none of the available tyrosine kinase inhibitors (ITKs), imatinib (IM), dasatinib or nilotinib, is active against it. T315I mutation has been found in 2 to 20% of CML resistant patients, depending on the phase of the disease, the method used to determine the mutation and the pattern of resistance (primary or secondary). The aim of this work was the study of T315I mutation in patients with chronic phase CML who become resistant to first-line therapy with IM. A quantitative analysis of the mutation at different times since the diagnosis has been performed. *Patients and methods*. Eighty-three chronic phase CML patients treated with IM as first-line therapy were included in the study. The European LeukemiaNet criteria (Baccarani et al, 2006) were used to define treatment failure. T315I mutation was retrospectively analyzed by allele-specific real-time PCR in those patients who accomplished resistance criteria. Quantification was expressed as a normalized ratio between the mutated and the wild type ABL1 copies. The sensitivity of the assay to detect the mutation was established at 0.1%.

Table 1. Quantification of T315I mutation before allogeneic transplantation.

Ratio T315I-ABL1 / ABL1 wild type						
	Diagnosis	3 months	6 months	12 months	15 months	18 months
Patient 1	<0.1%	<0.1%	3%	28%	59%	70%
Patient 2	<0.1%	<0.1%	<0.1%	0.12%	1%	9%

Results. Fourteen out of 83 CML patients (17%) showed cytogenetic resistance to first-line IM treatment, and T315I mutation could be detected in two of them (14%). Patient 1 failed to reach cytogenetic response after 6 months of treatment, and was also resistant to dasatinib and nilotinib. He received a reduced intensity allogeneic transplantation, with autologous recovery and maintainance of the T315I mutation at three months of follow-up post-transplant. Patient 2 lost cytogenetic response after 12 months of treatment with IM. He received a myeloablative syngeneic transplantation and remained in complete molecular response eight months after transplantation. T315I levels at diagnosis, and after 3, 6, 9, 12 and 15 months of treatment with ITKs are shown in table 1. *Conclusions*. In our series, the incidence of T315I mutation in chronic phase CML patients with cytogenetic resistance to IM was similar to that reported in previous publications. The quantification of mutated copies along the patient follow-up would have allowed an early detection of T315I mutation (still at low levels at the beginning), and would have shown how the resistant clone became selected and progressively raised during the TKIs treatment.

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THE TREATMENT OF CHRONIC MYELOID LEUKEMIA BY IMATINIB MESYLATE GENERIC: ABOUT 26 CASES

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Background. Chronic myeloid leukemia (CML) is a chronic myeloproliferative disorder. It's characterized by the presence of chromosomal translocation (9.22), which is transcripted at chimeric protein tyrosine kinase. Its prognosis has been transformed since the advent of Imatinib Mesylate (GLEEVEC®) a specific tyrosine kinase inhibitor (TKI) Some generics (copies) of this molecule are used in many countries, including Morocco, but no study of their effectiveness has been conducted. *Aims*. The study of the efficacy of treatment with imatinib mesylate. Methods. It is a retrospective study conducted between April 2005 and May 2010. Within the Department of clinical haematology of Military Hospital in Rabat (Morocco), this study included all the cases of CML treated by imatinib mesylate generic. Results. Twenty six patients were examined. Their median age was 43,3years (16 to 75 years). The sex-ratio H/F was the 0,85. The average time of diagnosis was 3 months (0 to 12 years). The clinical presentation was characterized by splenomegaly in 22 patients (85%). The hepatomegaly was found in 2 patients (8%). In 3 cases (12%), the discovery was coincidental. The leukocytosis was over than 105/mm3 in 16 patients (70%), the hemoglobin was less than 11 g / dL in 9 patients (35%) and thrombocytosis was detected in 8 patients (31%). The diagnosis was confirmed by conventional cytogenesis in 24cas (92%) and the molecular biology is performed in 2 cases. Additional abnormalities were found in 2 patients. 21 patients were in chronic phase, 4 in accelerated phase and only 1 in blastic phase. The Sokal score was low in 6 cases and intermediate in 16 patients. All patients received treatment with a generic (copy) of Imatinib Mesylate, produced in India (400mg per day for those in chronic phase and 600 mg per day for those in accelerated phase or transformation). The treatment was stopped in two patients because of intolerance in one and pregnancy in the other. The complete hematologic response at 3 months was 96% (23/24). The major cytogenetic response at 18 months was 77% (17/22) and the major molecular response was achieved in 2 cases. Two patients progressed to blastic phase and 2 others required the use of treatment with TKI of the second generation after the detection of mutations in the tyrosine kinase domain. *Conclusions*. Despite the small number of patients and limited resources in our serie, our results in terms of hematologic and cytogenetic responses were close to the international series.

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CD 68 EXPRESSION IN PATIENTS WITH HODGKIN LYMPHOMA: DOES IT REALLY HAVE A PROGNOSTIC SIGNIFICANCE?

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Background. Even if classical Hodgkin Lymphoma (cHL) is a curable neoplasia, 20 % of patients die from progressive disease. The international Prognostic Score (IPS) and most reported biomarkers are not sufficient to predict individual patient's evolution. Tumor-associated macrophages have been associated with an unfavourable outcome. In this study, we compare the CD68 status of patients with refractory or relapsed cHL and patients who achieved a durable complete remission (CR). *Methods*. Immunohistochemical analysis of CD68 expression was performed on initial lymph node biopsies from 28 patients with refractory or relapsed HL and 27 in persisting CR1 recruited in a single institution between 1995 and 2009. CD 68 positive cells were counted in representative areas containing Reed Steinberg Cells (RSC), without fibrosis and necrosis. Three groups were defined based on published data and correlation with clinical groups were realised. *Results.* In refractory (18) and relapsed (1O) cHL, median age was 42.5 years (range: 15-70), 19 patients had advanced disease (stage III-IV). IPS was 0-2 in 10 patients, 3-4 in 13 patients, >5 in 3 patients and in 2 patients IPS was not available. Patients received conventional chemotherapy (23 ABVD, 1 MOPP, 4 MOPP/ABV). FDG-PET was performed in 8 patients; two were negative after two courses of chemotherapy. Ten patients died, 5 from toxic death, and 5 from progressive disease. Eighteen patients are alive (13 in CR and 5 with progressive disease). CD68 expression was <5% in 2 patients, 5-25% in 10 patients and > 25% in 16 patients. Among the 27 patients who achieved and maintained a first CR, median age was 39.5 years (range: 17-61). Seventeen patients had advance stage (III-IV). Sixteen patients had IPS score 0-2, 9 patients had IPS 3-4, and 2 patients had IPS >5. All patients but one were treated with ABVD +/- radiotherapy. The remaining patient was treated wit BEACOPP. FDG-PET was available in 7 patients and 5 were negative after two courses of chemotherapy. All patients are alive and in CR. CD68 expression was <5% in 3 patients, 5-25% in 8 patients and >25% in 16 patients. No significant statistical correlation could be found between CD68 positivity and age, stage, IPS, treatment response, event free survival and overall survival. Because of the small number of FDG-PET evaluation, correlation was not performed. *Discussion*. In this small retrospective cohort, we could not demonstrate any prognostic impact of macrophages infiltration. We could not correlate with early FDG-PET because of the small number of patients. Prospective evaluation of the CD68 positivity impact has to be proposed in clinical trials.

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HODGKIN'S LYMPHOMA ASSOCIATED WITH NEPHROTIC SYNDROME

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Background. Minimal change glomerulopathy has commonly been associated with Hodgkin's disease. Method and results. We report three cases of nephrotic syndrome accompanied by renal insufficiency (RI) and Hodgkin's disease. The first patient is a 26-year old man, who was diagnosed with moderate RI and nephrotic syndrome. Renal biopsy disclosed minimal change nephropathy. He was treated initially with steroids and a partial remission of the nephrotic syndrome has been achieved after 3 months of treatment. The second patient , a 62-year old man, was diagnosed with nephrotic syndrome. The renal biopsy disclosed glomerulonephrytis with minimal messangial proliferation. He was treated with cyclophosphamide and steroids without complete remission. Nephrotic syndrome was followed by acute renale insufficiency. The third patient , a 47-year old man, was diagnosed with nephrotic syndrome. Renal biopsy disclosed minimal change nephropathy without renal insufficiency. Six , nine and five months respectively after the beginning of renal disease, nodular sclerosis Hodgkin's lymphomas were diagnosed to all the three patients. After chemotherapy (6 ABVD cycles) at all patients, a complete remission of nephrotic syndrome as well as a normalisation of renal function was achieved. *Conclusions*. An extensive evaluation for a lymphoproliferative disease could be advisable in adult patients developing minimal change nephropathy with steroid resistant syndrome.

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THE SERUM LEVEL OF TARC AND IL6 IN CLASSIC HODGKIN LYMPHOMA

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Background. The citocines and chemokines have become important in the diagnosis and prognostic evaluation of malignant diseases. "Thymus and activation-regulated chemokine"(TARC) is an 8 kDa volume polypeptid. It is expressed in large quantities by Hodgkin and Sternberg-Reed (HSR) cells and the secretion of Interleukin-6 is also observed in HSR cells. Our aim was to investigate the serum level of TARC and IL-6 in classic Hodgkin lymphoma (cHL). Methods. IL-6 and TARC level was measured by ELISA. The results were evaluated by MedCalc statistical programme. *Results*. The serum level of TARC was defined in 108 (49 active, 59 remission), the IL-6 concentration in 64 cases (20 active and 44 remission). The median value of TARC concentration in the active goup (n=49) was 2069 pg/ml [95 % confidence interval (CI):1892,67-2561,57 pg/ml]. In remission (n=59) the median value was 418 pg/ml (95% CI: 363,03-491,50). The Mann-Whitney test proved significant ($p < 0,0001$) difference between the two groups of patients. The median serum level of IL-6 was 8,48 pg/mL (95% CI: 6,43-15,23 pg/mL) in the active group (n=20), but in remission it was 2,20 pg/mL (95% CI: 2,05-3,07 pg/mL). The difference was again significant ($p<0,0001$). *Conclusions*. IL-6 and TARC level showed significant connection with the disease state (active or remission). Further investigations are needed to assign their prognostic value and their capacity to forcast relapse.

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BLEOMYCIN INDUCED LUNG TOXICITY IN PATIENTS WITH HODGKIN'S DISEASE TREATED WITH BLEOMYCIN CONTAINING REGIMENS (QATARI PROSPECTIVE)

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Background. Bleomycin pulmonary toxicity (BPT) has been known since the early clinical trials of bleomycin in the last century. Postulated risk factors include cumulative bleomycin dose, reduced glomerular filtration rate (GFR), and raised creatinine, older age, supplemental oxygen exposure. Cigarettes smoking, preexisting lung disease, radiotherapy the mount of hydration patient received. And among those with toxicity the supplemental oxygen, corticosteroids use and intervention with bronchoscopy may play a role in outcome. *Aims*. To evaluate the postulated risk factors for Bleomycin Induced Lung Toxicity in patients with Hodgkin's disease treated with Bleomycin Containing regimens in Qatar. Patients and Methods. From our retrospectively collected data of 72 patients diagnosed with Hodgkin's disease treated at Al-Amal Cancer Centre (Qatar) with bleomycin-containing regimens (ABVD, BEACOPP, STANFORD V) for Hodgkin's Lymphoma between January 2002 and December 2008, with median follow up of 30 months to identify those with BPT. Results. Eleven out of seventy two patients with Hodgkin's disease (15%) had BPT, range from mild dyspnea to respiratory failure and even death. And radiologicaly, X-ray/CT ranges from normal to overt fibrosis. There were two deaths out of the treated patients directly attributed to BPT. The median time from the start of bleomycin administration to documented lung toxicity is 5 months (range 3 - 8 months). the following risk factors were evaluated among patients with BPT the patients age were (16-61 year) with median age of 43 year, gender (eight males and three females), none of the patients with BPT is a smoker, weight upon starting bleomycin in patients with toxicity (62-107kg) with a median of 73 kg. Subtypes of HD (6 patients with nodular sclerosis and 5 with mixed cellularity), stage of the disease (6 patients with stage II, 3 patients stage III, and two patients with stage IV), chemotherapy protocols (ten out of eleven patients with BPT received ABVD while the eleventh patient received Stanford V), the cumulative dose of bleomycin ranged from (68-262 international units) with median of 136 IU. Calculation of Creatinine Clearance revealed that none of our patients had grade 2 or more chronic kidney disease. Radiological evaluation of the chest before starting bleomycin containing regimens by CT scan revealed that none of them had pre-existing parenchymal lung disease or fibrosis. *Conclusions*. In our evaluation for BPT we found toxicity occurring more in males, younger age group and those with nodular sclerosis and mixed cellularity subtypes. It seems that direct toxicity could happen regardless of the dose, and certain races maybe more susceptible to Bleomycin Pulmonary Toxicity, keeping in mind the small number of patients, we feel that further studies are needed to confirm the above mentioned findings.

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AUTOLOGOUS TRANSPLANTATION IN REFRACTORY OR RELAPSED HODGKIN'S DISEASE

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Background. Relapsed or refractory Hodgkin's disease is still a challenging problem for hematologists. The standard management of these patients should include the use of salvage chemotherapy followed by autologous stem cell transplant in patients who are chemotherapy sensitive. *Aims*.- We present our experience in 14 patients with relapsed or refractory Hodgkin's disease who received platinum based protocols as salvage chemotherapy followed by an autologous transplantation as a consolidation therapy. *Methods*. Fourteen patients (5 males, 9 females), mean age 19.3 years (17-48) are presented. Histologic subtypes included 1 case of lymphocyte predominance subtype, 11 nodular sclerosis and 2 mixed cellularity. Five cases were relapsed and 9 resistant to previous therapies (1 patient radiotherapy; three patients ABVD+radiotherapy; ten patients ABVD). *Results*. Mobilization scheme was salvage chemotherapy (platinum based protocols) plus G-CSF in 13 patients while the other received only G-CSF. The mean number of these cycles was 2.8 (2-4; median 3). Responses to these cycles and situation of the disease previous to transplant was 5 (35.7%) complete responses (CR), 7 (50%) partial responses (PR) and 2 (14.3%) no response (NR). In all cases the source of progenitors was peripheral blood. Mean number of aphereses performed was 1.35 (1-2; median 1) and no significative problems were registered during the procedures. In all patients enough number of progenitors could be collected. No other mobilization agents (plerixafor) should be used. All patients were invariably conditioned with BEAM protocol. Mean CD34+ cells infused was 6x106/Kg (3-18; median 6). After transplantation 12 (85,8%) patients achieved CR, 1 (7,1%) PR and 1 (7,1%) NR. Both patients with no response to salvage therapy achieved CR after transplantation but one of them early relapsed (4 months). Only 1 additional patient (8.3%) relapsed at 9 months after transplantation. Mean overall survival was 25.3 months (4-55+; median 19.5). *Conclusions*. In our experience, autologous transplantation is a useful and safe procedure in patients with relapsed or resistant Hodgkin's disease. Salvage therapy with platinum-based protocols plus G-CSF allowed the collection of enough progenitors in all patients with a low number of aphereses. Overall complete response rates after transplantation is high (including one case not responding to prior salvage therapy) and the number of relapses low. Mean overall survival in our series is, up to now, slightly higher than two years.

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RECOVERY OF SPERMATOGENESIS (SP) AFTER BEACOPP14 (B-14) PROTOCOL IN HODGKIN'S LYMPHOMA (HL) YOUNG MALES

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Background. The B-14 protocol is one of the latest cure strategies in advanced stages of HL. There is no medical information on B-14 and gonadal toxicity in young males. *Aims*. To evaluate the frequency of sperm disorders and infertility, to measure sex hormone concentrations (LH, FSH, Ts) as well as the number of patients who succeeded in achieving fatherhood with and without assisted reproductive technologies .Patients and Methods: from 2008 to 2011, 25 men of mean age 23yrs (18-29) with IIB-IV sts of classical HL were examined after cessation of treatment with 6-8 of B-14 and IFI irradiation in some pts with bulky mediastinum. All patients had the established fact of paternity (spouse pregnancy) and/or had cryopreservation of sperm prior to treatment. Time from the end of chemotherapy to the beginning of the survey averaged 16 mos (2-42). *Results*. Recovery of Sp was established in 60% pts (15 of 25) with mean time of recovery 16 mos (2-42). Azoospermia was observed in 40% pts (10 of 25) with mean 17 mos (4-42) of observation. Levels of sex hormones were evaluated in 24 of 25 pts, changes were noted in 71% pts (17 of 24). In Sp recovery group the following trends were observed: Ts decrease and LH, FSH increase - 1pt, FSH increase-1, Ts and LH decrease - 1, Ts decrease -3, LH decrease-1, no changes -7 pts. In azoospermic group: Ts decrease- 1pt, FSH increase-6, Ts decrease and FSH increase - 2, LH increase - 1 pt. All patients with recovered Sp have some deviations of Sp: oligozoospermia (O)/astenozoospermia (A)/nekrozoospermia (N)/ theratozoospermia(T): with A-1 pt, T-1t, AT-1, AN-1, OAT-2, ANT-3, OANT-2. 3 healthy children were born to 3 pts without assisted reproductive technologies. *Conclusions*. 6-8 courses of B-14 causes azoospermia in 40% of cured young male pts. This fact makes semen cryopreservation before HL therapy a necessary procedure to all reproductive males. Changes in levels of sex hormones (LH, FSH, Ts) are not always associated with azoospermia. Increased FSH levels are most often associated with azoospermia. Low Ts level is not linked with functional impairments. Spontaneous recovery of Sp is always associated with its deterioration. The probability of spontaneous recovery of Sp, improvement of sperm parameters after recovery, as well as necessity of Ts replacement therapy require further study.

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TREATMENT OF COMPLICATED AND COMPOUNDS FORMS OF HODGKIN´S DISEASE

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Background. Treatment of Hodgkin´s disease (HD) is well established in "standard"cases; front-line therapy with ABVD or ABVD-like regimens +/- radiotherapy are the cornerstone treatments of disease. The problem appears when HD is associated with another disease: another tumour, infection, autoimmune disease,… or when the illness itself relapse after several lines of therapy including bone marrow transplantation. In this cases and "wide"and "creative"management of the disease is compulso-

ry. AIMS: we try to describe and explain the management of special forms of HD in which we have to make a decision of treat 2 serious diseases simultaneously with a curative approach, or the specific treatment of HD in an out of protocol approach. *Methods.* We describe 5 cases of Hodgkin lymphoma of different subtypes: mixed cellularity (2) and nodular sclerosing(3), stage IA to IVB with complicated onset or evolution, in four of this HD was associated with others diseases, the other was a third relapse, after autologous bone marrow transplantation (BMT), without compatible donor. *Results.* In all cases the initial treatment (alone or combinated) was standard dosage ABVD without reduction or discontinuation, in 4 cases was associated simultaneously or sequentially with another/s treatment/s depending on concomitant disease: Case 1 was associated with VIH infection: treatment with antiretroviral therapy was simultaneous, consolidation with gemcitabine-vinorelbine was added because of a PET-TAC positivity after 3º cycle. Case 2: advanced stage prostate cancer, diagnosed simultaneously and treated with a regimen active for both of them: ABVD (contains an antracycline)+radiotherapy. Case 3: a combined lymphoma treated sequentially with maintenance therapy. Case 4: haemolytic autoimmune anaemia glucocorticoids-resistant treated with rituximab a dosage of 100 mg/week x 4 doses. Case 5 was a relapse of HD after BMT without compatible donor treated with a *sandwich* regimen: Gemcitabine-vinorelbine(x6) + local radiotherapy + Gemcitabine-cisplatin(x2). (table 1).

Hodgkin lymphoma	Diagnostic (2)	Gender/ age	Initial treatment	Adjuvant therapy	Results
1 -Mixed cellularity IIIA	VIH infection	Male/28	ABVD(x6)	Gemcitabine-vinorelbine (x3) + antiretroviral therapy	CR 24 months
2 -Mixed cellularity IVB	Prostate adenocarcinoma	Male/57	ABVD(X8)	RT+ bicalutamide	CR 23 months
3 -Nodular sclerosing IIIA	Follicular lymphoma	Male/69	ABVD(X6)	Maintenance clorambucyl x 6 months	CR 14 Months
4 -Nodular sclerosing IIA	Relapse after BMT	Female/ 32	ABVD(X6)	Gemcitabine-vinorelbine (x6) +RT+Cis-Pt	CR 60 Months
5 -Nodular sclerosing IA	AIHA/ PTI	Male/56	ABVD(X6) RT	Steroids Rituximab (x4)	CR 40 Months

Summary. The ABVD and ABVD like regimens are the basis of treatment of HD even in compounds and complicated forms, It´s a well-known regimen with a well-known toxicity profile, so it is possible associate another treatment without serious side-effects. As in all complicated/compounds diseases in these cases it is essential to innovate and adjust the treatment to the characteristic of the patient and illness, because actually there are not validated guides adapted to each possible case.

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HODGKIN LYMPHOMA - PROGNOSIS AND EVOLUTION

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Background. Hodgkin's lymphoma is a malignancy of lymphocytes and their progenitors and young people age 15-35years are usually affected. *Aims*. In this study we aim to discover prognostic factors to identify high-risk group of patients and treatment response rate. We performed a retrospective study of 85 patients diagnosed with Hodgkin's lymphoma in our Clinic of Hematology. Evaluation criteria of patients were: age, sex, number of lymph nodes affected areas, determining mediastinal, extranodal determinations, clinical stage of disease, histological type of disease, this splenomegaly, anemia, lymphopenia, serum iron, LDH, ALKP, response to treatment . RESULTS: The study group was found predominance of females. Mediastinal mass were met in 28 patients. Most cases were in stage at diagnosis II / III. Primary extranodal Hodgkin lymphoma affecting lung in one case, a case with pericardial tumor and spinal disease in one case was found.13 cases had onset splenomegaly. In terms of histological type in our study group was found predominance nodular sclerosis subtype. In the study group there were two cases of composite lymphoma (Hodgkin's lymphoma and non Hodgkin's lymphoma). Most patients showed B symptoms. In terms of biological evidence found biological inflammatory syndrome in most patients, low sideremia was present in one fourth of patients, anemia in half the patients in the study, increased LDH in 58 of the cases. CONCLUSIONS: All patients in the study group followed ABVD combination chemotherapy such as first-line treatment with obtaining complete remission in 27 of patients. Remission period was from 1 to 7 years. Relapsed appeared in some cases ~ 4 months after ABVD courses. Salvage chemotherapy was offered to patients who relapse or with refractory disease.In evolution three cases had pulmonary evolving, determining pericardial three cases, one case of chest wall tumor, and one case jugular vein thrombosis. Eight patients in the study group did autotranspant of which seven patients were favorable evolution and a bad case of post-transplant evolution, disease progression and patient exitus.Two allotransplant performed, one with good evolution.Advanced disease stage, extranodal disease, B symptoms, anemia, more affected lymph node areas are poor prognostic factors.

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FERRITIN EVALUATION AT DIAGNOSIS CAN IMPROVE THE CHEMOSENSITIVITY AND RESPONSE ASSESSMENT OF EARLY STAGE HODGKIN LYMPHOMA PATIENTS

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Background Inflammation dominates both the clinical and histological pictures of Hodgkin Lymphoma (HL). The interim-PET findings seem to reflect the chemo-sensitivity more than the real tumor eradication, related to the ability of chemotherapy to reduce the inflammatory microenvironment. *Aims*. In the present study, we evaluated the role of Ferritin in predicting the interim-PET results and the outcome. Methods Forty-four patients with early stage HL with unfavorable prognosis were treated with ABVD as first line and PET assessment was performed at diagnosis, after two cycles (interim-PET), and at the end of treatment. PET images were interpreted visually according to Dann *et al*., 2010 and 5 of them (11.36%) had a positive interim-PET. Response assessment was valuable for forty patients; three out of five patients with positive interim-PET (60%) progressed or relapsed although their early shift to BEACOPP regimen. Three more patients relapsed although their interim-ET was negative. The median follow-up was 17.1 months with a range between 0.4 and 65 months. Informed consensus has been signed according to Good Clinical Practice and Helsinki declaration. Results Ferritin levels showed a wide variation with a median of 142 ng/mL and a range between 11.3 and 1530 ng/mL. We found a significant correlation between ferritin levels at diagnosis and interim-PET positivity, having patients with an interim-PET assessment levels of ferritin greater than patients with a negative interim-PET (p=0.05). Additionally, we found that patients with high ferritin levels at diagnosis tend to have a reduced PFS compared to subjects with normal or moderately increased ferritin (p=0.06), independently from IPS score (respectively, 17.48 months vs not achieved median). *Summary/Conclusion*. Taken together, our observations suggest how the evaluation of ferritin at diagnosis can add prognostic information in early stage HL patients helping in suspecting the interim-PET assessment and the response to treatment.

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THE RESULTS OF GPOH-HD 95 PROTOCOL IN CHILDREN AND ADOLESCENTS WITH HODGKIN'S DISEASE: SINGLE INSTITUTION RESULTS

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Background: Hodgkin's disease (HD) is a curable malignancy in over 90% of patients in childhood using chemotherapy and radiotherapy. But long term side effects of combination therapies are serious and may impair the patients' quality of life. In our institution, children with newly diagnosed HD have been treated with GPOH-HD 95 chemo-radiotherapy regimens in order to to decrease therapy related side effects since 2000. In this study, the treatment results of the patients who had been treated with GPOH- HD 95 protocol were presented. Patients and methods: During the period 2000-2010, 38 children and adolescents with HD were reffered to our institution. Patients were excluded from the study if they were diagnosed in another center or their admission was for relapsed HD. So we retrospectively analyzed the epidemiological characteristics, clinical and laboratory datas and primary endpoints of the 28 previously untreated patients. Results: 21 males (75%) and 8

females (25%) (male/female ratio : 3; age : 3-15 years, median age : 9) were treated with GPOH-95 protocol. The lymph node regions involved at the diagnosis were 20 cervical (71%), 5 mediastinal (18%), 2 supraclavicular (7%), 1 inguinal (4%). Erythrocyte sedimentation rate (ESR) was high in 84% of the patients at diagnosis and in three patients admitted with the diagnosis of relapsed HD. Staging was as follows: stage I : 2 (7%); II : 15 (54%); III : 9 (32%); and IV : 2 (25%). Histopathology was nodular sclerosis in 15 patients (53%); 10 had mixed cellularity (MC; 36%) and 2 lymphocyte depleted (7%) and one patient (4%) lymphocyte-rich classic Hodgkin disease. Constitutional symptoms : 16 patients were asymptomatic (A; 43%) and 12 had constitutional complaints (B; 57%). Eleven patients (39%) were allocated into risk group 1, 7 (25%) risk group 2, and 10 (36%) risk group 3. Twenty seven patients (%96) received radiotherapy (20-25 Gy). Three (10%) patients relapsed over a period of 10 years (median follow up 40 months) and the autologous bone marrow transplantation was performed in one of them but two early - relapsed patients died. The EFS and OS rates at 5 years were 88.4% and 87,4%, respectively. Conclusion: The high number of early stage patients at diagnosis compared to our previous studies, was hopeful for the awareness of the society for malignancies. In contrast to our previous studies, nodular sclerosis was the predominant histologic subtype, while mixed cellularity was the most frequent histologic subtype beforehand. The investigation of this switching between the histologic subtypes may clarify the physiopathology of HD. Also, the importance of erythrocyte sedimentation rate persists in primary diagnosis and relapsed patients. It seems that, GPOH-HD 95 protocol is efficient, easy to use and has low toxicity. But the rates of OS and EFS are lower than the results of the DAL/GPOH-HD 95 These results may be due to low number of patients, high rates of patients with B symptoms and the lower socioeconomic level of the society.

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FEASIBILITY OF TANDEM AUTO/REDUCED-INTENSITY T-REPLETED HAPLOIDENTICAL BONE MARROW ALLOGRAFT FOR RELAPSED/REFRACTORY HODGKIN'S LYMPHOMA

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Background. Tandem auto/reduced-intensity allograft (auto-SCT/RIC-allo) is a feasible approach in poor prognosis Hodgkin lymphoma patients (HL). However, a minority of patients have an available matched related or unrelated donor. For those patients, haploidentical donor could be an viable alternative donor. Recently, post-transplantation cyclophosphamide (Cy) is effective to prevent graft versus host disease (GVHD) and graft rejection, using T-replete bone marrow. *Aims*. To investigate feasibility of tandem auto-SCT followed by RIC-haplo with high-dose post-transplantation Cy in poor prognosis HL patients. *Patients and Methods*. From January 2009, 6 HL patients were treated with auto-SCT followed by RIC-haplo, with high-dose post-transplantation Cy. Patients not in FDG-PET complete remission (CR) after 2 chemotherapy lines were included. No patients were relapsed after previous HDC. High dose chemotherapy (HDC) consisted of Melphalan 200 mg/m2 in 5 patients, and BEAM in 1 patient. RIC-haplo conditioning consisted of fludarabine (30 mg/m2 x 5 days), Cy (14.5 mg/kg/day x 2 days, and TBI (2 Gy). Unmanipulated bone marrow cells were infused (target MNC dose 4x 108/kg of recipient). GVHD prophylaxis consisted of Cy 50 mg/mKg/day for 2 days (d +3 and 4), and tacrolimus and MMF (starting from d +5). Results. At time of auto-SCT, four patients had progressive disease (PD), one stable disease and one partial response (PR). After auto-SCT, 3 patients achieved CR and 3 patients PR. Median time between transplant procedures was 2.5 months (range 1-3). Sorror score was 0 before transplantation. Median number of MNC, CD34+ and CD3+ cells in the graft were 4 x 10^8/kg, 3.1 x 10^6/kg and 40 x 10^6/kg respectively. Median time to myeloid and platelet engraftment was 22 (17-32) and 32 (22-43) days, respectively. Median hospitalization time was 33 days (range 23-48). Between day 30 and 45 days after RIC-haplo, all patients achieved a full, donor chimera chimera. No graft failure occurred. After tandem procedure 6/6 patients obtained CR. After a median observation time of 400 days (97-916), 5/6 patients are alive in CR and 1 died in PD. Two patients developed skin acute GVHD (one grade I and one grade II). No chronic GVHD was observed. Infectious complications were depicted in Table. *Conclusions*. Our results suggest that tandem auto-SCT/RIC-haplo with high-dose post-transplantation Cy is feasible and well tolerated, with no enhanced toxicity. However, these preliminary data should be confirmed in more patients.

	N= 6
Viral infections:	
CMV	4/6
EBV	2/6
BK	1/6
Bacterial infections:	
Pneumonia	3/6
Gram negative sepsis	2/6
Gram negative cystitis	1/6
Fungal infection:	
Candida non albicans diarrhea	1/6
TRM	0

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IT IS NECESSARY A SURVEILLANCE IMAGING IN HODGKIN LYMPHOMA PATIENTS IN EARLY REMISSION?

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Background. The majority of patients with Hodgkin lymphoma (HL) achieve disease remission after primary therapy. Early response evaluated by positron emission tomography/computed tomography (PET/CT) alter two chemotherapy cycles has demonstrated a predictive value in several studies. Nevertheless, there are not consensus exists about perform postremission surveillance imaging. *Methods.* Retrospectively, we have analyzed 40 adult patients with classic HL treated in our center between January 2005-December 2010. Patients were treated with ABVD schedulle for stages I, II, IIIA and BEACOPP14 ifor stages IIIB and IV. In all cases a PET/CT was performed after two cycles and after therapy was completed. Events were defined as recurrent HL or secondary malignancies. Primary outcome was positive predictive value (PPV) of surveillance PET/CT alter two chemotherapy cycles and secondary outcomes were costs and radiation exposures of surveillance scans. *Results.* Seven (17.5%) recurrent HL cases were detected during a median follow-up of 30 months. In 33 patients early PET/CT performed alter two cycles demonstrated non hypermetabolic disease activity. All cases with early negative PET/CT showed absence of metabolic activity when another PET/CT was performed after completed therapy were. Factors that were found to significantly improve the PPV of scans in detecting recurrent HL included PET/CT concordance involvement of a prior disease site, or the occurrence of systemic symptoms. There were too few events to determine whether event detection by PET/CT or the presence of symptoms at the time of event detection affected overall outcomes. The analysis of cost to detect a single event is high and radiation exposure to detect a single event should be considered. *Conclusions*. For patients with HL in first disease remission, surveillance PET/CT appears to be expensive, with limited clinical impact.

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THE SIGNIFICANCE OF EBV VIREMIA IN KOREAN PATIENTS WITH HODGKIN LYMPHOMA

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Background. Epstein-Barr virus (EBV) is known to be frequently associated with Hodgkin's lymphoma (HL). However the significance of EBV infection reguarding the clinical outcomes of HL patients has been unclear. Furthermore, due to relative paucity of the disease in Asian countries, the role of EBV-DNA infection in pathogenesis and prognosis of HL has not been explored yet. Several recently studies have reported the presence of EBV-DNA in blood of patients with EBV-positive HL. Aims: The aim of this study is to investigate the frequency of detection of EBV-DNA in blood of Korean HL patients and the clinical characteristics of patients with EBV viremia. *Methods*. Between October 2007 and

May 2010, a total of 34 patients with newly diagnosed HL were tested for EBV-DNA titer in blood before treatment, using real-time quantitative PCR. *Results*. Among them, 6 (17.6%) patients had a detectable EBV-DNA. *In situ* hybridization (ISH) for EBV-encoded RNA (EBER) in tumor tissues was performed in 5 of the 6 patients and all of them were positive. EBV-ISH could not be perfomed in one patient as tissue was not available. Patients with EBV viremia seemed to be older (median 62 years, range 47-68 years), compared with those without EBV viremia (median 31 years, range 14-77 years). In patients with EBV viremia, the proportions of advanced stage (Ann Arbo stage III-IV, 83.3% vs. 46.4%), extranodal involvement (66.7% vs. 39.3%), and international prognostic score (IPS) more than 3 (83.3%, vs. 18.5%) seem to be higher than in patient without EBV viremia. Monitoring of viral load was performed in 4 patients. After treatment, EBV-DNA was undetectable in two patients with initially low titers (6250 and 1075 copies/mL), and other 2 patients with relatively higher titers at baseline showed decrease in titers(12250 to 3500, and 14500 to 8000 copies/mL). These 4 patients achieved complete response while the one lost to follow-up and the other one is still under the treatment. *Summary/Conclusions*. Although these observations were based on very small number of patients, our data suggest that the detection of EBV-DNA in blood may be associated with risk factors indicative of a poor prognosis such as older age, advanced stage and higher IPS. Furthermore, serial monitoring of EBV-DNA titer may help to predict response to treatment in Korean HL patients.

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RELEVANT FACTORS FOR OUTCOME AND PROGNOSIS IN HODGKIN LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Background. Although Hodgkin lymphoma (HL) is associated with high curability rates reaching 90 % in children and young adults, unfortunately in a reduced number of patients the outcome is unfavorable. *Aims*. Therefore we aim to study the factors influencing outcome and prognosis in HL. *Methods*. In a retrospective unicentric study we included 35 patients with ages between 3 and 25 years diagnosed with HL during a 5 years period in the IIIrd Clinic of Pediatrics Timisoara. At onset of disease we analyzed the influence of the following factors: patient age, presence of *B symptoms*, level of hemoglobin (Hb) and serum lactic acid dehydrogenase (LDH) level, clinical stage and histological type. We also evaluate the state of disease and 5 years survivals by Kaplan-Meyer curves after classical chemo/radiotherapy and/or autologous hematopoietic stem cell transplantation (HSCT). Results: In 66 % of cases the patients' age was between 6 and 18 years. 60 % had "B symptoms"at onset and 74 % were diagnosed in late stages (III and IV). The serum level of LDH was elevated in 23 % of cases and the level of Hb was over 10 g% in 57% of patients. Histological 54 % were diagnosed with HL with nodular sclerosis. In all patients was administered chemotherapy, 85 % received additional radiotherapy and 40 % had also undergone an autologous HSCT. After initial treatment, 74 % were in complete remission, 23 % in partial remission, 3 % had stable disease. 51% relapsed after initial therapy. Survival curves revealed a predictive overall survival (pOS) at 5 years of 100% in mixed cellularity and lymphocyte depleted histological types compared with 83 % in nodular sclerosis and 67% in lymphocyte predominance types. Taking into account the clinical stage of disease pOS at 5 years was 93% in CS III, 89 % in CS II and 73% in CS IV. Serum LDH level had not influenced the pOS. In the group with age at onset under 6 years the pOS was 100%, in children between 18-25 years pOS was 97%, and in age group over 18 years the pOS was only 81 %. Predictive event free survival (pEFS) at 5 years was significantly influenced by Hb level: 71 % in the group with Hb>10 g% comparative with 50 % in the patients with Hb<10 g%. Conlusions: In our study, favorable predictive factors for outcome and prognosis were: age under 6 years, Hb level over 10 g % and histological types with lymphocyte depletion and mixed cellularity. Serum LDH levels did not influenced the chemo/radiotherapy response. Initial response to chemo and/or radiotherapy is defining for prognosis. HD in advances clinical stage at onset, refractory or relapsed HD has favorable outcome if treatment protocol includes autologous HSCT.

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AUTO-IMMUNE CYTOPENIAS IN NON RELAPSING HODGKIN'S DISEASES

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Background. Association between auto-immune cytopenias and Hodgin's disease (HD) is described (1, 2). AIC could occur before or after the diagnosis of lymphoma or during relapse of the disease. Few data are available in patients with AIC and no symptoms of relapse. We report 3 cases of AIC occurring a long period after Hodgkin's disease treatment in patients still in remission. Cases report: Case 1: immune thrombopenic purpura in 37 years old man ITP occurred 9 years after HD diagnosis, stage IIAa (cervical lymph nodes). Treatment of HD was chemotherapy (3ABVD) and radiotherapy. No relapse was found to explain ITP (18FDG-PET/CT scan, bone marrow biopsy). ITP was treated with corticosteroid and intravenous immunoglobulin (IGIV) but sustained response was not observed. Romiplostim was efficient at maximal dose. Case 2: 77 years old woman was treated in 2009 for stage IVBb HD's with COOP/ABV. In January 2010 she developed auto-immune haemolytic anaemia and severe bleeding due to ITP (platelets 2.10^9/L, haemoglobin 88g/L). No relapse was found to explain ITP (18FDG-PET/CT scan, bone marrow biopsy). Initial corticosteroid therapy and IVIg was successful but relapse was observed. Treatment with Rituximab was efficient in 3 weeks. Case 3: A 16 years old man with history of HD staging IV Ab by lung and liver diagnosed 2 years ago (December 2004), and treated with chemotherapy type OPPA/COPP associated with radiotherapy with complete response, was affected by ITP. Clinical and biological findings were cutanous purpura, thrombocytopenia at 50.10^9/L, and a peripheral origin on bone marrow aspiration. secondarly appeared auto immune haemolytic anemia with warm antibodies with a minimum value of haemoglobin level at 58 g/L. Treatment by corticosteroids was unsuccessful and RITUXIMAB was introduced with an increased of the 2 lineages obtained in few days. *Conclusions*. autoimmune cytopenias could affect patients with a history of Hodgin's disease in the absence of relapse. In all cases cytopenias were resistant to the first level of treatment. Severe symptoms allow us to use major therapy of ITP or AIC as Rituximab or Nplate to avoid life-threatening complcations.

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UNDIAGNOSED SARCOIDOSIS MANIFESTING AS PERSISTENT FDG-PET POSITIVITY AFTER TREATMENT OF LYMPHOMA

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Background. Positron emission tomography (PET) with [18F]fluorodeoxyglucose (FDG) scanning has become an increasingly important tool in decision-making in patients with high-grade non-Hodgkin's lymphoma (NHL) and Hodgkin's disease (HL). Sensitivity and specificity of FDG-PET for detection of residual disease after completion of first-line therapy is reported as 84% and 90% for HL and 72% and 100% for high grade NHL, respectively. *Aims*. We report two patients with persistent positivity on FDG-PET scanning after treatment for lymphoma that suggested persistent active lymphoma but was subsequently diagnosed as sarcoidosis. *Methods*. Retrospective chart review to collate clinical details, laboratory results and radiology reports. Results. Patient 1 is a 23 year old female diagnosed with biopsy-proven nodular sclerosing Hodgkin's disease, stage III. The initial biopsy material (cervical lymph node) did not show any evidence of granulomatous inflammation. She did not have a PET scan at diagnosis. She received 6 cycles of doxorubicin/bleomycin/vincristine/dexamethasone (ABVD) combination chemotherapy. However, she had persistent mediastinal PET positivity at interval imaging and given that a positive early interim FDG-PET is highly predictive of progression in patients with advanced-stage HL (Hutchings et al, Blood 2006), her treatment was escalated to ifos-

famide/etoposide/vincristine (IEV) combination chemotherapy. She proceeded to stem cell mobilisation and collection. Several attempts at that time to biopsy the areas of persistent PET positivity failed because of morbid obesity. She remained otherwise very well and a watch and wait approach was adopted. However, progressive widespread PET positive lymphadenopathy developed and biopsies were again attempted 2 years later. Pathology confirmed extensive non-caseating granulomatous inflammation. There was no evidence of residual Hodgkin's lymphoma. Serum angiotensin converting enzyme level was normal at 21 IU/L (normal range 8-52 IU/L). Patient 2 is a 72 year old female diagnosed with extensive diffuse large B-cell lymphoma (DLBCL), with bulky para-nasal sinus involvement. She received 8 cycles of rituximab/cyclophosphamide/doxorubicin/vincristine/prdnisolone (R-CHOP) combination chemotherapy with CNS prophylaxis with inthrathecal methotrexate. An FDG-PET scan at the end of treatment showed persistent FDG avid mediastinal lymphadenopathy. Endobronchial ultrasound guided biopsy of these lymph nodes was performed and samples from the two nodes biopsied showed non-caseating granulomata. There was no evidence of residual high-grade lymphoma. Serum angiotensin converting enzyme level was just above the normal limit at 55 IU/L (normal range 8-52 IU/L). *Conclusions.* FDG-PET scanning is fast becoming a cornerstone in the management of certain lymphomas. However, use of this imaging modality for detecting residual disease during and after treatment may be confounded by other reasons for positivity including sarcoidosis. Biopsy confirmation of suspected residual disease may be important before proceeding to high-dose salvage chemotherapy.

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IS COMBINATION OF FOUR CYCLES OF EBEACOPP AND FOUR CYCLES OF BBEACOPP APPROPRIATE TREATMENT OPTION FOR PATIENTS WITH ADVANCED STAGE HODGKIN´S LYMPHOMA?

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Introduction. HD-12 trial of the GHSG de-escalated therapy of advanced stage of Hodgkin lymphoma (HL) by comparing 8 cycles of escalated BEACOPP with 4 cycles of escalated BEACOPP and 4 cycles of baseline BEACOPP. We evaluate efficacy and toxicity of this schema used in our institusion since 2001. *Patients and methods.* A total 78 patients with newly diagnosed HL - advanced stage were treated with this approach between December 2001 and December 2010. 62 of them (median age 31 years, range 18-61 years) with minimal follow-up of 12 months from the end of therapy were finally evaluated. Initial stage II/III/IV disease was found in 12/32/18 patients, respectively. We analyzed this group of patients for early toxicity, outcome in interim restaging and treatment outcome. Results: A total of 59 (95%) patients achieved complete remission. One patient progressed on treatment, one had stable disease. One patient died during treatment. Radiotherapy was given to 10 patients with residual PET positivity. Three patients experienced disease relapse 13/22/22 months after the beginning of the therapy. Relapse or progression occured in only 5 patients (8%). With the median of follow-up (FU) 59.5 months FFTF for all patiens is 92 %, OS is 93 %. Toxicity: Preplaned 8 cycles of the therapy completed 54 patients (87%). 5 patients didn´t complete treatment due to adverse events (AE), 2 due to noncompliance. Major toxicities were hematologic. The grade 3-4 anemia has occured in 26 patiens (42 %), grade 3-4 neutropenia in 55 (89 %) and grade 3-4 thrombocytopenia in 23 (37 %) patients. G-CSF support in baseline BEACOPP was needed in 41 (66 %) patients, 9 patients were hospitalized due to febrile neutropenia. Other examined early averse events were aseptic necrosis of the had of femur, venous thrombosis, soft tissue absces, chronic osteomyelitis, peripheral neuropathy, pneumonitis and osteoporosis. During the FU period 1 patient had secondary malignancy, 1 patient myelodysplasia following salvage high-dose chemotherapy with stem cell rescue. *Conclusions.* These data showed that combination of escalated and baseline BEACOPP chemotherapy seems to be effective treatment with acceptable acute toxicity, very promising effectivity. Longer follow-up is needed for evaluating of late toxicity of this regimens.

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PATIENTS WITH MYELODYSPLASTIC SYNDROMES SHOW REDUCED FREQUENCIES OF CD4+CD8+ DOUBLE POSITIVE T-CELLS

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Background. Even though the expression of CD4 and CD8 on thymocytes is considered mutually exclusive, CD4+CD8+ double positive T-cells (DP) represent a small subset of T-lymphocytes which have been described in the peripheral blood of normal individuals as well as in some pathological conditions. In particular an age dependent accumulation of monoclonal DP has been shown in elderly healthy subjects. From the functional point of view DP are able to act as differentiated effector memory cells with specific antiviral functions. However, considering the concomitant expression of granzyme B, Foxp3, interleukin 17 as well as of both Th1-type and Th2-type cytokines, it is very likely they are able to mediate different functions, not only in the peripheral blood but also in other sites. *Aim and methods*. Although a DP cell population has been described in the lymph nodes of patients with nodular lymphocyte predominant Hodgkin's Lymphoma, the relative representation of this cell subset has never been analyzed in patients with myeloid malignancies. As myelodysplastic syndromes (MDS) are a group of clonal disorders characterized by a marked immune dysregulation specifically involving the T-cell compartment, we evaluated the frequency of DP in the peripheral blood of 41 patients with MDS and 40 age-matched normal controls by using flow cytometry. Results: We showed that MDS patients when compared with normal controls had reduced frequencies of DP (0,99% ± 0,72% vs 1,38% ± 0,80% calculated on total lymphocytes; p<0,05). We then looked at the possible impact on DP frequencies of several patient- and disease-related factors but, after stratifying patients by WHO adapted Prognostic Scoring System (WPSS), cytogenetics, hemoglobin levels, neutrophil and platelet counts, transfusion dependence and coexistence of autoimmune phenomena, we could not detect any statistically significant difference. However by comparing DP frequencies in patients belonging to different WHO subclasses, we demonstrated a further reduced frequency of DP in patients with refractory cytopenia and multilineage dysplasia (CRDM) than in patients belonging to the other WHO subclasses (0,98% ± 0,43% vs 1,00% ± 0,68%; p<0,005). *Conclusions*. Our data further suggest that an abnormal activation of the T-cell compartment may be deeply involved in the pathophysiology of MDS, especially in subtypes such as RCMD which are more likely characterized by the functional inhibition of hematopoietic precursors mediated by autoaggressive T-lymphocytes described in these disorders. The very recent evidence that Myb is a fundamental promoter of DP survival, along with the demonstration that this gene is typically down-regulated in MDS patients due to the abnormal expression of specific micro-RNA, could well explain the reduced frequency of DP we observed in our patients.

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THE *EX VIVO* CLONAGENIC POTENTIAL OF SELECTED CD34+ CELLS FROM PATIENTS WITH MDS APPEARS PRESERVED

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Background. The myelodysplastic syndromes are a heterogeneous group of haematological malignancies characterized by cytopaenias and ineffective haemopoiesis. A role for the immune system in the pathogenesis has been suggested and research has implied that T-lymphocytes inhibit colony growth of early progenitors leading to variable degrees of cytopaenia. However, previous investigations have shown that the cells undergoing apoptosis are the mature CD34- progenitors and that when separated from stromal and bone marrow accessory cells the colony growth of the CD34+ cells is similar to normal. Aim: Our aim was to develop a unique dual layer culture system which would serve to examine the effects of activated and non-activated immune cells on selected bone marrow clonogenic progenitors from MDS patients, which were devoid of stromal and bone marrow accessory cells. Methodology: 17 patients with myelodysplasia were studied. All patients and normal individuals were required to sign a consent form according to University of Cape Town guidelines. The autologous peripheral blood mononuclear cells were separated and activated using phorbol 12-myristate13 acetate (PMA) and thereafter analysed, for expression of the activation antigen CD69 employing standard flow cytometry. The effect of these autologous immune cells populations on clonogenic marrow cell growth was then studied by culturing selected CD34+ cells with both PMA activated and non-activated autologous lymphocytes using a double layer culture technique. In this system the CD34+ cells were isolated from the accessory cells in the bone marrow and incubated in dose response with lymphocytes immobilized in an agar underlayer. Thus, physical contact with the activated immune cells was prevented. Clones containing more than 40 cells were scored and compared to age matched control samples. Results: In MDS patients the median percent of CD3+

T-cells expressing CD69 was reduced in comparison to age matched controls (p=0.025). The effects of the immune cells on clonagenic cell growth was heterogeneous, however when the results were compared with control cells there was no difference in the colony stimulating capacity of autologous PMA stimulated or unstimulated lymphocytes from MDS patients. In addition the number of CFU-GM increased similar to control values, as increasing numbers of lymphocytes and monocytes were added (p=0.02). MDS CD34+ cells cultured with PMA activated immune cell populations had higher numbers of colonies than those cultured with non activated cells (p=0.05) Conclusions: Our investigations suggest that soluble factors secreted by lymphocytes and monocytes, when not in direct contact with CD34+ progenitors, have the ability to stimulate colony growth of autologous CD34+ cells. In addition, in contrast to previous reports, these experiments also show that when the CD34+ progenitors are separated from the bone marrow environment and the local accessory cells, their proliferative capacity is preserved. Therefore the role of accessory cells such as stromal and antigen presenting cells in the pathogenesis and the maintenance of an abnormal environment in MDS require further investigation.

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MATURE MONOCYTE DERIVED DENDRITIC CELLS HAVE ABNORMAL ANTIGEN EXPRESSION BUT APPEAR TO STIMULATE AUTOLOGOUS CD8+ T-CELLS IN MDS PATIENTS

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Background: An activated immune environment has been described in MDS and it is hypothesized that T-cells are reacting to antigens present on the surface of the malignant cells leading to inhibition of colony growth and apoptosis. However it has been demonstrated that selected CD34+ cells in the presence of cytokines and the absence of accessory cells in the bone marrow have colony growth similar to normal. It has therefore also been suggested that T-cells are not solely responsible for the characteristic cell death observed in the bone marrow and that other cells such antigen presenting cells could also play a role. Dendritic cells have been shown to be clonal and abnormal in MDS and therefore their defective interaction with T-cells could be important and requires investigation. Aims: The aim of this study was to examine the ability of antigen presenting cells to mature, express co-stimulatory molecules and activate both allogeneic and autologous CD4+ and CD8+ T-cells. Methodology: 5 patients with MDS were studied. All patients and normal individuals were required to read and sign a consent form according to University of Cape Town ethical guidelines. Monocyte derived dendritic cells (MoDC) were generated by culturing peripheral blood monocytes with GM-CSF and IL-4 for 5 days. The immature MoDC were then activated using LPS and TNFα and thereafter analysed for the expression of co-stimulatory and activation antigens (CD80, CD86, CD83, HLA-DR, CD11c, CD1a) using standard 4 colour flow cytometry. The activated antigen presenting cells were then cultured with both autologous (from the same patient) and allogeneic (donor) T-cells. After 72 hours of culture, CD4+ and CD8+ T-cells were examined for expression of the activation antigen CD69 using standard flow cytometry. The results were compared to 9 normal controls which were cultured in the same manner. Results: After activation with LPS and TNFα the percentage and mean fluorescent intensity of expression of HLA-DR (p=0.04), CD11c (p=0.03), CD80 (p=0.05) and CD86 (p=0.03) was reduced in 4 out of the 5 patients with MDS when compared to the normal MoDC. CD1a and CD83 expression was however similar to normal. The mature MoDC had a reduced ability to stimulate allogeneic donor T-cells but the activation and CD69 expression of autologous CD3+ and CD8+ T-cells was increased when compared to normal controls. Conclusion: This study confirms that antigen presenting cells in myelodysplasia do not mature normally, have reduced expression of co-stimulatory molecules and are unable to effectively stimulate allogeneic T-cells. However, the results also imply that their capacity to activate autologous T-cells is enhanced indicating that that they are able to present antigen. These findings suggest that both T-cells and dendritic cells could be utilised to develop anti-tumour immune based therapies in myelodysplastic patients.

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AGE-ASSOCIATED ALTERATION OF GAMMA/DELTA T CELL REPERTOIRE AND DIFFERENT PROFILES OF ACTIVATION-INDUCED DEATH OF V-DELTA1 AND V-DELTA2 T CELLS

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Background. Although γδ T cells have been suggested to be involved in certain autoimmune disorders, the role of γδ T cells in bone marrow failure syndrome remains largely unknown. Since age and race have been reported to influence the γδ T cell repertoire, appropriate reference data matched for age and race are absolutely required for clinical studies. *Aims*. The principal aim of this study was to establish reference data on γδ T cell repertoires in a healthy Japanese population and to identify the mechanisms shaping the γδ T cell repertoire. *Methods*. We examined the γδ T cell repertoire in 120 healthy volunteer donors by flow cytometry. Flow cytometry-based analysis of peripheral blood T cell subsets was performed using a whole blood lysis technique in order to avoid the potential loss of certain T cell subsets. Lymphocyte counts were calculated from the white blood cell count and the percentage of lymphocytes determined by flow cytometry. The monoclonal antibodies (mAbs) used in this study were as follows: anti-CD3 (SK7); anti-TCR-α/β-1 (WT31); anti-TCR-γ/δ-1 (11F2); anti-Vδ1 (TS8.2); anti-Vδ2 (Immu389); anti-NKp46 (9E2/NKp46); anti-CD56 (MEM188); anti-CD95 (Fas/APO-1) (DX2); control mouse IgG1-PE (679,1Mc7); mouse IgG1-FITC (DAK-GO1) and mouse IgG1-PerCy5.5 (X40). Activation-induced apoptosis and expansion of the γδ T cells in vitro were also analyzed. Expression of Intracellular Bcl-2 family proteins was examined by staining the permeabilized cells with anti-Bcl-2 and anti-Bim mAbs. This study was approved by the institutional review board at Akita University. Results: The average numbers of T lymphocytes in blood from all donors examined (n=120) were as follows: 1,084 ± 369 (SD) αβ T cells, 68 ± 44 γδ T cells, 16 ±12 Vδ1 T cells and 43 ± 36 Vδ2 T cells (/μl). The absolute numbers of γδ T cells were reduced in association with aging (R=-0.378 , p<0.001). The decrease of γδ T cells was a result of age-related reduction of Vδ2 T cells (R=-0.419, p<0.001) but not of Vδ1 T cells (R=-0.098, p=0.299) . As a result, the Vδ2/Vδ1 ratio showed an age-dependent decrease. Gender also affects the γδ T cell repertoire, and the numbers of Vδ2 T cells were significantly higher in male donors than female donors (p=0.007). The numbers of αβ T cells, Vδ1 T cells and NK cells were significantly higher in CMV-seropositive donors than CMV-seronegative donors. The Vδ2 T cells but not Vδ1 T cells showed a rapid reduction in cell numbers against mitogen stimulation, and exogenous addition of IL-2 did not rescue the Vδ2 T cells to die. Annexin-V binding was increased at 6 hours of PHA stimulation in all T cell subsets, and the Vδ2 T cells showed the strongest Annexin-V binding. Bcl-2 protein expression was down-regulated in mitogen-stimulated Vδ2 T cells but not in Vδ1 T cells. Summary/conclusions: These results indicate that age and gender have great impact on the γδ T cell repertoire in Japanese donors as well as European and American donors. Age-related decrease of Vδ2 T cells might be explained by their susceptibility to activation-induced cell death.

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SIMULTANEOUS DETECTION OF GENOMIC REARRANGEMENTS IN MYELODYSPLASTIC SYNDROMES (MDS) USING THE MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) ASSAY

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Cytogenetic analysis of the bone marrow is indicated in MDS not only for diagnostic purposes, but also to assess individual prognosis according to the IPSS scoring guidelines and to plan tailored therapy. Conventional cytogenetic (CC) analysis is performed in clinical practice to detect chromosomal abnormalities. It has been reported that fluorescence in situ hybridization (FISH) is a more sensitive approach, however this analysis is limited to detection of the more frequent abnormalities on chromosomes 5, 7, 8, 11, and 20, and conflicting data are reported in literature. A new method has recently been described for the meas-

urement of the gene/chromosome copy number using genomic DNA: Multiplex Ligation-dependent Probe Amplification (MLPA). The purpose of this study was to compare the results of the MLPA assay with the CC data obtained in a series of 38 MDS patients (M: 28, F:10, median age 70 years, range 44-87). In accordance with the WHO classification, 10 cases were classified as RA, 10 as RCMD, 4 as RAEB-1, 8 as RAEB-2, 1 as MDS_U, and 5 as CMML. The MLPA assay contains 61 specific target sequences for chromosome regions commonly involved in MDS: 5q (9 probes), 5p (1 probe), 7q (8 probes), 7p (2 probes), 8q (8 probes), 8p (2 probes), 11q (8 probes), 12p (6 probes), 17q (2 probes), 17p (4 probes), 20q (5 probes), 20p (1 probe) and 21q (5 probes). The MLPA was performed on all samples according to the manufacturer's recommendations (MRC-Holland).

Table I

Sample	MLPA	Karyotype
1	No anomalies	46, XX
2	No anomalies	46, XY
3	No anomalies	46, XY
4	Del 5q	46, XY, Del 5q, t(1;12)
5	Del 7	46, XY, Del 7
6	No anomalies	46, XX
7	No anomalies	46, XY
8	No anomalies	46, XY
9	No anomalies	46, XY
10	Del 5q	46, XY, Del 5q
11	Del 5q, Del 11q23	46, XY, Del 11q23, Del 15, der 5 t(5;15) [10] / 46, XY [4]
12	No anomalies	46, XX
13	Trisomy 8	47, XY, Trisomy 8
14	Del 7q, Del 12p, Del 20q	No metaphases
15	Del 11q23	46, XX, Del 11q23 [5] / 46, XX, Del 9q22-23, Del 11q23 [15]
16	No anomalies	46, XY
17	No anomalies	46, XY
18	No anomalies	46, XY
19	No anomalies	46, XY
20	Del5q, Del 12p, Del17p	42~47, XX, Del 1p34 [3], Del 3 [6], t(4;18) [10], Del 5q [18], der 9 [3], Del 12 [10], t(13q;17q) [3], der 15 [4], Del 20 [3], Del 21 [2], Trisomy 22 [3], +m [10] [cp18] / 46, XX [2]
21	Del 20q, Trisomy 21q, Trisomy 15p, Trisomy 8	48, XY, Trisomy 15, Del 20q, Trisomy 21[12] / 46, XY, Trisomy 8, Trisomy 15, Del 20q[8]
22	No anomalies	46, XY
23	Trisomy 11q23 (MLL gene)	46, XX
24	No anomalies	46, XX
25	No anomalies	No metaphases
26	No anomalies	46, XX
27	No anomalies	46, XY,add(11)(p15)[10] / 46, XY[10]
28	No anomalies	46,XY
29	No anomalies	No metaphases
30	Del5q22-33	46, XY, Del5q15-33[8] / 46, XY[7]
31	No anomalies	46, XY
32	No anomalies	No metaphases
33	Trisomia 11	47, XY, Trisomy 11
34	No anomalies	46, XY
35	No anomalies	46, XY
36	No anomalies	46, XY
37	Del 7q22	46, XY
38	No anomalies	46, XY

The CC study was performed following standard protocols and at least 20 metaphases were analyzed. Our study showed a good correlation between the MLPA and CC results (Table 1), since most of the alterations being detected by both techniques. Discrepancies were found in 7 samples (18.5%). MLPA analysis did not detect the presence of a chromosomal (chr.) translocation (sample n°4); a chr. deletion and a chr. translocation (sample n°11); a chr. deletion (sample n°15); several chr. translocations and deletions (sample n°20); a chr. gain (sample n°27). In fact, the MLPA assay is not able to detect chr. translocations but only chr. loss or gain; it can only analyse the chr. regions commonly involved in MDS (5,7,8,11,12,17,20,21); it can reveal chr. abnormalities providing the percentage of cells carrying the alterations is about 30-35% and it do not show mosaicism. On the other hand, CC analysis did not show a small deletion in 2 samples: a deletion of the MLL gene in 11q23 (sample n°23) and deletion of 7q22 (sample n°37). With CC we also observed a karyotype failure (no metaphases) in 4 samples, while the MLPA assay showed three chr. deletions (sample n°14), but no anomalies in the other 3 samples (25, 29, 32). MLPA proved rapid, cost effective, relatively easy to perform, had high throughput and enabled simultaneous analysis of many samples by automated data processing. MLPA and CC resulted complementary techniques, MLPA being particularly useful in MDS cases with karyotype failure and for identifying small rearrangements.

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SETTING-UP OF THE FCM OGATA PROTOCOL FOR DIAGNOSIS OF MDS: EXAMPLE OF A FRENCH EXPERIENCE

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Background. Initial evaluation of patients with suspected MDS requires among other a careful assessment of their peripheral blood smear and blood counts, as well as morphology of bone marrow cells and specific diagnostic marker. Diagnosis of low-grade MDS without conventional markers (excess of blasts, cytogenetic abnormalities, Ring sideroblasts...) is more difficult to assess. It is necessary to take into account the clinical history and to rule out others conditions which are associated with dysplastic myeloid cells (viral infection, heavy metal, deficiency of vitamin, drug, ethanol...). In these cases, flow cytometry (FCM) is recognized by the WHO classification as a useful diagnosis tool. Aims. Our aim was to adapt the simple three color FCM protocol developed by Ogata and applied on a Japanese and an Italian cohort (*Ogata and al. Haematologica 2009; 94(8), 1066-10*). The protocol is based on CD34, CD45, CD10 labeling. We have added labeling of the CD19 marker to sensitize the detection of B-cells precursors. *Methods*. Fifty one bone marrow samples, including 17 proven MDS, 13 control patients (2 normal subjects and 11 patients with non-clonal cytopenia), 21 patients with suspicion of MDS but without enough evidences to firmly assess the diagnosis, were included. All the samples were treated within 16 hours. The nucleated cells were stained with four antibodies CD45-FITC, CD10-PE, CD19-PC5, CD34-PC7. At least 100000 cell events were acquired. We used the reference range (RR) published by Ogata. We thus analyzed four parameters: (1) the percentage of CD34+ myeloblast in all nucleated cells (RR<2.4%): (2) the percentage of CD34+CD19+ cells in all CD34+ cells (RR>5%); (3) the lymphoblast/myeloblast ratio (RR=4-7.8); (4) the granulocyte/lymphocyte SSC peak chanel ratio (RR>6). One point was given for each abnormal parameter. *Results.* One patient of the control group had a score of two. In that case, diagnosis of drug toxicity was obvious. Among proven MDS, 11/17 (65%) of patients had a score of 2 or more (specificity=93%, sensitivity= 65%). 23% of patients with suspicion of MDS had a score of two or more. Interestingly, the two most frequently abnormal parameters, were the SSC ratio and the percentage of CD34+CD19+ cells. *Summary/Conclusions.* The Ogata FCM protocol is a simple FCM method that was easily adapted to four colors by including the CD19 marker. In an attempt to improve the diagnostic power of the Ogata's score, we will work out our own reference range. Furthermore, all the cases in the group of suspicious MDS will need to be reevaluated. However, it is already very striking that, using the same reference range of Ogata *et al*., we reached the same sensitivity and specificity that the one published.

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FAS AND FAS LIGANT (FASL) EXPRESSION IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background. The Fas/Fas ligant (FasL) system dysfunction has been associated with hematological and non hematological diseases. In PNH, evidences suggest that this system could select and maintain the proliferation of the mutational clone by promoting normal cells apoptosis. *Aims:* The aim of this study was to analyze the expression of Fas and FasL in neuthophils and monocytes from PNH patients. We tried also to detect a possible relation between these expressions and clinical subtypes of this disease. *Methods*. We studied 16 patients followed at Universidade de Sao Paulo (USP), Universidade Federal de Sao Paulo (UNIFESP), and Hospital do Servidor Publico Estadual (HSPE). The patients were clinically classified as classic PNH (PNH/CL: 9 patients) and PNH in the setting of another bone marrow failure syndrome (PNH/BF: 7 patients). The expression of Fas and FasL in neutrophils and monocytes were analyzed by flow citometry (FACScalibur, Cell Quest software - BDB). In PNH, the neutrophils were divided according to the CD59 expression (59+/Nt or 59-/Nt), and in monocytes according to the CD14 expression (14+/Mo or 14-/Mo). The Fas and FasL expression in neutrophils and monocytes were also analyzed in 10 normal individuals as control group (59/CG and 14/CG, respectively). *Results.* No statistical difference was found in Fas expression among the neutrophils 59+/Nt, 59-/Nt and 59/CG. The same result was observed among monocytes 14+/Mo, 14-/Mo and 14/CG. FasL expression in 59-/Nt was significantly lower than in 59+/Nt (p=0.008), but there were no statistical difference between

14+/Mo and 14-/Mo. When we analyzed the data according with clinical subtypes, PNH/BF patients had lower Fas expression on both 59-/Nt (p=0.041) and 14-/Mo (p=0.014) than PNH/CL. In addition, when we analyzed PNH/BF group, we observed that 59-/Nt expressed less FasL than 59+/Nt (p=0.031). CONCLUSIONS: Previous studies had been show higher expression of Fas in CD59+/stem cells than CD59-/stem cells in PNH. In this study, PNH/BF patients had less Fas expression on 59-/Nt and on 14-/Mo than PNH/CL patients. This data suggest that 59+/Nt and 14+/Mo cells could be more susceptible to apoptosis, which is in agreement with the PNH clone survival advantage hypothesis. FasL expression was higher on 59+/Nt than on 59-/Mo, especially in the PNH/BF group. We could speculate, with this data, that FasL in excess could act in bone marrow, initiating the stem cells apoptosis process, what explain the characteristics bone marrow alterations found in PNH. Although the importance of FasL in apoptosis, this is the first study to analyze its expression on PNH.

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MARROW CELLS OF VITAMIN B12 DEFICIENCY CASES SHOW A PHENOTYPIC PROFILE SIGNIFICANTLY DIFFERENT FROM MYELODYSPLASTIC SYNDROME

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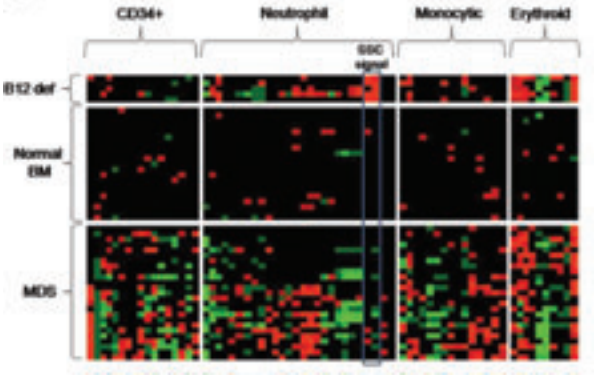
Background. The diagnosis of myelodysplastic syndromes (MDS) relies on documentation of morphologic dysplasia in bone marrow (BM) cells. Many reports have proposed flow cytometry (FC) as an useful integrating technique in this subset. Nonetheless some non-malignant disorders, such as vitamin-B12 deficiency (B12 def), mimic MDS causing macrocytic anemia and megaloblastic-like abnormalities. *Aims*. We compared the immunophenotypic characteristics of B12 def patients' BM cells to normal BM and MDS samples. Our aim was to verify if FC was able to differentiate between the diseases and to identify any phenotypic feature useful to suspect a deficiency-related pathogenesis. Methods. 2x106 BM cells were stained with quadruple combinations of a wide panel of monoclonal antibodies; 50,000 events of global BM cellularity were acquired; if required, a second step was performed to collect 1x103 CD34+ cells at least. Data acquisition was performed using FACSCalibur flow cytometer and CellQuestPro software (Becton Dickinson). For data analysis, Infinicyt (Cytognos) software was used. Our approach was adapted from what described by Matarraz et al (Leukemia 2008). Some major BM compartments were identified on the basis of forward (FSC) and sideward (SSC) light-scatter and reactivity for CD45/CD34. Sixty-nine phenotypic parameters were expressed as percentage of positive cells within a compartment and/or mean fluorescence intensity (MFI;arbitrary relative linear units, scaled from 0 to 104). Phenotypic aberrancies were quantified considering deviations from normal profile, defined by mean value ± two standard deviations. The phenotypic data were also displayed through a color code (Figure 1): black corresponded to normal expression; red and green to higher and lower than the mean, respectively; color intensity was proportional to deviation degree.



Results. BM of 5 patients with proven B12 def were analyzed, and 25 newly-diagnosed MDS patients; according to WHO, they were classified as: RA, 7 patients; RARS, 1; RCMD, 5; RAEB-1, 8; RAEB-2, 4. CD34+ cells of B12 def showed no significant phenotypic difference from controls. Conversely, neutrophil compartment had several deviations from normal pattern. As highlighted in Figure 1, SSC resulted significantly higher for B12 def (median value 291 vs 226,p=0.011); this finding appeared more evident restricting the analysis on stage-IV mature granulocytes (395 vs 250,p=0.0002), likely expressing the phenotypic counterpart of hypersegmented neutrophils. Moreover, in MDS subset, stage-IV granulocytes showed a significant reduction of SSC in a substantial fraction (24%) of patients, as expression of hypogranularity and pseudo-Pelger abnormality. Monocytic lineage showed an increased FSC for B12 def (560) compared to controls (462,p=0.0006) without any other relevant difference. Erythroid subset was featured by several alterations, primarily regarding an increase on global cellularity (30.3% vs 9.9%,p=0.0002). The cells revealed higher FSC (283 vs 226,p=0.0002) and SSC (30 vs 20,p=0.0084), while the antigenic profile showed a weaker CD36 (1125 vs 1704,p=0.04) and CD71 (1077 vs 3207,p=0.0245) expression. As depicted in Figure 1, the phenotypic aberrancies of B12 def within the erythroid compartment resembled what found in MDS. *Summary/conclusions*. Through a multiparameter and systematical approach, we highlighted some aberrancies shared by patients with B12 def and defined a useful profile to distinguish it from clonal disorders.

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THE SIGNIFICANT ASSOCIATION BETWEEN PRIMARY MYELODYSPLASTIC SYNDROME AND SINGLE NUCLEOTIDE POLYMORPHISMS

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Background. Myelodysplatic syndrome (MDS) represents a heterogeneous group of clonal disorders with ineffective hematopoiesis and its etiology has not been fully explained. Additionally, MDS may be induced by some chemotherapeutic toxins or mutagenic environment factors but the association with certain genes has yet not been detected. *Aims.* To expand the understanding of MDS etiology we sought to identify genes and polymorphisms associated with MDS using multiplex genotyping. *Methods.* We used the Illumina Cancer SNP Panel containing 1421 single nucleotide polymorphisms (SNPs) derived from 408 genes thought to be involved in cancer. We conducted a case-control study of 189 patients with primary MDS and 262 controls in Czech population. Firstly, the quality control for all SNPs and samples was implement and then, Chi2 p-value, odds ratio (OR) and upper and lower limits of 95% confidence interval of OR were calculated. When applying Bonferroni correction for multiple testing, the p-value is supposed to show significant association of SNP with the phenotype. *Results*. Ten SNPs showed significant case-control differences at the level of p< 0,0001. Findings included an increased risk associated with variants in the anion exchange gene SLC4A2 (p-value=0,0000000001;OR=2,64;95% Cl=1,97-3,55), two ATP-binding cassette transporters genes ABCB1 and ABCC2 (p-value=0,0000005;OR=3,38;95% Cl=2,08-5,48 and p-value=0,00001;OR=2,11;95% Cl=1,05-2,95), the DNA ligase I LIG1 (p-value=0,0000008;OR=2,10;95% Cl=1,57-2,82) the aurora kinase A STK6 (p-value=0,000003;OR=2,51;95% Cl=1,70-3,71), the progesterone receptor PGR (p-value=0,000008;OR=2,14;95% Cl=1,54-2,99), DNA mismatch repair protein MSH3 (p-value=0,00008;OR=6,71;95% Cl=2,49-18,08) and the DNA repair gene RAD52 (p-value=0,00006; OR=1,88;95% Cl=2,38-2,55) and decreased risk associated with the ROS1 gene (p-value=0,000002;OR=0,44;95% Cl=0,31-0,61). These findings are biologically plausible since association of SNPs in SLC4A2, ABCC2, STK6, LIG1, GPX3 and RAD52 genes with some kind of cancer were described. *Conclusions.* We observed a significant association between MDS and the common genetic variants described above. This evaluation of genetic polymorphisms identifies SNPs which may be involved in the pathogenesis and biology of this disease. Specific genes associated with risk may have particular relevance for gene function and/or carcinogenesis.

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MYELODYSPLASTIC SYNDROME. EGYPTIAN EXPERIENCE

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Background: Myelodysplastic syndromes (MDS) incidence is unclear because of historical lack of population-based registration and possibly because of under diagnosis. Purpose: To present some retrospective data

on the epidemiology of myelodysplastic syndrome (MDS) in Egypt, as reflected by a single center based Registry which is the largest tertiary referral center in Egypt. Patients and Methods: Patients diagnosed with MDS and referred to Clinical Haematology unit of Internal Medicine Department Cairo University, Egypt between 2007-2010 were identified. Complete demographic and clinical data, laboratory results, treatment modalities were collected and analyzed. Results: 69 patients with MDS were identified. 39 (57%) females, 30(43%) male. Mean age was 55 years. 9 (13%) patients were positive for HCV. Mean ferretin level was 844 ng/ml and mean blood transfusion units were 12 units. 26 (38%) patients were RCMD, 15 (22%) patients with RA-EB, 10(14%) patients with hypoblastic MDS and 18 (26%) patients with RA and RARS. 12(17%) patients were less than 40 years, 4 (5%) of them had RA-EB. There were a strong correlation between ferretin and ALT (r=0.415 P:0.002), ferretin and blood units (r=0.26 P:0.046) and negative correlation between ferretin and age (r=-0.27 p:0.03). 48 (70%) patients were from rural areas. Summary and Conclusion: We consider this number of diagnosed MDS is a large one and reflects the increased awareness of the disease and improved methods of diagnosis. The young age of diagnosis and appearance of RAEB in young patients may reflect the impact of environmental pollution especially water and soil (70% were from rural areas) on the development of genetic mutation. Iron overload is a permanent feature of MDS. The higher prevalence of HCV is part of the problem of increasing hepatitis among Egyptians and could be related to blood transfusion during course of treatment though the strict measures in blood banks . The correlation between ALT with ferretin reflected the impact of under treatment of those patients with iron chelation therapy on progression of liver disease especially in presence of HCV.

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INTRAVENOUS IRON SUPPORT VS ORAL LIPOSOMIAL IRON SUPPORT IN PATIENTS WITH REFRACTORY ANEMIA TREATED WITH EPOα. MONOCENTRIC PROSPECTIC STUDY

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Background. Intravenous iron support simultaneous to erythropoietin administration improve response to erythropoietin in myelodysplastic patients. In fact intestinal absorption of common commercial oral iron compounds is considerably impaired. Moreover, in MDS patients, absorbed iron is frequently stored in tissues and is'nt bioavailable. Oral liposomal iron, bypassing normal intestinal mechanism of absorption, shows an increased haematic absorption, better than usual commercial oral iron compounds. *Aims*. Aim of this study is to verify if in MDS patient support with oral liposomal iron is not inferior to iv iron support. Methods. Between july 2008 and december 2010, 24 patients affected by refractory anemia were studied. Median follow-up was 12 months (R10-24). Patients were randomized 1:1 to receive in group A sodium ferrigluconate 62.5 mg iv in NS 100 ml in 1 h/day in the day when patient received α erythropoietin 40000 IU sc/week + calcium levofolinate 7.5 mg/day orally + Vitamin B12: 400 mg/day orally. In group B patient received lipofer 14 mg 2 tablets orally/day + α erythropoietin 40000 IU sc/week + calcium levofolinate 7.5 mg/day orally + Vitamin B12: 400 mg/day orally. In group A median age was 70 years (R65-75), M/F: 4/8. In group B median age was 66 years (R60-70), M/F: 6/6. Caryotype was normal in group A and B patients. Median level of haemoglobin was 9 g/dl in group A (R8.5-11) and 8.8 g/dl (R8.5-11.5) in group B. *Results*. Group A patients increased Hb level of 1 g/dl after a median time of 4 weeks (R4-7) and after a median time of 5 weeks (R4-8) in group B. Most frequent side effects in group A were erythema in site of injection in 4 patients (33%), hypotension in 1 patient (8%). Most frequent side effects in group B were grade 2-3 diarrhoea in 4 patients (33%). During median follow-up time patients of A and B group gaigned near 3 g/dl of Hb. *Summary/Conclusions*. Oral liposomial iron supporting erythropoietinic therapy seems to be safe, feasible and substantially not inferior to intravenous iron support in patients affected by refractory anemia.

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ASSOCIATION OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND PAROXYSMAL NOCTURNAL HEMOGLOBINURIA WITH A SMALL CLONE: A CASE REPORT

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Background. Paroxysmal nocturnal haemoglobinuria (PNH) is a haematologic disorder characterized by a clonal expansion of haematopoietic stem cells bearing a deficiency of the glycophophatidylinositol (GPI)-linked proteins due to an acquired mutation. Usually PNH clones are detected in patients with aplastic anaemia although non systematically ; they are rarely observed in monoclonal gammopathies of undetermined significance (MGUS). Here is reported the case of a 77-year old woman with both a MGUS and a small PNH clone which remained steady during several years Case report : A monoclonal IgG lambda gammopathy with a PNH clone was diagnosed in 2001. In 2008, no plasmacytosis was observed on the bone marrow smear and there were no clinical or radiological symptoms of myeloma. Methods : Three markers on red blood cells (RBC) were studied by flow cytometry (CD55, CD58 and CD59) also two markers on granulocytes (GN) (CD55 et CD59) and one on monocytes (MN) (CD14). The direct antiglobulin test (DAT) was performed on RBC by gel filtration. Results: In April 2008, CD55-, CD58- and CD59- RBC were at 2.9%, 2.1% and 0.6% respectively. On GN, CD55- and CD59- cells were at 0.9% and 1.8% and on MN, CD14- cells at 5.4%. The haemoglobin (Hb) level was at 94g/L, the haptoglobin and total bilirubin levels were normal at 0.9 g/L and 9.0 micromole/L respectively. The DAT was negative. In July 2009, the size of the PNH clone was still low (CD55-, CD58- and CD59- RBC were at 1.8%, 0.6% and 0.6%; CD55- and CD59- GN at 1.7% and 2.8%; CD14- MN at 3.5%). The Hb level was at 107g/L, the lactate dehydrogenase (LDH) level at 330 IU/L, total bilirubin level at 6 micromol/L, the DAT was negative but the haptoglobin level decreased to 0.2 g/L (normal higher than 0.7g/L). In April 2010, the same pattern was observed on PNH cells : CD55-, CD58- and CD59- RBC were at 0.7%, 0.6% and 0.6%; CD55- and CD59- GN at 1.2% and 2.0%; CD14- MN indicated doubtful results. No sign of haemolysis was noted, only a low level of haptoglobin (0.2 g/L). Nevertheless, in June and December 2010, CD14- MN began to increase to 6.4 and 10.1% without change for the other blood cells. *Summary/conclusions*. This case combines an unusual association of two blood disorders. When first observed the size of the PNH clone was low and remained such for several years without sign of haemolysis but with a low level of haptoglobin. No sign of myeloma has been observed during the clinical course.

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LOW RISK MDS: 5-AZACITIDINE, WHAT´S ELSE?... LENALIDOMIDE!

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Alternative treatments are poor and limited effect, in low risk MDS patients. However, everybody have some younger patients, that they could receive 5-azacitidine or lenalidomide when it failed. Patients: We reported 4 patients have been treated with 5-azacitidine, they researched a good erithroid response, but they lost it. In this moment, we offer them to initiate therapy with lenalidomide. Case 1: Male 50 years-old was diagnosed of SRA (low IPSS, normal cytogenetic) 3 years ago. He had a transitory respond to EPO. 6 months later, he received 5-aza with a good respond 4 months later. This was mantened to nine cycles. Later, he has initiated Lenalidomide (initial dosage was 10 mg/d, actually he intakes 5mg/48h), he has managered the independent transfusional. Initial Hb was 4.8 g/dL, and now Hb is 10 g/dL, after 6 months of treatment. . At 5 months, he has lost the respond (Hb 5.2g/dL). He has completed 14 cycles. Case 2: Female 81 years-old was diagnosed of SRA (int-1 IPSS, normal cytogenetic) 2 years ago, she had a transitory respond to EPO. 18 months later, she began 5-aza therapy, with good respond 2 months later. She did keep up to 14th cycle. Later, she has initiated Lenalidomide at dosage 10 mg/d, actually she intakes 5mg/48h), she has managered the independent transfusional. Initial Hb was 6.0 g/dL, and now Hb is 11 g/dL, after 10 months of treatment. At present, she has Hb 9.8 g/dL after 12 months of therapy. Case 3: Male 78 years-old diagnosed of SRA (low IPSS, normal cytogenetic) 1 year ago. He had a transitory respond to EPO. 2 months later, he began 5-aza therapy, with good respond 3 months later. He did keep up to 15th cycle. Later, he has initiated Lenalidomide at dosage 10 mg/d, he has managered the independent transfusional. Initial Hb was 6.0 g/dL, and now Hb is 9 g/dL, after 8 months of treatment. Case 4: Female 63 years-old was diagnosed of SRA (low IPSS, normal cytogenetic) 20 years ago. She began 5-aza therapy, with good respond 3 months later, and this was maintained 24 months later. Later, she has initiated Lenalidomide at dosage 10 mg/d. At present, she hasn´t respond yet, after 5 months of therapy. Results. the three patients reached the independent transfusion, but we were

obligated to tapering dosage and we must wait 6-10 months to research the respond. We reported hematologic toxicity: G3-4 neutropenia and thrombocytopenia. We used G-CSF treatment by 48-72h. Conclusions. 1) In low risk MDS patients, non 5q-, lenalidomide is an alternative therapeutic, when 5-aza has failed; 2) Lenalidomide used dosage, is lower than recommended, by hematological toxicity; 3) The response, if it happens, is later than we hope (between 6-10 months).

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A CASE REPORT OF WHIM SYNDROME (MIELOKATHEXIS) IN BRAZIL - CLINICAL FEATURES AND BONE MARROW MORPHOLOGY

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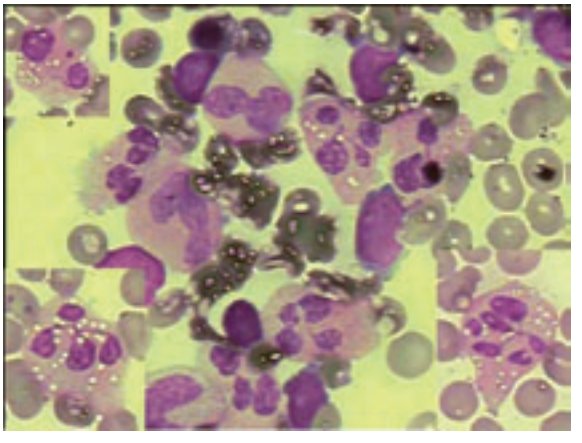
Background. WHIM syndrome is a very rare congenital disorder with approximately 40 cases described until now. This syndrome is characterized by severe chronic neutropenia, retention and apoptosis of mature neutrophils in the bone marrow (myelokathexis), hypogammaglobulinemia and recurrent bacterial infections. *Aim*. To report a case of WHIM syndrome in a brazilian patient.



Methods. Clinical and key laboratory data were recorded during the follow up of the patient before and after treatment. *Results*. A 2-year old girl was evaluated at our hematology clinic with a past history of recurrent infections (3 episodes of pneumonia and 5 episodes of urinary tract infection) and persistent low neutrophil counts, ranging from 64 to 650/μL since she was 10 months old. There were no abnormalities on physical examination. Investigational tests showed hypogammaglobulinemia (gamma globulin=0,29g/dL) on protein electrophoresis and the examination of the bone marrow aspirate revealed a hypercellular bone marrow with granulocytic hyperplasia, characterized by an increased number of mature neutrophils with hypersegmented nuclei and cytoplasmic vacuolization. Neutrophils showed nuclear lobes often separated by long strands of chromatin (Figure 1). These typical bone marrow morphologic findings of myelokathexis in association with the clinical picture were consistent with the diagnosis of WHIM syndrome. Treatment with G-CSF (5mg/Kg per day subcutaneously) was initiated after diagnosis confirmation, leading to an increase in neutrophil count to 550/μL after 12 days of therapy and to 1300/μL after 24 days. No side effects or new episodes of bacterial infection were observed after therapy initiation. *Summary/Conclusions*. (i). Careful morphological examination of bone marrow aspirate is determinant for diagnosis of myelokathexis, especially in clinical settings where genetic and molecular characterization is unavailable. (ii). Response to G-CSF is in accordance with previous reports.

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CLINICAL AND BIOLOGICAL CHARACTERISTICS, EVOLUTION AND IMPLEMENTATION OF THE NEW PROGNOSTIC SCORE IN A SERIE OF PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML)

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Background. CMML is a heterogeneous clonal hematologic neoplasm with both myeloproliferative and myelodysplastic features. The International Prognostic Scoring System (IPSS) is of limited usefulness. Recently a new prognostic model (*Kantarjian et al, Cancer 2008*) has been proposed that predicts better survival in these patients. Aims and Methods. Retrospective analysis of clinical and biological characteristics, evolution and application of a new prognostic model for patients with CMML diagnosed in a community hospital between January 2003 to May 2010. Results. Thirty nine patients were identified. They were 28 (72%) male and 11 (18%) women; median age was 75 years (range 55-93). In 18% the performance status measured by ECOG scale was higher than 2. Average and extreme analytical values at diagnosis were as follows: Hb 112 g/L (44-175), WBC 35.1x10^9/L (3.5-82), monocytes 3.6x10^9/L (1-17.9), platelets 151x10^9/L (17 - 750). At the time of diagnosis, thirteen patients (33%) had leukocytosis >13x10^9/L, 8 (20%) had peripheral blasts (range 2-11), 19 (49%) had mielemia and 5 (13%) had splenomegaly. Thirty five (90%) of the 39 patients had CMML type 1 and 4 (10%) had CMML type 2. Eight (20%) patients had cytogenetic abnormalities (+8 (n=2), 11q (n=1), -4 (n=2), -7 (n=2), -5q (n=1)). Seven patients (18%) had previously required blood transfusions. The distribution of patients according to the IPSS was 25 (64%) low risk, 10 (26%) intermediate-1 risk, 4 (10%) intermediate-2 risk and no patient had high-risk IPSS. The distribution according to the score proposed by Kantarjian et al was: 22 patients (56%) low risk, 4 (10%) intermediate-1 risk, 6 (15%) intermediate-2 risk and 7 (18%) high risk. Four patients (10%) of the 39 patients progressed to acute leukemia. Of these, 3 had cytogenetic abnormalities and all showed intermediate-1 IPSS while 3 had a score intermediate-2 or high risk under the new score. With a mean of 23 months (0-84), 19 patients (49%) survived, 17 (43%) have died and over 3 their status is unknown. In 60% the cause of death was secondary to CMML. The medians and ranges of survival (months) of patients stratified by IPSS were as follows: 16 (0-75) in the low risk group, 22 (0-52) in the intermediate-1, 5 (2-6) in the intermediate-2. Applying the new score they were: 22 (0-75) in the low risk, 21 (12-30) in intermediate-1, 16 (0-24) in the intermediate-2 and 5 (2 - 24) in high-risk. Conclusions: The CMML affects elderly patients. Their clinical and biological features are heterogeneous. A minority presents cytogenetic abnormalities. While the small number of patients in this serie, the new prognostic score seems to be better than the IPSS stratifying patients into different risk groups.

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TRANSFUSIONS IN HOME CARE PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background. The majority of patients diagnosed with myelodysplastic syndromes (MDS) are individuals of older age often afflicted by several comorbid conditions for which they are generally unsuitable for disease-modifyng treatments. The treatment of anemia is an essential part of the global management of most MDS patients. In erythropoietin-failed patients or in those unsuitable for this option, red blood cell (RBC) transfusions remain the only available measure. For this category of frail patients and for their families, home care (HC) represent a valuable option allowing to preserve the patient's quality of life and to avoid useless hospital admissions. *Aims*. To evaluate the management of RBC transfusions at home during the last two years. Methods. There were 68 MDS (27 male) with a median age of 86 (69 - 98) years. Patients were followed at home for a mean of 8.8 (1 - 24) months. Therapy with erythropoietin stimulating agents was used in 41 pts (60%). *Results*. Overall, 55 (81%) patients required transfusions, for a total of 927 RBC units; RBC units / transfused pt were a median of 10 (1-68). RBC units monthly requirement in transfused pts were a median of 1.5 (0.05-5.7). A lower baseline Hb concentration and the time for the primary diagnosis of MDS strongly correlated with the number of transfused RBC units. All transfusions were safely administered at home without any untoward effect. *Conclusions*. QoL is a particularly important issue for older MDS patients. With this regard, management of chronic patients requiring multiple and repeated admissions to receive RBC transfusions may be a concern for the affected individual and for its family. Our experience demonstrated that the administration of RBC transfusion at home is a feasible, reliable and effective in our older MDS patient, avoiding social

and economic costs due to an inappropriate removal from his domestic environment. In conclusion, in our experience domiciliary management of RBC transfusions represented an important added value to home care program, allowing the best humanization of this procedure for our patients.

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EVALUATION OF CIRCULATING COLONY-FORMING PROGENITOR CELLS AND TREATMENT RESPONSES IN PATIENTS WITH APLASTIC ANEMIA: A SINGLE CENTER EXPERIENCE

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Aplastic anemia (AA) is a life-threatening hematopoietic disorder characterized by bone marrow failure with *empty* marrow and peripheral pancytopenia. Standard treatments include hematopoietic stem cell transplantation (SCT) and immunotherapy (IST) with antithymocyte globulin (ATG) and cyclosporin-A (CSA). However, not all patients can be cured with SCT or ATG/CSA. Only a few, more or less robust, prognostic factors predicting long term relapse-free survival in AA are available. We have retrospectively analyzed a cohort of fifty patients with AA (27 males and 23 females) treated in our department between 1987 and 2007. The median age was 37 years (range 14-70 years). Fourty two patients received ATG/CSA, and 7 were transplanted upfront using a matched sibling donor. One patient was treated with CSA and growth factors only. In the group of transplanted patients, one patient died from multi-organ failure and 6 are alive and are in continuous complete remission (CR). Of the 43 patients receiving ATG/CSA or CSA, 28 patients (65%) achieved a CR including the one patient who was only treated with CSA, 7 (16%) entered a partial remission (PR), and 8 patients (19%) did not respond to treatment. Eight patients (19%) relapsed after IST. To evaluate possible prognostic factors predicting responses to IST, patient age, karyotype, existence of PNH clones, pre-treatment blood counts, progenitor cell counts, and outcome were evaluated. In this analysis, we found that in IST-treated patients with AA, the numbers of colony-forming progenitor cells increased but remained below the normal range of healthy controls in all patients. We also found that the numbers of granulocyte-macrophage colony-forming progenitor cells (CFU-GM) increased during successful therapy and were higher in those who underwent SCT than in patients who received IST ($p<0.05$). However, we were unable to detect a prognostic risk factor that would predict long term responses or relapse-free survival in these patients, which may be because of the low number of patients examined. In summary our data show that an increase in the numbers of circulating colony-forming progenitor cells is associated with regeneration of bone marrow function in AA patients successfully treated with SCT or IST, although normal CFU levels are not reached. Prospective studies with more patients are required to define prognostic factors predicting relapse-free survival in these patients.

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CYTOLOGICAL FINDINGS OF PAGET'S DISEASE DIAGNOSED BY BONE MARROW ASPIRATION

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Paget's disease is estimated to occur in 1-3% of individuals older than 45-55 years and in up to 10% in persons older than 80 years. Because early diagnosis and treatment is important, a range of specific imaging and laboratory studies are essential. Clinical diagnosis often is difficult and a bone biopsy may be needed, especially to exclude a metastatic bone disease. Only one case of Paget's bone disease initially diagnosed by bone marrow aspiration has been reported in english literature. We report the case of a 53-year-old man who presented with vertigo and hypertension of two days duration. On x-ray and CT-scan of the skull osteoblastic bone lesions were noted and a bone scintiscan followed which revealed a multifocal osteoblastic activity with lesions on the skull, vertebrae, pelvis and long bones of the lower leg. A bone marrow aspiration was performed and in the aspirate smears were identified multiple osteoblasts mainly in small aggregates and few osteoclasts, findings which were consistent with Paget's disease. The core biopsy showed an abnormal bony architecture with osteolysis accompanied by osteoblastic bone formation with an increase in the number and activity of the osteoblasts and replacement of the normal marrow with fibrous tissue, findings which confirmed the diagnosis of Paget's disease. In conclusion, we describe the second case of Paget's disease diagnosed by bone marrow aspiration which proves to be a relatively simple, cost-effective, and accurate method in the diagnosis of a clinically suspected disease, confirm the radiological impression and may exclude the presence of a malignant process.

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5-AZACITIDINE IN PATIENTS WITH MYELODYSPLASIA AND ACUTE MYELOID LEUKEMIA: A SINGLE CENTRE EXPERIENCE

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Background. Hypometilating agents have recently been shown to prolong overall survival and improve quality of life in patients either with INT-2 and high IPSS risk myelodysplasia (MDS) or with low bone marrow blast count acute myeloid leukemia (AML). Aims. The aim of our retrospective analysis was to evaluate the efficacy and the feasibility of 5-azacitidine therapy in a cohort of patients for whom no alternative therapy is available. Methods. Since September 2008 we have been treating with 5-azacitidine 33 patients affected by acute myeloid leukemia (18 patients), MDS (13 patients) or chronic myelomonocytic leukemia (2 patients). The median age of patients at treatment starting time was 70 years (range: 51-82). Azacitidine was administered subcutaneously (75 $mg/m^2/d$) for 7 days of every 28-day cycle until loss of response or disease progression. Patients received a median number of 6.5 cycles of therapy (range 1-24). According to International Working Group MDS and LMA criteria, in the overall study population we evaluated overall improvement (CR + PR + HI), the best response obtained and adverse events. In the subgroup of patients who received at least 6 cycles of therapy (13 patients) we also evaluated overall survival (OS) and progression free survival (PFS). *Results*. In the AML cohort, after a median number of 4.6 cycles (range 1-17), we observed a complete response in 5% of patients, a hematological improvement (HI) in 22% of patients, a stable disease (SD) in 39% of patients and a lack of response in 33% of patients. In the MDS cohort, after a median number of 8.5 cycles (range 2-24), we observed a complete response (CR) (including a complete cytogenetic response) in 31% of patients, a partial response (PR) in 7,5% of patients, a hematological improvement in 46.5% of patients and a stable disease in 15% of patients. The overall improvement (CR + PR + HI) was 28% in AML cohort and 84.5% in MDS cohort. In the CMML cohort, after a median number of 10.5 cycles (range 9-12), we observed a partial response in 50% of patients and a hematological improvement in 50% of patients. In the subgroup of patients who received at least six cycles of therapy, the overall survival was 14 months and the progression free survival was 11.5 months. In the overall study population, only two patients (6%) discontinued treatment as a result of adverse events. *Conclusions*. The limited number of cases and the short period of follow-up don't allow us to evaluate overall survival and progression free survival in the whole study population. In the subgroup of patients who received at least six cycles of therapy, we observed that 5-azacitidine plays an important role in treatment of MDS and low bone marrow blast counts AML, particularly with prolonged OS and good safety profile. Further trials should assess the number of cycles required for treatment, the role of hypometilating agents in low-risk MDS and in patients with AML and a bone marrow blasts counts > 30%

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THE IMPORTANCE OF COMPLEX BLOOD COUNT ASSESSMENT FOR DIAGNOSTICS AND PROGNOSIS IN MDS PATIENTS

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Background. The myelodysplastic syndrome (MDS) manifests as mono-, bi- or pancytopenia in peripheral blood and therefore the full blood count (CBC) play a key role in its diagnostics and diferential diagnostics. The careful evaluation of all parametres of blood count can contribute to fast estimation of MDS prognosis. Aims: The goal of retrospective analysis of 100 MDS patients examined within the period from 2000 - 2008 was evaluation of initial parametres of blood count for the risk of leukemia development and overall survival (OS). Methods: Clinical and laboratory data at diagnosis were analysed to assess the prognosis and the risk of leukemia development, were observed the course of disease and overall survival. The cohort consited of 100 patients (pts), 51 males

and 49 females (ratio 1:1), the age was 28 - 90 year (median 65 years). Results:According FAB clasification was OS (mean and median) for RA 68,7 months (median unobtained), for RARS 39,2 and 52,7 months, for RAEB 22,9 and 13,6 months (p=0,0003). The mean time to AML development was 77,6 months for RA, 50,5 months for RARS and for RAEB 17,4 (median 10,3), p<0,0001. 41% pts transformed to AML. According WHO 2001 classification to AML transformed: 1 pt from 8 RARS, 1 pt from 8 5q- syndrome, 5 pts from 30 RCMD, 3 pts from 8 RCMD-RS, 13 pts from 20 RAEB-I, 18 pts from 23 RAEB-II. 58 pts died - the reason was: AML - 25 pts (43%), infection - 6 pts (10%), 1pt brain haemorrhage, 1 pt myocardial infarction, reason unknown was in 25 pts (43%). Initial parameters of blood count were: Hb 44 - 149 g/l (Me 93), leucocytes 1 - 30.10^9/l (Me 3,5), neutrophiles 0,1 - 17.10^9/l (Me 1,7), lymfocytes 0 - 5.10^9/l (Me 1,3), MCV 77 - 125 fl (Me 96), trombocytes 3 - 702.10^9/l (Me 113), plt mass (MPVx trombocytes) 0 - 6,8 ml/l (Me 1,6), peripheral blasts 0 - 12 % (Me 0). Statistically significant unfavourable prognostic factors for OS showed trombopenia, neutropenia, presence of blast cells in peripheral blood, peripheral blast cells ≥ 5% and low platelet mass. The favourable factors were macrocytosis and lymfopenia. The level of hemoglobin (Hb), MPV and RDW were not shown as statistically significant for the prognosis. The risk factors of leukemia development were trombopenia, low or normal platelet mass, normal or low MCV, presence of blast cells in peripheral blood, neutropenia, lymphocytosis. The another parametres of CBC (Hb, RDW, MPV) were not statistically significant for leukemia development in our group. Summary/conclusion: This analysis confirms the importance of CBC not only as a basic diagnostic laboratory test but also as a prognostic indicator for MDS patients.

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DURABLE ERYTHROID AND CYTOGENETIC RESPONSE AFTER SHORT-TERM LENALIDOMIDE TREATMENT IN TWO PATIENTS WITH MYELODYSPLASIA AND DEL(5Q)

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Background . Lenalidomide is a immunomodulatory drug that has an impressive effect on myelodysplasia with deletion of the long arm of chromosome 5, leading to transfusion-independence, a complete erythroid and cytogenetic response in over 65% of cases. Aims. In this report we describe unexpected erythroid and cytogenetic responses in two del(5q) MDS patients treated with lenalidomide for less than 12 weeks. Little is known about these types of responses. Methods. Since November 2008 we have been treating two patients with myelodysplasia (MDS) and a deletion of the long arm of chromosome 5 [del(5q)] with lenalidomide. The monthly transfusion requirement before starting treatment was 6 packed RBC units. Lenalidomide was started at a full dose (10 mg/day for 21 days every 4 weeks). Results. Patient 1 received a diagnosis of *5q- syndrome*. She stopped therapy after only 10 days due to severe agitation and panic attacks: however, one month later we observed a progressive reduction of transfusion need. Two months later, the patient was transfusion-free and the response had been ongoing for 6 months; she obtained a very good partial cytogenetic response. After 6 months the anaemia worsened again; we started lenalidomide 10 mg/day, but after 15 days the therapy was stopped due to the same side effects. Forty days later, we observed transfusion-independence for another 6 months, and a very good partial cytogenetic response. When patient's condition worsened again, she was treated with lenalidomide 10 mg on alternative days with a better tolerance. After the first cycle of therapy she presented the same side effects. Again the karyotype analysis showed a very good partial cytogenetic response. In Patient 2 the morphologic analysis was compatible with RAEB-1 and the cytogenetic analysis showed a [del(16)(q22)] together with del (5q). Lenalidomide was stopped after 15 days due to renal impairment, cardiopulmonary and cardiac failure; she was then given only palliative therapy, but four weeks after she achieved a complete erythroid response and remained transfusion-free for one year. In January 2010 the patient worsened and started Lenalidomide again (10 mg/day for 21 days every 4 weeks) with good tolerance, a complete hematologic response and a very good partial cytogenetic response. In these two patients, after a very short treatment with lenalidomide we observed a complete erythroid response and a very good partial cytogenetic response. The median time to achieve the response was 6 weeks. Transfusion-independence was durable (24 and 38 weeks respectively). One patient experienced grade III hematologic toxicity during the first cycle of therapy; despite this, we did not observe severe infectious nor hemorrhagic complications. Conclusion. We observed unexpected effects of lenalidomide in myelodysplastic patients with del(5q) and a low-int1 IPSS risk who were treated for a very short period; additionally, this response was observed again at least twice when treatment was begun again: although these patients obviously represent only selected cases, the good erythroid and cytogenetic responses suggest that some patients with MDS and del(5q) may benefit from lenalidomide treatment even if this must be discontinued.

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INTERSTITIAL LUNG DISEASE: UNCOMMON BUT POTENTIALLY SEVERE EFFECT OF HYPOMETHYLATING AGENTS

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Background. Hypomethylating agents represent gold standard therapy in high risk myelodysplastic syndromes (MDS) not eligible for intensive chemotherapy or stem cell transplantation. Since drug approval, Azacitidine in USA and Europe, and Decitabine in USA, 3 cases of interstitial lung disease were published, two after treatment with Azacitidine (1, 2) and one after Decitabine therapy (3). Purpose: we report three new cases of interstitial lung disease (ILD) involving patients treated with hypomethylating agents for high risk myelodysplastic syndromes (MDS). Cases: Case 1 70 yrs old man, progressive dyspnea occurred after 12 cycles of treatment, chest X ray and tomodensitometry showed interstitial lung disease, diagnosis was confirmed by bronchioloalveolar liquid analysis. Treatment was discontinued. Lung function recovered within few months. Case 2 76 yrs old woman, fever and interstitial lung disease occurred after 6 cycles of treatment infectious causes were excluded. After drug discontinuation and corticosteroid therapy lung status improvement was observed. Case 3 76 yrs old man, after one cycle of treatment while anemia was corrected, he developed dyspnea due to ILD. Drug discontinuation was performed and lung function was recovered. *Conclusions*. Between 2007 and 2010 three cases of ILD associated with hypomethylating agents were published. Clinical presentation includes cough, dyspnea, and less commonly fever. Risk factors of lung interstitial disease associated with hypomethylating agents remain unknown as well as the possibility to use the other member of this therapeutic class after such reaction. Lung function evolution is often favorable after drug discontinuation. Corticosteroid therapy could be usefull. In our center approximately 150 patients received this treatment during the same period so estimated prevalence of this effect is 2%. Larger exposure to drug reveals new uncommon adverse events. In one patient Decitabine was used without lung fibrosis relapse.

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EVALUATION OF PERIPHERAL BLOOD SMEAR FOR MYELODYSPLASIA IN BREAST CANCER PATIENTS WHO RECEIVED ADJUVANT ANTRACYCLINE

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Therapy-related myeloid neoplasms (t-MN) account for approximately 10% to 20% of all cases of AML, MDS, and MDS/MPN. Topoisomerase-II inhibitors significantly associated with an increased risk of AML/MDS. Anthracycline-containing chemotherapy is currently the gold standard in breast cancer patients. In our study, we evaluated peripheral blood smear samples and hemogram values in breast cancer patients, receiving adjuvant anthracycline regimen that are in remission. Material and method. The patients, receiving anthracycline based adju-

vant chemotherapy treatment were evaluated. A total of 78 patients from Kayseri Research and Training Hospital and Mersin State Hospital were enrolled in the study. Their adjuvant treatments had been completed at least 18 months before. No patient had either chronic or infectious diseases. Patients with abnormal ferritin, vitamin B12 or folate levels were excluded from the study. Results. Two patients complained of anemia (2,2%) (Hb<11mg/dl); leucopenia was observed in seven patients (7,7%) (Leukocyte<4000/mm3); thrombocytopenia was observed in four patients (4,4%) (PLT< 150.000/mm3). It was established that ovalomacrocytes were 14%, macrocytes 37%, acanthocytes 1%, stomatocytes 12%, teardrops 12% , nucleated erythrocytes 1%, basophilic stippling 14% and Howell-Jolly bodies 1%. There were 38% hypo-granulation, 26% Pelger-Huet abnormality, 20% hypersegmentation, 8% immature granulocytes and 6% blasts. We have established 50% of giant platelets and 19% of platelets hypogranulation. Discussion. According to the peripheral blood smear assessments of our study, we suggest that breast cancer patients should be evaluated for MDS at the early stage, starting from Month 18, even if the automated blood counts are normal.

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PATIENT WITH COPPER DEFICIENCY MIMICKING MYELODYSPLASTIC SYNDROME (MDS)

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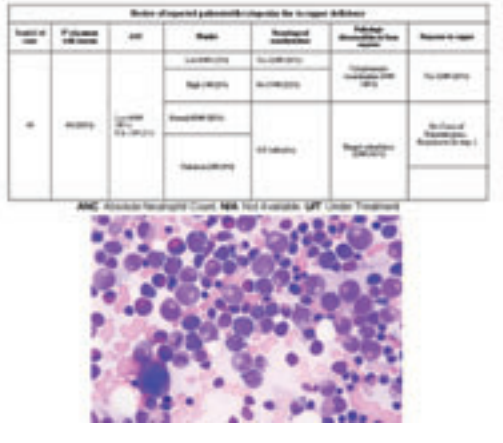
Myelodysplastic syndromes (MDS)are a group of heterogenous clonal stem cell disorders characherized by variable degree of cytopenias, cytogenetic abnormalities and evolution to acute myeloid leukemia. I n the low risk categories of MDS without cytogenetic abnormalities diagnosis relies on morphology and exclusion of other possible causes of cytopenias such as Vit B12 or folate deficiency. Copper deficiency is ussualy not taken in account in the study of a possible MDS. , several cases of bone marrow failure due to its deficiency have been reported. We present a case of Copper deficiency mimicking MDS. Case report and methods A 65 years old male with anemia (Hb=59gr/L VCM=94) and severe neutropenia (WBC=1.1X10^9/L, neutrophils=320/mm^3) and normal platelet levels was seen at our institution. The patient had progressive gait disturbance. A neurological exam was consistent with subacute combined degeneration as seen in Vit. B12 deficiency. Serum levels of B12, folate and ferritin were in the normal range. A bone marow exam disclosed dysplastic features in granulocyte and erythroid lines with a prominent vacuolization of progenitors of both lines(Picture 1) and 16% ring sideroblasts. A standard cytogenetic analysis did not show any clonal abnormality and FiSH was also negative for del 5q, monosomy 7; trisomy 8 and del 20q. A presumptive diagnosis of MDS refractory cytopenia with multilineage dysplasia of the WHO classification was established. The patient was treated with growth factors with anemia improvement but neutropenia persisted. Due to the vacuolization of marrow progenitors and the presence of ring sideroblasts Copper, Zinc and lead levels were determined.



On two separate samples copper was very low <9 μg/dl (80-155) and Zinc was increased 239μ g/dl (60-150).The patient has just started oral copper sulfate therapy 3mg 3 times daily *Discussion*. To our knowledge 49 patients have been described to date, including ours, and most of them had also neuropathy, vacuolated marrow progenitors and many of them ring sideroblasts (Table 1). Copper has a role in iron absortion, transfer from the reticuloendothelial cells and cytochrome oxidase activity in mitochondria, all possible mechanism for the anemia associated with its deficiency.. Copper deficiency is ussualy seen in malnourished patients, parenteral nutrition and after gastrectomy. In some cases, like ours, no cause could be found. Our patient , like others, had hyperzyncemia without known exposure, a fact believed by some authors to be an epiphenomenon. Zinc induces metallothionin production in enterocytes for which copper has a great affinity displacing Zinc. Copper is therefore acumulated in gastrointestinal tract and being eliminated with cell shedding. All the patients described normalized blood counts with copper therapy or Zinc withdrawall but neurological symptoms did not improve. Our patient has just begun treatment to evaluate response but the clinical, morphology and serum levels of Copper and Zinc are concordant with other cases described. Some of the patients described in the literature were detected before a bone marrow trasplant. We and others suggest that Copper deficiency should be routinely ruled out before establishing a diagnosis of MDS in the low risk categories specially if they have vacuolated marrow progenitors, ring sideroblasts or neuropathy to avoid potentially harmful therapies.

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NEUTROPENIA IN MYELODYSPLASTIC SYNDROMES BASED ON THE DATA OF A SINGLE CENTER ROMANIAN REGISTRY

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Background. The myelodysplastic syndromes (MDS) are a group of clonal disorders with an annual incidence of 4 cases/100000 in the general population. Neutropenia is a common cytopenia in MDS. *Aims.* The purpose of this study is to evaluate the incidence of neutropenia in MDS and the overall survival of these patients in our department. Methods: this retrospective, epidemiological study incudes 560 patients diagnosed and treated in our department during 1982-2010. Among them 184 cases with neutropenia were found. The registration form was kindly provided by the MDS Foundation (USA) and the diagnosis of MDS was based on well-accepted FAB minimal diagnosis criteria. Neutropenia was dfined as a neutrophils count < 1500/dL and was divided into three subgroups of severity (mild 1500-1100/dL, moderate 1000-500/dL, severe <500/dL). The parameters age, sex, rural/urban location, temporal trend and distribution by FAB subtypes were analyzed comparatively in neutropenic and nonneutropenic MDS cases. We also analyzed the complications appeared and the evolution of the disease. Results: the lot includes 184 patients (89 females and 95 males) with an average age of 63 years. The distribution by rural/urban location was grater for urban (114 patients) than for rural location (70 patients). According to FAB criteria, the distribution of neutropenia in MDS patients was: 12.50% RA, 8.69% RARS, 37.50% RAEB, 13.04% RAEB-T, 3.81% CMML and 24.45% U-MDS. Regarding the severity of neutropenia we found 32.06% patients with neutrophils between 1500-1100/dL, 36.41% cases between 1000-500/dL and 31.52% under 500/dL. Severe infectious events were found in 31.60% patients; the site of infection was commonly pulmonary (42 cases), cutaneous (5 cases) and digestive tract (11 cases). 11 patients required intensive unit care transfers due to acute respiratory distress syndrome, and 10 died. Overall mortality rate was 15%. *Conclusions*. Neutropenia in MDS is a challenging finding because, when severe, it is an important cause of death by life-threatening infections. The interest in MDS-related neutropenia increases when we are thinking to the economic aspects of its management.

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SAFETY AND EFFICACY OF COMBINATION THERAPY WITH DEFERASIROX AND DEFEROXAMINE FOR MANAGEMENT OF IRON OVERLOAD IN MULTITRANSFUSED HEPATOPATIC PATIENT WITH MYELODYSPLASTIC SYNDROME: A CASE REPORT

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Myelodysplastic Syndromes (MDS) are a heterogeneous group of haematopoietic stem cell disorders characterized by cytopenia and hyperplastic bone marrow: in this context red blood cell transfusions represent a life-saving treatment for patients with chronic anaemia. Iron overload is the consequence of a long term transfusion therapy thus it

is necessary to prevent this complication by applying a correct iron chelation therapy. 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 preexisting hepatic diseases. Here we report the case of a 62-year-old man affected by HCV positive cirrhosis and MDS (Refractory Anaemia, IPSS 0.5). Recombinant Erythropoietin therapy was ineffective and a RBC transfusion program was started (2 blood package pro month). At a ferritin serum concentration near 700 ng/mL iron chelation therapy with deferoxamine was proposed 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. 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. At the present time, the patient receives 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. No serious adverse event has been observed. In conclusion we suggest that combined therapy with deferasirox and deferoxamine could be considered a safe and useful therapeutic choice in the management of critical transfusion dependent iron overload in old MDS patients with hepatic disease.

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MYELODYSPLASTIC SYNDROMES AND CHRONIC MYELOMONOCYTIC LEUKEMIA (ABOUT 23 CASES)

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Background. Myelodysplastic syndromes (MDS) are a set of oligoclonal disorders of hematopoietic stem cells characterized by ineffective hematopoiesis that manifest clinically as anemia, neutropenia, and/or thrombocytopenia of variable severity. Since 2001, the WHO classification considers chronic myelomonocytic leukemia (CMML) as an intermediate entity between Myelodysplastic and Myelproliferative syndromes. Aims. The aim of this study is to report the experience of the clinical hematology department, of the Military Hospital Mohammed V, in MDS and CMML. Patients and methods. This is a retrospective study including all patients who consulted for MDS or CMML since the creation of clinical hematology department in 2006 until December 2010. The patients with MDS were classified according to 2008 WHO classification. Prognostic scores used are the IPSS and WPSS (CMML excluded). Response criteria adopted are those of: modified IWG Criteria response in myelodysplasia (2006). Results. Twenty-three patients are included; the median age at diagnosis is 63 years (32 to 98 years). The sex ratio [H / F] is 1.55. The distribution of the twenty patients with MDS according to WHO classification 2008 is as follows: 1 refractory anemia (RA), 3 refractory anemia with ringed sideroblasts (RARS), 8 refractory cytopenia with multilineage dysplasia (RCMD), 1 RCMD and ring sideroblasts, 4 refractory anemia with excess blasts type 1 (RAEB1) and 3 RAEB2. In addition to two patients with CMML1 and one patient with a CMML2. Seventeen patients of twenty three had a karyotype (74%), which is normal in 88% and intermediate risk in 12% cases. Concerning the IPSS score: 29.4% are classified as low risk, 41.2% as intermediate 1 risk and 29.4% as intermediate 2 risk. 14 patients are included in the WPSS score: 50% are low risk, 14% intermediate risk and 35.7% high risk. 6 patients benefits from 3 to 12 cures of Azacitidine with achieving a complete remission in 2 cases, partial remission in 2 cases and no response in 2 cases. 8 patients received erythropoietin (EPO) alone (5 cases) or associated with G-CSF (2 cases). With achieving transfusion independence in 62.5% and reduced transfusion requirements in 37.5% of cases. The therapeutic abstention was indicated in 8 patients, 50% had never been transfused and 50% are under transfusion support. The transformation to AML occurred in 3 patients (at 18 months, 4 months and 2 months). During the study period 5 patients died: 2 by transformation, 2 by sepsis and one patient by hemorrhagic shock. Conclusions. The management of MDS has been shaken in recent years by the emergence of new therapies. The availability of Azacitidine in Morocco is recent and its use still limited because of its high cost and low coverage of health insurance in the country. This study is one of the first to have evaluated Azacitidine in Morocco.

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MYELODYSPLASTIC SYNDROMES WITH DEL(5Q) AND JAK2 V617F MUTATION: A CASE OF HEMATOLOGIC RESPONSE TO LENALIDOMIDE THERAPY

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Deletion of long arm of chromosome 5 (del5q) is one of the most common cytogenetic abnormalities in Myelodysplastic Syndromes (MDS). The JAK-2 V617F somatic mutation is the molecular marker most frequently detected in the BCR/ABL negative myeloproliferative neoplasms (MPN). Here we describe simultaneous occurrence of JAK-2 V617F mutation and del(5q) in a case of MDS successfully treated with Lenalidomide. A 62-year-old woman had been treated with hydroxyurea for 5 years because of a diagnosis of Essential Thrombocytemia (ET) before admission to our hospital for normocytic anemia (Hb 9,5 g/dL) thrombocytosis (PLT 853x10V/L) and mild leucopenia (WBC 2,9x10^9/L). Bone marrow aspirate showed increased number of megakaryocytes with dysplastic features, dyserythropoiesis and dysgranulopoiesis, no ringed sideroblast was observed. Cytogenetic analysis performed by Fluorescence in Situ Hybridation (FISH) revealed an isolated interstitial deletion of the long arm of the chromosome 5. Polymerase Chain Reaction (PCR) was positive for JAK-2 V617F mutation. Treatment with Lenalidomide (10mg/day on days 1 through 21 of repeated 28 days cycles) was started after red blood cell transfusions. After seven cycles a complete recovery of hemoglobin and platelet concentration was obtained (Hb 13 g/dL, PLT 180x10^9/L), while worsened leucopenia needed supplementation with growth factor. Several authors have already reported the presence of del(5q) in MPN as well as MDS with JAK 2 V617F somatic mutation, furthermore some cases of ET with concurrent presence of del(5q) and JAK 2 mutation have been referred and finally Ingram and coll. have described six cases of MDS arboring del(5q) and JAK2 alteration. There is not yet an univocal evaluation of the prognostic significance concerning the association of JAK 2 mutation and del(5q) in MDS or in MPN, in future probably new entities will be identified with the more extensive application of molecular investigation. Here we underline the very good response of this patient to Lenalidomide therapy with normalization of thrombocytosis, previous resistant to the Hydroxyurea administration, and recovery of hemoglobin concentration with no further need of transfusion therapy.

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EXTRA-MEDULLARY LOCALIZATION IN HIGH RISK MYELODYSPLASTIC SYNDROMES: A NEW MODALITY OF RELAPSE AFTER THERAPY IN ELDERLY

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Background: extra-medullary localization in myelodysplastic syndromes (MDS) is a rare complication (1) but represents a pattern of relapse following allogenic bone marrow transplantation in about 20% of acute myeloid leukemia (AML) (2). Features of relapse following hypomethylating agent therapy in high risk MDS involved usually bone marrow leading to AML transformation. Purpose: we report 3 cases of extra-medullary localization following hypomethylating agent treatment in high risk MDS. Cases: Case 1 after 15 cycles of Decitabine, a 73 years old man developed mental impairment including confusion, somnolence and desorientation; Weight loss and disability with falls increased in few days. While blood analysis showed no features of relapse, cerebral fluid analysis revealed massive presence of blasts cells. Case 2 after 14 cycles of Azacitidine, a 72 years old man developed pulmonary symptoms (cough). Biopsy of a tumoral lung lesion was confirmed MDS relapse. Case 3 after 13 cycles of Azacitidine, a 80 years old woman developed diplopia due to ocular muscle impairment. MRI showed tissular lesion of the right caverous sinus. Bone marrow aspira-

tion showed no relapse of RAEB. Massive blasts infiltrate was detected in cerebral fluid analysis. *Conclusions*: extra-medullary localization in high risk MDS is a new modality of relapse following therapy. Diagnosis could be difficult, poor outcome is the rule. Role of hypomethylating agent in this issue should be studied.

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IRON CHELATION IN MYELODYSPLASTIC SYNDROMES: A DUAL CENTER EXPERIENCE

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Background. Iron overload is common in patients with myelodysplastic syndromes (MDS) who are treated with red blood cell (RBC) transfusions. Transfusion dependency is associated with leukemic progression and shorter survival. Guidelines recommend iron chelation therapy for management of iron overload, but little is known about chelation patterns in daily clinical practice. *Aims*. The objective of this dual centre study was to evaluate iron status and management in MDS patients, especially the utilisation of iron chelation therapy and chelation patterns Method. A total of 77 patient records between January 2006 and December 2009 were analysed for this retrospective, cross-sectional, observational study. *Results*. Median age at diagnosis was 75 years. There were no statistically significant differences between chelated and nonchelated patients in terms of International Prognostic Scoring System score(IPSS score). Fifty-three patients had an IPSS score of low/intermediate-1 and hence eligible for assessment of iron chelation. Eighteen patients had received more than 25 RBC units in the past 12 months and therefore were eligible for iron chelation therapy. Medium serum ferritin was 1930μg/L. Eleven patients (61%) did not receive any iron chelation therapy. Their median ferritin was 1930μg/L. Reasons for not receiving iron chelation therapy were refusal (2), malignancy (1), parkinsonism (1), stroke (1) and age (98yrs) (1). No reasons were documented in five patients. Seven patients (39%) received iron chelation therapy with either deferoxamine (1), deferiprone followed by deferasirox (3) and deferiprone (3). Their median ferritin was 2534μg/LNone of the patients on deferiprone had a therapeutic response. *Summary/Conclusions*. Iron chelation therapy may be underutilized in transfusion-dependent MDS patients. This can be reduced by using a combination of clinical judgment, a serum ferritin level >1,000 μg/L and/or two or more RBC transfusions per month for the past year. The key reasons for not initiating iron chelation therapy were related to poor patient prognosis, patient age, and comorbidities. Deferiprone was not an effective iron chelation agent in this study and warrants further study to demonstrate its effectiveness.

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THROMBOTIC EVENTS IN DEL(5Q)ASSOCIATED MYELODYSPLASTIC SYNDROMES.

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Background. Thrombocytosis is the hallmark of the *5q-syndrome* but subsequent risk of thrombotic events remains unclear. JAK2 V617 mutation clones and del(5q) in myelodysplastic syndromes could be associated (1). Recent publications suggest that JAK2 and del(5q) arise in discordant clones (2). Whether if thrombotic events could be linked to JAK2 occurrence moreover than del(5q) is an unresolved question. Patients and methods: thrombotic events assessment in a cohort of myelodysplastic syndromes (more than 800 patients) localization, risk factors evaluation (including thrombophilic factors, JAK2), ongoing treatments. Correlations between platelet level, cytogenetic features, treatments of the disease (EPO, lenalidomide) were evaluated. *Results*. 6 thrombotic events occurred in del(5q) associated MDS 2 deep venous thrombosis (DVT), one deep venous thrombosis with pulmonary embolism, 2 intra-abdominal thrombosis and one transient ischemic stroke. Mean age was 70 years (59-85). In 50% of patients thrombosis occurred before the diagnosis of MDS. Risk factors of thrombosis (except thrombocytosis) were JAK2 mutation in 2 cases (1 DVT and 1 intra-abdominal thrombosis), post-surgery period in 1 case and lenalidomide treatment initiation in 2 cases. In the two reminding patients no risk factor was found. During the same period 1 case of DVT and 1 ischemic stroke occurred in non del(5q) MDS. *Conclusions*. Spontaneous risk of thrombosis in del(5q) seems to be higher than in overall MDS population. Some additional risk factors have to be evaluated as JAK2 mutation especially if intra-abdominal thrombosis was observed. Thrombotic risk prevention could be discussed in the field of del(5q) treatment.

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PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) IN THE CANARY ISLANDS: DESCRIPTION OF NINE CASES

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PNH is a very rare clonal stem cell disorder due to an acquired mutation of PIG-A gene which causes deficiency of glycosy lphospahatidyli nositol and several proteins linked to it,. Some of these proteins play a central role in protecting cells from the lityc activity of complement, therefore rendering blood cells highly sensitive to complement.. PNH is a chronic disease with predisposition for thrombosis and variable degrees of bone marrow failure. We present the data of 9 patients diagnosed and treated in our community Material and methods . Median age at diagnosis was 48y (33-70), Male/female ratio was 2. Blood counts median were Hb =91gr/L, Plateletes=135x10E9/l and WBC=4.1X10E9/L. Median LDH=1855UI/L (n<247) ,bilirrubin 1,7 mg/dl (N<1.4) and creatinine 1.15mg/dl (N<1.4). All patients, except one with aplastic anemia , had classical PNH. 7 of 9 had moderate to severe quality of life impairment that interfered with laboral and social life due to chronic invalidating fatigue or abdominal pain. 4 cases had one thrombotic episode in different territories: one right leg deep vein thrombosis, one portal vein, one suprahepatic veins and one CNS vein stroke. All patients are since then on oral anticoagulation. 4 cases had chronic renal damage and 2 others experienced acute renal failure during a crisis. Diagnostic was performed by flow citometry analyzing CD16, and CD55 expression in granulocytes and CD55 and CD59 in red cells. 8 of 9 cases had a granulocyte size clone over 50%. Bone marrow cellularity was increased except in the case with aplastic marrow. No cytogenetic abnormalities were detected in any case. All patients received folic acid suplementation and iron if deficiency was detected. 5 cases were transfusion dependent regularly and 2 were ocasionally transfused during an acute attack. Due to the cost of Eculizumab health authorities in our community encouraged the design of a therapeutic guide by hematologists for PNH patients. In summary according to our guide, therapy with eculizumab was indicated in adult patients if they were transfusion dependent or poor quality of life and had a size clone type III>10% with overt hemolysis (LDH>1.5 over normal range) and preserved marrow function (platelets>30.000) . Chronic renal damage (not justified by other causes) or thrombotic episode were by themselves indication for therapy. 7 patients started eculizumab after meningococceal vaccination last year . No adverse reaction was seen during infusion. 3 of the patients became transfusion independent and the rest had a dramatic reduction in transfusion requirements. One patient with chronic renal failure normalized serum creatinine (2.3mg/dl to 1.3) In one patient with breaktrough haemolysis the dose was increased to 1200mg every 2 weeks with good response. No Coombs positive extravascular haemolysis has been observed so far. All patients wether transfusion dependent or not experienced a marked improvement in quality of life Conclusions The prevalence of PNH in our community is 0.43 by 100.000 inhabitants. Clinical data does not differ essentially from other series. Although folow up in patients with eculizumab in our community is too short tolerance end response seems to be excellent. Due to the cost of eculizumab clinical guidelines could help in decision making

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DEMOGRAPHIC AND SURVIVAL DATA OF INCIDENT CASES OF MDS/CMML IN MANITOBA DURING 2006-2007: DIFFICULTIES IN RETROSPECTIVE PROGNOSTICATION

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Background. With the availability of newer, but more expensive therapies for MDS, demographics and prognostic scoring are increasingly important to allocate resources for health planning. We have earlier reported on the Incidence of MDS/CMML in Manitoba during the years 2006-2007 (*Kumar et al Blood 2009; 114: Abstract#245*). This cohort provided us with an opportunity to study prognostic characteristics of this cohort. *Aims*. To study the demographic and prognostic characteristics of MDS patients in Manitoba for health care planning. Methods: After obtaining ethics sanction, the clinical records of all incident cases of MDS/CMML diagnosed during a two year period (2006 and 2007) were retrospectively studied to categorize the subtype according to the WHO classification and determine the prognostic features. The Manitoba Cancer Registry was used to study survival data. Kaplan-Meier (KM) curves were used for estimates of median overall survival. Log rank test was used to compare KM estimates between groups. All analyses were conducted using SAS version 9.1.Last date of follow up was Apr 30, 2010. Results: The newly diagnosed cases of MDS/CMML in the study period of two years consisted of 43 males and 37 females with a median age of 73 yr (range 45-90). Nine patients were <60 yr (11%). The following subtype categories and number of patients were identified: RA- 9; RARS-11; RCMD-22; RCMD-RS- 2; RAEB1- 5; RAEB2- 7; MDS del(5q) -1; MDS-U- 12; MDS/CMML - 7 and MDS/MPD- 4. Bone marrow cytogenetics were available in only 17 patients and could be classified in the following categories: Good risk -10, Intermediate -2, Poor -5. IPSS Score was determined in 17 Pts - Low risk: 5, Int-1: 3; Int-2: 7; High risk: 2. Blood counts at initial diagnosis were available in 75 patients with the following median values: Hb 91g/L (range 55 -140); Platelets $124x10^9$/L (range 5 - 1108); ANC $3.3x10^9$/L (range 0.12 - 31) with ANC < $0.5x10^9$/L in 11%. There was little data on ferritin values and the WPSS scoring was not possible in 65 Pts (81%). The median OS was 14.4 months (95% CI 9.96-25.22). Median OS for females was 12.04 months and 18.40 months for males (difference not significant p= 0.45). *Conclusions*. This retrospective analysis showed a poor OS in the cohort. It could not accurately determine the prognostic group of most patients due to inadequate investigations or records. For population based prognostic scoring, there is a need to create MDS Registries and collect data prospectively.

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PNH CLONAL EXPANSION FOLLOWING BONE MARROW TRANSPLANT: CASE REPORT

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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. Patients experience debilitating quality of life (QoL) issues, including fatigue, shortness of breath, erectile dysfunction, and abdominal pain; a number of these symptoms have been shown to increase TE risk, thereby increasing risk of mortality. The complement inhibitor, eculizumab, has been shown to reduce the incidence of TE and CKD, and improve survival in PNH patients by inhibiting chronic hemolysis. While bone marrow transplantation (BMT) 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 BMT die or develop graft-versus-host disease. *Aims*. Presented here is a case report of a PNH patient whose PNH clone reappeared and expanded following un-related BMT. Case Report: A 39 year-old male patient first presented with PNH symptoms in 2001, with hemolytic anemia, thrombocytopenia, and elevated LDH. He was transfusion dependent beginning in September 2004, at which time he experienced a deep vein thrombosis and was treated with warfarin and prednisolone. PNH diagnosis was made in March 2005, following flow cytometry analysis showing loss of CD55/CD59 expression on granulocytes, monocytes, and lymphocytes (Table 1). In June 2005 he presented with fatigue, *coke-colored* urine, decreased hemoglobin, and a positive Ham's test. With no related donor, 3 compatible umbilical cord blood units were located in New York. He received treatment with fludarabine, melphalan, and thymoglobulin with prophylactic tacrolimus and serolimus. He underwent BMT in September 2007. Post- BMT, he required almost 5 months hospitalization for severe anemia requiring RBC transfusions, infections with urinary focus, and furunculosis. In March 2008, secondary bone marrow failure was detected; he experienced a severe hemolytic crisis with anemia requiring multiple RBC transfusions, leukopenia, indirect hyperbilirubinemia, and high corpuscular volume. The patient described strong abdominal pain, back pain, severe headaches, fatigue at rest, and hematuria. Flow cytometry in July 2008 showed loss of CD55/CD59, indicating reappearance of PNH (Table 1). Due to severe symptomatology, he was placed on eculizumab therapy in July 2009, and experienced sustained hematologic response. He continues therapy with eculizumab, cyclosporine, folic acid, and ferrous fumarate without complications. *Conclusions*. This is a unique case detailing the reappearance and expansion of PNH clones following BMT. Although BMT is considered a potentially curative option in PNH, it is associated with a high risk of mortality and morbidities, and may not be curative in all patients. In light of the improved survival of PNH patients on eculizumab from clinical trials and single-center studies, this case highlights the potential benefits of eculizumab and reinforces the complexity of considering potentially dangerous treatment options such as BMT in PNH patients.

Table 1. Flow Cytometry Before and After BMT

	March 2005	July 2008
% Cells Deficient for CD55		
Granulocytes	22.3%	94.0%
Monocytes	0.56%	NA
Lymphocytes	10.0%	NA
Platelets	NA	90.0%
% Cells Deficient for CD59		
Granulocytes	14.26%	99.0%
Monocytes	2.76%	NA
Lymphocytes	19.96%	NA
Platelets	NA	95.0%

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APLASTIC ANEMIA IN CHILDHOOD: 8-YEARS EXPERIENCE AT A SINGLE INSTITUTION

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Background. Aplastic anemia (AA) is a rare acquired or inherited syndrome of bone marrow failure and early stem cell deficiency characterized by peripheral pancytopenia, bone marrow hypoplasia, and mild macrocytosis due to stress erythropoiesis. Chemical exposure, medicines, viral infections and immunodeficiencies are the aetiological factors responsible. Immunosuppressive therapy (IST) and allogeneic bone marrow transplantation (BMT) have improved the outcome of AA in childhood, though the best treatment options remain to be established. Aims: Diagnostic evaluation, treatment, short and long term follow up and outcome of children with AA who were hospitalized at a single Pediatric Hematology Oncology Unit. *Materials and Methods*. Retrospective analysis of children with the diagnosis of moderate or severe AA in the last 8 years (aetiological factors, treatment response, and overall survival rates). Results: Seven children were diagnosed with AA with mean age 9.2 years (± 1.6), of which five (71%) were girls. The main initial symptoms were pallor (75%), bleeding with ecchymoses (69%) and mucosal bleeding (46%). At diagnosis, the mean values of WBC, Hb and PLT were $2x10^9$/L, 7.2 g/dL and $21x10^9$/L respectively. Exposure to known etiologic agents was found in 4 children (57%) (recent parvo

B19 infection in 3 children, recent HCV infection in 1 child). One child suffered from inherited AA (Fanconi anemia). Of all the children, two received only supportive treatment (28.6%), while the remaining five (71.4%) received combination therapy with cyclosporin A, anti-thymocyte globulin (ATG) and G-CSF. The response rate to treatment was 28.5%. Mean follow up was 3.8 years. Four out of seven children (57%) underwent finally bone marrow transplantation (75% response). The overall survival rate was 57.1%. *Conclusions*. The course of the disease to recovery is variable one but is similar to that reported in the international literature. The role of etiologic factors and the best treatment options in childhood AA is not fully disambiguating and needs further investigation.

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DYSKERATOSIS CONGENITA A POTENTIAL CAUSE OF BONE MARROW FAILURE

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Background. Dyskeratosis congenita (DC) is a genetic syndrome including usually abnormal cutaneous pigmentation, nail dystrophy and mucosal leukoplasia. In addition, an increased risk of neoplasia, myeloid hemopathy, organ fibrosis (lung, liver) and dysimmunity is described. Patient survival is strongly impaired by bone marrow failure. Pleomorphic presentation of the disease leads to frequent misdiagnosis. We report the case of a 53 years old man with bone marrow hypoplasia and severe lung fibrosis. The diagnosis of DC was performed and a new TERT (Telomerase reverse transcriptase) mutation was described. Familial history of cirrhosis (brother) and association between bone marrow failure and lung fibrosis were significant parameters to assess the diagnosis. Conclusion: New modality of transmission of DC were recently described. Recessive X-linked (DKC1 mutation) and recessive autosomal form (TERC mutation) were first described. More recently, mutations with regard to telomerase complex's proteins (TERT, NOP10, NHP2) and its steady proteins were reported. These new mutations allow us to explain different clinical pathways and transmission's forms of the disease.

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CYCLOSPORINE A MONOTHERAPY IN CHILDREN WITH SEVERE APLASTIC ANEMIA :SINGLE CENTER EGYPTIAN EXPERIENCE

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Background. Immune suppression (IS) therapy has provided an opportunity of cure or improvement in the aplastic anemia patients who are not feasible candidates for bone marrow transplantation. Although the combination of cyclosporine A (CSA) and antithymocyte globulin (ATG) is superior to either agent alone, CSA monotherapy is an easily available, safe and cheap (IST) option. These advantages are particularly valuable in developing countries where ATG is frequently not available. *Methods.* In the Hematology Department at Children's Hospital Ain Shams University, 35 patients (24 males and 11 females), with a median age of 8.5 years with severe aplastic anemia (SAA) were prospectively identified and managed with CSA monotherapy at a dose of 6-10 mg/kg/day (divided every 12 hrs) during the period between January 2006 and January 2011. Seven patients(20%) expressed PNH-like clones at diagnosis, 3 patients (8.5%) had non-A, non-B, non-C hepatitis 3-6 weeks before presentation, one patient gave history suggestive of drug toxicity, 5 patients had a preceeding upper respiratory tract infection, and in the remaining patients, no possible cause was identified (idiopathic). *Results.*30 eligible patients giving parental consent were treated with cyclosporine for at least 6 months . After 6 months of therapy, 4 patients (13.3%) achieved complete remission (CR) , 9 patients (30%) achieved partial remission (PR) .After one year of therapy 10 patients(33.3%) achieved CR and 11 (36.7%) remained in PR. Two patients (1.5%) lost follow up and 2 patients (1.5%) died of serious septic and bleeding events before the fourth month. Discontinuation of CSA before the sixth month occurred in one patient for serious neurotoxicity, otherwise, other side effects were modest and easily monitored. Short term steroid therapy was used in frequently transfused alloimmunized children. Hospital admission was more frequent during the first 3 months of IST and was mainly for febrile neutropenia and serious infection .Younger age, shorter interval between diagnosis and treatment, lower monthly requirement of platelets transfusion, higher hemoglobin levels before IST therapy, and, higher bone marrow cellularity were positively associated with response. *Conclusions.* In developing countries where facilities are modest and most patients cannot afford adequate treatment of SAA, CSA monotherapy can provide an available easily monitored immunosuppressive tool for patients with SAA.

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IRON CHELATION THERAPY WITH DEFERASIROX IN TRANSFUSION DEPENDENT MYELODYSPLASTIC SYNDROME PATIENTS

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Background. The majority of low-intermediate risk patients (pts) with MDS require red blood transfusions, which can result in iron overload and its clinical sequelae. The recent development of a safe and efficient once daily oral iron chelator (Deferasirox, Exjade) made possible regular chelation therapy in transfusion dependent MDS patients. *Methods*. Fifty-nine transfusion dependent IPSS low-intermediate1 risk MDS patients were studied. Baseline characteristics were the following: median age was 67 years (range 25 - 84); 28 were IPSS low risk and 31 Intermediate1; duration of transfusion dependency before treatment was 24 months (12-36) corresponding to 58 (22-72) packed red blood cells transfusions received. Baseline serum ferritin was 2000 ng/ml (1471-3500). Mean duration of transfusions 3.5 years (1-5) MDS therapy include hydroxiuree, anagrelide and growth factors. Patients started treatment with the standard 20 mg/kg Deferasirox dose but dose adjustments on clinical indications were done. *Results*. Over 12 months the mean dose of Deferasirox was 20mg/kg/day, and the mean transfusion rate was 3.4 units/months. The reduction in feritin level was achieved after 3 months of treatment. Hematological improvement by IWG2000 criteria was achieved in 7 pts (11.8%); erythroid response in 5; platelet in 1 and neutrophil in 1. *Conclusions*. In these MDS iron overloaded pts, Deferasirox was generally well tolerated. Serum feritin behavior confirms Deferasirox efficacy. The serum feritin reduction was more evident in the more heavily overloaded population indicating successful iron depletion in this group of patients as clinically requested.

1289a

CAN BLOOD FILM ANALYSIS OF HEALTHY INDIVIDUALS BE USEFUL IN DETECTING EARLY SIGNS OF MYELODYSPLASTIC SYNDROME; A POPULATION BASED STUDY OF 922 FILMS IN THE IRISH POPULATION

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Background. Our subjects (aged 45-75+) took part in the SLAN national survey of lifestyle, attitudes and nutrition enrolling 10,364 participants nationwide. Data on the relevant variables were available on 1,191 participants. *Aims.* We aimed to analyse blood films for reproducible evidence of myelodysplasia and haematinic deficiency in a population based cohort of 922 blood films taken from 1,191 subjects participating in a sub-study of the Irish national SLAN survey. *Methods.* We analysed 922 blood films for 19 agreed features of myelodysplasia, B12 deficiency and iron deficiency. We assessed for morphological changes including blasts, red cell stippling, monocytosis, anisopoikilocytosis, hypogranular neutrophils, atypical lymphocytosis and EDTA change. All 922 films were analysed by one observer (Ob 1) and two further observers (Ob 2 & 3) analysed a subset of 52 randomly selected films. 48 films with significant morphological findings were sent to an external morphology expert for validation. *Results.* Most films showed some degree of EDTA change as expected in EDTA blood collected from centres around the country and analysed centrally in Dublin. Evaluation of interobserver variation on 48 abnormal films demonstrated agreement on features such as hypogranular neutrophils and atypical lymphocytes was fair

(Kappa statistic 0.25-0.23) while on Pelger abnormality and anisopoikilocytosis moderate agreement was reached (Kappa 0.45-0.46). *Conclusions*. Evaluation of blood films in a cohort study is difficult as transport times to the laboratory adversely affect film quality. Interobserver concordance on early blood film features is poor - limiting the value of film analysis for early myelodysplastic features. Film analysis for features of haematinic deficiency - the differential for myelodyslastic syndrome was also unsatisfactory.

Table I. This table demonstrates the degree of interobserver concordance for each morphological feature identified.

Morphological Feature	Observer 1 % identified	Observer 2 % identified	Cohen's kappa measure	Agreement strength	P Value
Anisocytosis	0.3	1.7	0.663	Good	0.000
Macrocytosis	20.9	20.9	0.621	Good	0.000
Microcytosis	16.6	14.8	0.407	Moderate	0.01
Anisopoikilocytosis	22.9	29.2	0.457	Moderate	0.01
Fragments	0	6.3	*	poor	*
Hypersegmented Neutrophils	0	0	*	*	*
Hypersegmented Neutrophils	6.3	8.3	0.342	Moderate	0.234
Microcytosis	4.2	2.1	0.464	Moderate	0.063
Duplicate Hemocytes	2.1	0	*	poor	*
Thrombocytopenia	0	0	*	*	*
Thrombocytosis	16.7	23	0.502	Moderate	0.000
Hypogranular Neutrophils	6.3	8.3	0.231	Fair	0.052
Atypical Lymphocytes	12.5	14.6	0.252	Fair	0.111
Hemoglobin	8.3	2.1	0.379	Fair	0.013

*Indicates that a Kappa measure was not given as agreement was considered no better than chance thus no P value was given.

1290

LOW PERCENTAGE OF PERIPHERAL BLOOD DENDRITIC CELLS IN PATIENTS WITH MULTIPLE MYELOMA CORRELATES NEGATIVELY WITH INTERLEUKIN-6 AND BETA-2-MICROGLOBULIN LEVELS

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Several studies demonstrate the presence of quantitative and functional abnormalities in the dendritic cell subsets in patients with multiple myeloma (MM). The inhibitory effect of IL-6, TGF-β, IL-10 and beta-2-microglobulin is highly suspected. The aim of the study was to evaluate the myeloid and plasmacytoid dendritic cells (MDC and PDC) in newly diagnosed patients with MM in correlation with various biologic markers. Thirty patients with newly diagnosed MM were included in the study. All the laboratory parameters were obtained at time of diagnosis. Thee color flowcytometry with ILT3/lin/CD11c was used for the detection of the two peripheral blood DC subsets. The plasma levels of IL6 were detected by ELISA (standard curve range: 2-200pg/ml). The median age of patients was 61,5r (36-89). The mean M-protein concentration was 46,5±16,2 g/l. IgG kappa was detected in 15 patients, IgG lambda-in 4, IgA kappa-in 7, IgA lambda-in 3. The mean level of beta-2-microglobulin was 7,0±5,7mg/l (1,82 - 22,49 mg/l); β-2-microglobulin was used to determine the stage according to the ISS. The mean level of IL6 was 27, 73±21,47pg/ml 4,6-72,5 pg/ml). The percentage of MDC and PDC were significantly lower in the patients with MM in comparison to healthy subjects (0.08%±0.09% vs 0.21%±0.02% and 0.04%±0.03% vs 0.16%±0.01%, respectively). A statistically significant difference was found between the percentage of MDC and PDC in the different stages. There was a negative correlation between MDC and PDC and the levels of β-2-microglobulin (p=0.02 and p=0.02), as well as between MDC and the IL6 levels (p=0.04). No correlation was found between MDC, PDC, levels of M-protein and the type of paraprotein. Our results demonstrate the relationship between peripheral blood DC, IL6 and β-2-microglobulin and confirm the published data for the inhibitory effect of the two factors on DC differentiation and maturation *in vitro*. The monitoring of β-2-MG and IL6 may have clinical implication as a predictor of the immune system status as well as for the yield of harvested DCs for vaccination.

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ASSOCIATION STUDIES OF FUNCTIONAL ICOS GENE POLYMORPHISMS WITH MULTIPLE MYELOMA IN THE POLISH POPULATION

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Background. Inducible costimulatory molecule (ICOS) which is expressed on the T-cell surface after activation enhances all the basic T-cell responses to a foreign antigen, namely proliferation, secretion of lymphokines, the up-regulation of molecules that mediate cell-cell interaction, and effective help for antibody secretion by B cells. *Aims*. The study was undertaken to evaluate the association between three ICOS polymorphisms (which were recently described as functional ones) and susceptibility to multiple myeloma (MM) in a Polish population. Methods: A case-control study of 454 individuals including 201 MM patients was conducted on polymorphisms in the ICOS gene. Genotyping of the polymorphisms ICOSISV1+173T>C, ICOSc.1624C>T, and ICOSc.602A>C and ICOSc.1564 T>C was done using allelic discrimination methods with the TaqMan SNP Genotyping Assay. Results: We noted that frequencies of alleles ISV1+173T>C [T], ICOSc.602A>C [A], and ICOSc.1564T>C [T] were higher in MM patients than in healthy controls, but differences did not reach statistical significance (0.91 vs. 0.88, p=0.17; 0.82 vs. 0.79, p=0.19 and 0.83 vs 0.79, p=0.2, respectively). The distribution of alleles and genotypes for ICOSc.1624C>T polymorphism was similar in both groups. *Conclusions*. The result of present study do not strictly confirm the association of investigated polymorphisms with susceptibility to MM due to small size of studied group, but warrant further investigation through replication studies.

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PROTEIN Z CONCENTRATION IN MULTIPLE MYELOMA PATIENTS

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Background. The potential role of alterations of protein Z levels in the pathogenesis of thrombotic diseases has been investigated in several studies and has revealed conflicting results. A relationship between venous thrombosis and PZ plasma levels has been excluded in four case-control studies. In addition, the studies indicated that only very low levels of PZ, arbitrarily considered as lower than 5.0 percentile of controls have been associated with a moderate thrombotic risk. AIM: The aim of our study was to estimate the concentration of protein Z in patients with multiple myeloma (MM) and to assess a correlation between PZ levels and the thrombotic disturbances. Additionally we evaluated the influence of chemotherapy on the PZ concentration. *Materials and Methods.* Sixty five patients hospitalized at University Hospital in Bialystok, Poland, with a new diagnosed multiple myeloma were studied. The median age of patients was 53 (range: 40-66 years). Patients were not under the oral anticoagulant before treatment and had normal liver function. None of the patients had any thromboembolic complications at time of diagnosis. All patients were divided into three groups based on international staging system (ISS), twenty six patients at 1st stage, eighteen patients at 2nd stage and twenty one at 3rd stage. For all studied patients the induction regimens were thalidomide, dexamethason and cyclophosphamide (CTD), every 28 days (according to Polish Myeloma Study Group). The evaluation was done after the third and sixth cycle of chemotherapy with the same methods as at the diagnosis. Response was assessed according to the European, International, and Autologous Bone Marrow Transplant Registries (EBMT/IBMTR/ABMTR) criteria. The control group consisted of fifty healthy individuals, age- and sex-matched. Quantitative determination of protein Z in the plasma was done using commercial test (Asserachrom Protein Z Elisa Kits, Diagnostica Stago). The PZ assay methods were compared using T-student test for dependent and independent samples. The statistical significance of the measured differences was determined using an alpha index of 0.05. *Results*. The mean PZ concentration of MM patients (n=65) and healthy individuals (n=50) were with no statistical significant differences (1.50±0.77 μg/mL vs 1.58±0.42 μg/mL, p=0.6). According to ISS, we found significant differences of mean PZ concentration between the stage one of the disease (1.71±0.77 μg/mL) and other stages: 2nd (1.44±0.69 μg/mL) and 3rd (1.00±0.56 μg/mL), p=0.34 and p=0.007, respectively. The study revealed decreased concentration of PZ during

the treatment with statistical significant difference at month 3rd (1.18±0.46 μg/mL) and 6th (1.17±0.52 μg/mL), respectively p=0.04 and p=0.05. Venous thrombosis (DVT) occurred at the time of treatment in eight (12.3%) of MM patients. The study revealed tendency of a modulating effect of protein Z on the DVT in the entire group of patients, as well in the group with PZ concentration below 5th percentile, but not with the statistical significant correlation, p=0.13 and p=0.08, respectively. *Conclusions*. This reduction to the level below 5th percentile during IMIDS containing therapy may be an additional puzzle in explanation of the increased rate of thrombosis in MM patient treated with thalidomide.

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A NEW INDICATION FOR AN OLD DRUG; THERAPEUTIC POTENTIAL OF PROPRANOLOL ON HUMAN MULTIPLE MYELOMA CELLS

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Background. Propranolol, a nonselective -adrenergic receptor blocker, has been used for the treatment of the patients with hypertension for more than 50 years. Propranolol is also used in the treatment of angina pektoris, anxiety, tachycardia, arrhythmia, tremor, migraine, panic attack, and thyrotoxicosis. There are several in vitro and in vivo evidences that β-adrenergic receptor antagonists inhibit proliferation and angiogenesis, decrease tumor metastasis, and also increase apoptosis in breast, stomach, skin, and colon cancers. Multiple myeloma is the second most common hematological malignancy. Despite of the high dose chemotherapy and other treatment approaches, there is no efficient curative approach yet. *Aims*. In this study, we aimed to investigate the cytotoxic and apoptotic effects of propranolol, the nonselective β-adrenergic receptor blocker, on U266 human multiple myeloma cells. Methods. Time-dependent proliferation of U266 cells exposed to increasing concentrations of propranolol was determined by MTT cell proliferation assay. Apoptotic effects of propranolol on human U266 multiple myeloma cells were examined by analyzing the changes in caspase-3 enzyme activity, mitochondrial membrane potential, and also the localization of phosphatidylserine in the plasma membrane. For this purpose, caspase-3 colorimetric assay kit, JC-1 mitochondrial membrane potential detection kit and Annexin V-FITC apoptosis detection kit were used, respectively. *Results*. IC50 values (drug concentrations that inhibit the proliferation of cells by 50%) of propranolol in U266 cells were calculated as 141-, 100-, and 75 μM after 24, 48, and 72 hour propranolol exposure, respectively. Incubation of U266 cells with 50-, and 100 μM propranolol for 48 or 72 hours resulted in 10 and 18% or 46 and 50% increases in caspase-3 enzyme activity, respectively. The same concentrations of propranolol resulted in 8 and 35% increases in loss of mitochondrial membrane potential after 48 hours, and 73 and 77% increases in loss of mitochondrial membrane potential after 72 hours, respectively, as compared to untreated control group. To confirm these data we detected apoptotic cells by examining the localization of phosphatidylserine. The results revealed that propranolol induced apoptosis significantly in a time- and dose-dependent fashion in multiple myeloma cells. *Summary/Conclusions*. These results revealed that propranolol has antiproliferative and apoptotic effects on U266 human multiple myeloma cells. Being supported by *in vivo* analyses, propranolol can be a good and economical way to treat multiple myeloma patients.

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APOPTOTIC EFFECTS OF SUNITINIB ON MULTIPLE MYELOMA CELLS

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Background. Multiple myeloma is the second most comultiple myelomaon hematological malignancy characterized by abnormal increases in the number of plasma cells. The direct interaction of the multiple myeloma cells with the other cells within the microenvironment has a vital importance for the progression of the disease since these interactions triggers the release of some cytokines and growth factors, critical for signaling pathways for multiple myeloma cells and for the cells in the microenvironment. Different therapeutic agents and treatment protocols were used for the treatment of multiple myeloma and some of them could increase survival time and quality life of the patients. But, despite these succesful responses, multiple myeloma still remains to be an uncurable disease. Sunitinib is a multi-targeted receptor tyrosine kinase inhibitor that repress the activity of many receptors involved in important signaling pathways that regulate cell growth, proliferation and apoptosis. Aims. In this study, we aimed to desipher the cytotoxic and apoptotic effects of sunitinib on multiple myeloma cells and determine the roles of caspase-3 and mitochondrial membrane potential in sunitinib-induced apoptosis. Methods. Antiproliferative effect of sunitinib on U266 multiple myeloma cells was determined by XTT cell proliferation assay. Apoptotic effects of sunitinib on U266 cells were evaluated by the examination of changes in caspase-3 enzyme activity, mitochondrial membrane potential and Annexin V assays. These analyses were conducted by using caspase-3 colorimetric enzyme activity assay, JC-1 mitochondrial membrane potential detection kit and Annexin V-FITC kit, respectively. *Results*. IC50 value of sunitinib, the drug concentration which inhibits cell proliferation by 50% as compared to untreated control group, was calculated from cell proliferation plots and found to be 4 μM. There were 7, 17 and 20% increases in loss of mitochondrial membrane potential in response to 1, 5 and 10 μM sunitinib, respectively, compared to untreated control. The same soncentrations of sunitinib increased caspase-3 enzyme activity by 10, 30 and 160%, respectively comparing to control group. In order to confirm these results, apoptotic deaths were detected by AnnexinV and results revealed that 1, 5, 10 μM concentrations of sunitinib induced apoptosis in 9, 55 and 98% of U266 cell population, respectively. *Summary/Conclusions*. The results of this study showed that sunitinib can be an alternative treatment approach for multiple myeloma. These studies should be confirmed further by in vivo analyses.

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DETERMINATION OF CYTOTOXIC EFFECTS OF GOSSYPOL ON MULTIPLE MYELOMA CELLS

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Background. In multiple myeloma, plasma cells reproduce in an uncontrolled manner due to a number of molecular changes such as chromosomal translocations, point mutations and oncogene activation. Then, these plasma cells produce excess amount of a single type of immunoglobulin. Multiple myeloma is diagnosed with the detection of more than 30% plasma cells in bone marrow. Furthermore, the cancerous cells accumulate in the bones and bone marrow. In the treatment of multiple myeloma, different agents have been administered as standard for many years. Although some novel agents increased the quality of life and survival times of patients, cure is still not possible and serious side effects of the agents is still a big issue. Therefore, investigations to find out novel agents are very important. Gossypol is a natural phenol derived from the cotton plant. Gossypol can induce apoptosis through downregulating Bcl-2 and upregulating Bax. It also inhibits protein kinase D. Gossypol has antimalarial, antiviral activities in addition to its anticancer potential. *Aims*. In this study, we aimed to identify the cytotoxic and apoptotic effects of gossypol on multiple myeloma cells. Methods. Multiple Myeloma cells lines, ARH77, were cultured in RPMI1640 medium containing 10% FBS and 1% penicillin-streptomycin. The cytotoxic effects of gossypol on ARH77 cells were conducted via XTT cell proliferation assay. Then, apoptotic effects of gossypol on ARH-77 cells were tested by loss of mitochondrial membrane potential, increases in caspase-3 enzyme activity, and location of phosphotidylserine by using caspase-3 colorimetric enzyme activity assay, JC-1 mitochondrial membrane potential detection kit and Annexin V-FITC kit, respectively. *Results*. The XTT data showed that there were dose-dependent decreases in proliferation of ARH-77 cells. There were 29, 45 and 61% decreases in cell proliferation in response to 10, 20 and 50 μM gossypol, respectively, compared to untreated control group. Apoptotic analyses showed that there were 12 and 290% increases in loss of mitochondrial membrane potential in ARH-77 cells exposed to 5 μM and 20 μM gossypol for 72 hours, respectively, compared to control group cells while 5 and 20 μM gossypol increased caspase-3 enzyme activity in ARH-77 cells, respectively, comparing to control group cells. Phosphotidylserine location analyses by Anexin V staining also confirmed dose-dependent increases in apoptosis in ARH-77 multiple myeloma cells. *Summary/Conclusions*. All together these findings demonstrated cytotoxic and apoptotic effects of gossypol on human ARH-77 multiple myeloma cells. Gossypol induced apoptotsis through deformation in mitochondrial membrane potential and increases in caspase-3 enzyme activity.

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EFFECTS OF BRUCINE ON METABOLISM OF OSTEOBLAST AND OSTEOCLAST IN MULTIPLE MYELOMA

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This study was aimed to explore the influence of brucine on the early differentiation of osteoblast and the metabolic pathway of osteoclast in multiple myeloma(MM) and to eompare the effects between brucine and bontezomib on MM.MTT method was used to determine IC50 of brucine and bontezomib on the MM cell line U266.The supernatant of cultured U266 cell line was added to the culture system for incluing the differentiation of osteoblast cell line MC3T3-E1. Aftter aseptic assay,RT-PCR was used to determine the in RNA levels of alkaline phosphatase (ALP), osteocalcine (OC), osteoprotegerin (OPG) and osteoprotegrin ligand (RANKL). As a result, the median inhibitory concentration (IC50)of bortezomib on U266 cell line for 48h was 22.4 nmol/L,and that of strychnine was 0.16 nmol/L. The mRNA levels of ALP,OC and OPG in osteoblast co-intervened by brucine combined with the supernatant of MM cells ($p<0.05$). The degree of increasing or reducing was larger than the level of control group intervened only by bortezomid($P<0.05$)).The above-mentioned results indicated that the therapeutia effects of brucine on MM might be carried out through the regulation of osteoclast by osteoblast ,and the experiment confirmend that the theoapentic efteds of brucine on MM was superior to that of bortezonid.

1297

EVALUATION OF T REGULATORY AND TH17 CELLS RELATED-GENES EXPRESSION IN BONE MARROW ASPIRATES OF SOLITARY PLASMOCITOMAS AND MULTIPLE MYELOMA PATIENTS

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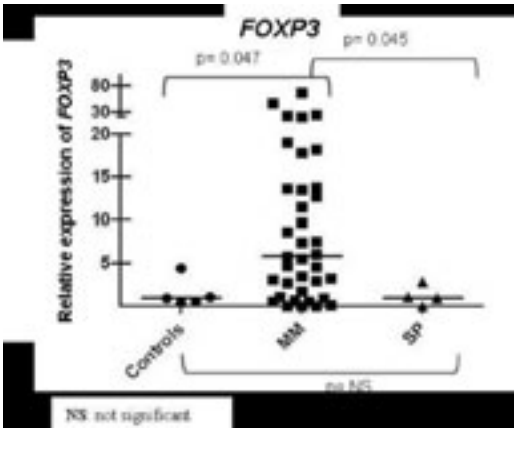
Introduction. Underlying biological mechanisms of multiple myeloma (MM) involve a series of genetic alterations and changes in the bone marrow microenvironment, favoring the growth of the tumor and the failure of the immune system in controlling it. T regulatory (Treg) cells play an important role in the maintenance of self-tolerance and modulation of overall immune responses against infections and tumor cells. Th17 cells have a critical function in eliminate extracellular pathogens and tumor cells. The balance between Treg and Th17 cells may be essential for maintaining homeostasis of anti-tumor immunity. In this scenario, the aim of this study was to characterize the expression of Treg- and Th17-related genes in bone marrow (BM) aspirates of MM and solitary plasmacytomas (SP) to evaluate their potential as therapeutic targets in this disease. Material and Methods: Expression of Foxp3 and ROR-γt genes, respectively associated with Treg and Th17 subpopulations, were determined by quantitative real-time PCR (RQ-PCR) in BM aspirates of 37 newly diagnosed MM patients, 04 newly diagnosed SP and 05 healthy controls (allogeneic transplant donors). Genes were considered overexpressed when RQ-PCR results showed values at least 2 times higher in cases than in normal samples.



Results. Foxp3 was overexpressed in 72% of MM cases. A 5.89-fold increase in Foxp3 expression was observed in MM patients compared to controls ($p=0.0476$, Mann-Whitney test). On the other hand, MM patients and controls showed equal levels of ROR-γt expression and the difference between groups was not significant. Also, SP BM aspirates showed Foxp3 and ROR-γt levels similar to controls. *Conclusions*: Overexpression of Foxp3 in MM cases suggests an accumulation of immunosuppressive Tregs in the tumor environment and/or an immediate involvement of this gene in the development and progression of myeloma. Therapeutic approaches that specifically target Foxp3-expressing Tregs may provide more focused treatment strategies for MM.

1298

POINT MUTATIONS IN KINASE AND PSEUDOKINASE DOMAINS OF JAK1 GENE DO NOT SEEM TO BE RESPONSIBLE FOR ACTIVATION OF JAK/STAT PATHWAY IN MULTIPLE MYELOMA

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Background: JAK/STAT pathway, which can be persistently activated in multiple myeloma (MM) patients due to constant stimulation by IL-6, was recently explored by Burger et al (2009) as potential therapeutic target in this still incurable disease: Janus Kinase (JAK) inhibitor INCB20 presented antiproliferative and apoptotic effects on human myeloma cells *in vitro* and *in vivo*. *Aim*s. To search for point mutations in JAK1 gene kinase and pseudokinase domains in an attempt to define any critical and recurrent alteration that could be used as therapeutic target for MM. *Patients and Methods*. We obtained RNA from purified CD138-positive cells from MM bone marrow samples using microbeads conjugated to monoclonal anti-human CD138 (sydecan-1) by the MACS methodology - Magnetic Cell Sorting of Human Cells (Miltenyi Biotec, Bergisch Gladbach, Germany) from 21 newly diagnosed patients MM, four healthy controls (one peripheral blood and three reactive tonsils) and four MM cell lines (U266 , Sko-007, SKMM-2, RPMI). After amplification of of JAK1 pseudokinase (exons 12-18) and kinase (exons 19-24) domains in cDNA samples, we performed automatic sequencing of fragments using forward and reverse primers. Results: 15 of the 21 (71%) MM cases showed at least one polymorphism, all synonymous SNPs, being: 12/15 in codon 733 (CCA>CCG), 6/15 in codon 683 (AGC>AGT), 4/15 in codons 1032 (AAG>AAA) and 659 (CGC>CGT), and 3/15 in codon 699 (GCC>GCG). All the four cell lines also presented only synonymous SNP: 4/4 in codon 683 (AGC>AGT) and ¼ in codon 733 (CCA>CCG). Among the four controls, one showed synonymous SNP in codon 733 (CCA>CCG), one in codon 683 (AGC>AGT) and one in codon 1032 (AAG>AAA). *Conclusions*. Mutations in kinase and pseudokinase domains of JAK1 gene do not seem to be important for activation of JAK/STAT pathway in multiple myeloma and other underlying mechanisms, besides IL-6 stimulation, must be investigated.

1299

THE INFLUENCE OF CYTOKINE INTERLEUKIN-16 IN MULTIPLE MYELOMA

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Multiple myeloma is a malignancy characterized by the expansion of a plasma cell clone that localizes to the human bone marrow. Myeloma cells and bone marrow stroma cells both produce soluble factors promoting the survival and progression of multiple myeloma. Interleukin-(IL)-16 is involved in regulating migration and proliferation of normal leukocytes, however, it has been unclear whether IL-16 also plays a role in the pathophysiology of human cancers. We found IL-16 to be strongly overexpressed in the bone marrow of myeloma patients. Myeloma cell lines as well as primary tumor cells from myeloma patients constitutively expressed IL-16 and its receptors CD4 and/or CD9 and spontaneously secreted soluble IL-16. Silencing of IL-16 had an anti-proliferative effect on the tumor cells which could be reversed by the addition of the C-terminal fragment of soluble IL-16. Most importantly, the application of a monoclonal antibody directed against IL-16 had a strong growth-inhibiting influence on myeloma cells. These findings indicate that cytokine IL-16 is an important growth-promoting factor in multiple myeloma and a candidate for novel diagnostic, prognostic and therapeutic applications for this incurable human malignancy.

1300

EFFECT OF 13Q DELETION ON IL-6 PRODUCTION IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Numerous studies have shown a correlation between 13q deletion and poor prognosis in multiple myeloma (MM), but the mechanisms are not fully understood. Earlier studies suggest that this lesion involves large segments or the entire long arm involving the retinoblastoma (Rb) gene. In myeloma, Rb gene is believed to downregulate interleukin -6 (IL-6) which plays a central role in the pathogenesis of MM. Therefore, it has been hypothesized that loss of Rb gene might be associated with very high expression of IL-6 and subsequent bad prognosis. *Materials and Methods*. Forty MM patients and 20 matched controls were included in this study. Interphase fluorescence *in situ* hybridization (FISH) analysis was performed using LSI 13q14-specific probe. Serum levels of IL-6 were determined by ELISA. All patients received conventional chemotherapy. Refractory patients received other therapeutic regimen of Thalidomide or Bortezomib. *Results*. Significant increase ($p<0.001$) of IL-6 production was recorded in patients with 13qdeletion compared to patients with normal chromosome 13q status .These patients were also refractory to conventional chemotherapy but showed striking response to Thalidomide or Bortezomib. *Conclusions*. This study suggests that 13q deletions are associated with increased production of IL-6 in MM and this could be a possible cause of the associated bad prognosis. In addition, the results also show the potential to improve responses in patients with refractory MM with the introduction of novel therapies.

1301

EVALUATION OF APOPTOTIC MARKERS IN MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Multiple myeloma (MM) is characterized by aberrant cellular responses to signals governing proliferation and apoptosis. Its' first pathogenic step is a pre-malignant monoclonal gammopathy of undetermined significance (MGUS). With progression from MGUS to MM, complex genetic and/or epigenetic events occur in the malignant plasma cells (PCs) and in the bone marrow microenvironment that contribute to cell signalling pathways deregulation. The knowledge of these mechanisms may provide new markers for disease progression and new molecular targets for therapy. Apoptotic cell death may be triggered by several mechanisms involving death receptors and/or by mitochondria (extrinsic and intrinsic pathways). The intrinsic pathway centers on mitochondria as initiator of cell death. Multiple signals converge on mitochondria causing these organelles to release cytochrome c (cyt c) and other apoptogenic proteins into cytosol. The B-cell lymphoma-2 (Bcl-2) family proteins are the most prominent of the intrinsic pathway. In this family some of them have pro-apoptotic properties, as Bax, Bad and Bid, while Bcl-2 and Bcl-Xl have an anti-apoptotic function. In contrast, the extrinsic apoptotic pathway relies on tumour necrosis factor (TNF) family death receptors for triggering apoptosis. TRAIL is a ligand that triggers and activates 4 receptors of TNF family, TRAIL-R1 and -R2, also known as death receptors, DR4 and DR5, and antagonistic or modulator receptors, TRAIL-R3 and-R4, also known as decoy receptors, DcR1 and DcR2. Many mechanisms may contribute to the complex effects of TRAIL, namely the differential expression of TRAIL-Rs or functional defects, like those related to cytoplasmatic inhibitors of apoptosis such as inhibitory apoptotic proteins as survivin. However, apoptosis deregulation during MGUS to MM progression is not yet clarified. AIMS: The aim of this study is to contribute to clarify the molecular mechanisms involved in MGUS and its evolution to MM, namely the involvement of apoptotic pathways.METHODS: For this purpose, we evaluated bone marrow PCs from 13 patients (6 MGUS and 7 MM). PCs were identified by flow cytometry by CD138 expression and malignant PCs were analyzed by gating the CD138+/CD19- population. In malignant PCs we evaluated the expression of apoptotic proteins Bax, p53, Fas/FasL, TNF/TNF-R1, TRAIL/TRAIL-R1-2, caspase 3 and the anti-apoptotic proteins TRAIL-R3-4, survivin and Bcl-2. Some of the proteins involved in survival, namely the transcription factor, NF-kB were also performed. RESULTS: The preliminary results from our study show that MM patients have highest levels of malignant PCs compared to MGUS patients (95% vs 67%, $p<0,05$). On the other hand, MM PCs show a statistically significant increase in p53, caspase 3, survivin and NF-kB ($p<0,05$) and a statistically significant decrease in BAX/BCL-2 ratio ($p<0,05$). These results may be related to the survival and apoptosis resistance of MM PCs. CONCLUSIONS: Our preliminary data suggest that in the progression from MGUS to MM a deregulation in apoptosis and survival pathways occur. The clarification of these mechanisms may contribute to the identification of new prognostic molecular markers and selection of patients for molecular targeted therapies.

1302

SEROTONIN STORED IN PLATELETS OF PATIENTS AFFECTED OF MULTIPLE MYELOMA REFLECTS THE ANGIOGENESIS SWITCH NEEDED TO SUBSTAIN BONE LESION

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Background. Physiological interactions between the serotoninergic and skeletal systems have been implicated by clinical observations. Hypoxic conditions modify plasma levels of serotonin, serotonin transporter activity, and expression of 5-HT1B and 5-HT2B receptors. Under low oxygen tension, cells increase the transcription of specific genes via stabilization of the transcription factor hypoxia-inducible factor (HIF)-1. In lung, the 5-HT2B receptor via phosphatidylinositol-3 kinase/Akt activates nuclear factor-kappaB, which is involved in the regulation of HIF-1 expression. Immunoglobulins have been shown to induce platelet release when participating in immune reactions as antigen-antibody complexes. While MM patients with evidence of osteolytic lesions have been shown to have elevated concentrations of serum tryptophan and serotonin the consequences of this elevation have not been previously studied in the bone itself. Methods We retrospectively measured bone remodeling signal pathway perturbations in 15 bone marrow core biopsies from patients diagnosed with MM, at different clinical stages, and correlated this with the presence of osteolytic bone disease. We measured circulating serotonin levels by ELISA in a pilot set of MM patients (n=20), in both serum and platelets isolated from peripheral blood to confirm observations obtained by RPMA. Finally, we collected platelets from 19 peripheral blood of subjects affected of monoclonal gammopathy (5 MGUS, 14 MM at different clinical stages and treatment with or without biphoshonates). Samples were lysed, protein extracted, printed and stained on Reverse-phase protein microarrays (RPMAs). In this last independent test, RPMAs were used to quantitatively map 45 cell signaling pathway endpoints, including oxygen sensors and transmembrane receptors proteins, in order to provide quantitative information regarding post-translational modifications (e.g. phosphorylation, cleavage, acetylation) and/or total cell signaling kinase levels, more sensitive and accurate of ELISA kits actually available in commerce. Results Bone marrow core biopsies exhibited significant elevation of Serotonin, RANK, MMP-11, TNFα, TNF-R1, and Ezrin Tyr353 by RPMA in patients with osteolytic lesions compared to patients without evidence of bone disease. Cytokines IL-1β, IL-6, and IL-10 were also significantly elevated in the bone marrow cores of patients with bone disease. Free circulating serotonin in MM sera was elevated compared to healthy controls. In particular, in the platelets we found a positive correlation between the content of serotonin and HIF-1-α ($r=0.74$, $p=0.0005$). Both analytes decreased after treatment for at least 3 months to Zoledronic Acid 4 mg i.v. (respectively $p=0.0014$ and $p=0.0317$), suggesting as a drug effective to control bone disease is able also to modulate the content of platelets grains. Anti-angiogenic molecule like PEDF was not related to serotonin levels and it was not modulated by Zoledronic Acid. However patients with MGUS or MM did not exhibit a differential activation status of platelets, with no appreciable differences in expression of CD9 and CD63. Conclusions. Taken together, our data confirm the role of peripheral 5-HT system in mediatingmediated signaling cascades related to the pathogenesis of MM-induced bone disease. This insight could provide strategies for reducing osteolysis with agents that regulate serotonin, either alone or in combination with other molecular targeted inhibitors.

1303

MULTIPLEXED PHOSPHOPROTEIN CELL SIGNALING ANALYSIS PREDICTS PATIENT-SPECIFIC THERAPEUTIC RESPONSE AND/OR OFF TARGET EFFECTS IN MULTIPLE MYELOMA

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Background Interaction of Multiple Myeloma (MM) cells with bone marrow microenvironment cells has a pathogenetic role in the disease and may confer tumor cell resistance to conventional therapies. A great need exists to understand the differential effect of treatment on myeloma as well as non-myeloma cells. We designed an ex vivo study to rapidly screen treatment combinations to predict treatment efficacy. Methods A panel of signal pathway inhibitor treatments was applied to fresh bone marrow aspirate samples from patients undergoing standard of care hematological work up for multiple myeloma. Bone marrow aspirates were immediately subdivided and treated ex vivo with a panel of molecular targeted inhibitors, including combinations thereof that target a wide range of cellular pathways (autophagy, proteosome, angiogenesis, protein degradation, proliferation/survival, insulin response, and translation). Up to 48 different treatment conditions can be studied from 5mL aspirate. After overnight incubation the samples were placed in a preservative that suppresses fluctuations in kinase pathway proteins. CD138+ plasma cells were separated from CD138 negative cells via immunomagnetic sorting. Reverse phase protein microarrays were used to quantify 60 cell signaling proteins in both cell populations. *Results* At baseline, patients with active myeloma (untreated before or relapsed) exhibited an increased pro-survival signaling (TNFR1, Akt Ser473, Akt Thr308, B-Raf Ser445) and ligands (IL6, IL10, TNFα) in both $CD138^+$ and bone marrow microenvironment cells. Only 1 case exhibited the activation of NfKBp65Ser536 in CD138+ plasma cells. Only in plasma cells, a subset of patients exhibited higher levels of Beclin1 and LC3B, suggesting a potential role of autophagy in MM aggressive phenotype. Untreated before and relapsed patients did not show a unique hyperactivated pathway, except in a patient treated with lenalidomide, relapsed after 6 months and subsequently bortezomib resistant. In this specific case, almost endpoints evaluated were pretty higher than the other subjects included in the study, except for autophagy markers and c-kit signaling. When we evaluated the response of this patient to the treatment ex-vivo with Lenalidomide 1uM and Bortezomib 100nM, alone or in combination, we were able to predict his refractariety, since the lack of protein profiling after treatment. Plasma cells isolated from patients previously exposed to Bortezomib did not differ from Lenalidomide-exposed ones. However, in vitro these drugs triggered pathways different. After 12 hours of exposure to lenalidomide we confirmed the Caspase8 upregulation, yet reported in literature, associated to increased levels of Bcl-2Ser70 and reduced Caspase3 Asp175 and Caspase9 Asp330. Conversely, Bortezomib was not able to induce Caspase8 and Bcl-2 Ser70. Conclusion Taken together, our data confirm the value of the RPMA assay to investigate improperly activated pathways converging on apoptosis triggering. The proposed assay conducted at beginning of the treatment is a valid mean to individualize therapy and improve combination strategies.

1304

THALIDOMIDE EFFECT ON ANGIOGENESIS AND IN CD57+ LYMPHOCYTE POPULATION OF MULTIPLE MYELOMA PATIENTS

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Background. The thalidomide (thal) action has not been fully elucidate. This drug has been consolidated as a promising therapeutic option for multiple myeloma (MM) because of its antiangiogenic and immunomodulatory effect. The microvessel density (MVD) and the amount of bone marrow (BM) CD 57+ lymphocyte population from (MM) patients are correlated with disease activity. Aims- To evaluate the bone marrow angiogenesis and CD57+ lymphocytes amount before and after 3 months on thal treatment. *Materials and methods*. We collected BM biopsies from 20 MM patients treated with Thal up front. The Thal dose was 200 daily. Samples were collected at diagnosis and after 3 months on thal treatment. The angiogenesis was accessed by CD 34 staining and was estimated by microvessel-density (MVD) and cytotoxic lymphocytes / NK cells counting by CD 57+. Microscopy was performed by two independent blinded pathologists to the treatment time. Photographs of three areas of high vessel concentration were selected for CD 34 counting with 400X magnification. Analysis of cytotoxic lymphocytes / NK was performed by direct counting on three areas of high concentration for the CD57 marker. We also correlated the response rate to the MVD and lymphocytes number. Wilcoxon's matched pairs test was used. Results - The median age was 64 (40-82y). The isotype identified was: IgG = 14, IgA=2, and light chain=4. DS stage: IIA=2, IIIA/B=15/3 and ISS 1/2/3=4/9/7. The schemes used were: TD=14, CTD=4, CTD +TD=1 and MPT=1. The response rate observed was: VGPR=8, PR=7, MR=2, progression in 1 case and 2 were not analyzed. The reduction in MVD after 3 months with thal was significant (p=0.04). There was no significant difference in the amount of CD 57 + pre and post treatment. The response rate was not correlate with angiogeneses reduction or with the amount of CD57+ lymphocytes. *Conclusions*. Evaluation after 3 months on thalidomide treatment showed a significant angiogenesis reduction. The number of patients may have been insufficient to evaluate the CD57+ lymphocytes population. Probably, the variations of lymphocytes occur in later treatment phases.

1305

INVESTIGATION QUALITY OF LIFE IN MULTIPLE MYELOMA PATIENTS WITH ANEMIA RECEIVED RECOMBINANT HUMAN ERYTHROPOIETIN

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Background. Anemia in patients with Multiple Myeloma (MM) is a frequent symptom and can influence the efficacy of antitumor 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*. This study was performed to find out the effectiveness Recombinant Human Erythropoietin in multiple myeloma patients with anemia and improving QoL. Methods. There was done prospective study to investigate the effectiveness of rHuEPO (epoetin beta) on Hb concentration, RBC count and QoL in multiple myeloma patients with anemia (n=44). The median age of patients was 67 years (range 26-80). If Hb concentration was <8.0 g/dl, the patients were performed red blood transfusions before rHuEPO treatment. Recombinant human erythropoietin beta was injected subcutaneously on 30.000 IU 1 times a week. Before start of rHuEPO treatment all patients have been received two or more cycles of chemotherapy. The target Hb level was 12 g/dl. The positive response was estimated as increasing Hb concentrating on 2.0 g/dl and so achieving target Hb level (12 g/dl). QoL was assessed by FACT-An questionnaire. Results. Mean baseline Hb concentration was 9.02±1.19 g/dl and RBC count was 2.78±0.43x1012/l. 10 patients had Hb concentration less 8.0 g/dl (5.6-7.9 g/dl) therefore they were transfused 2-5 units of red blood until Hb increased up to 8.0-9.0 g/l. The period of rHuEPO-therapy was from 4 to 16 weeks (mean 9.1±3.6 weeks and median follow-up of 9 weeks). During the study period in whole group, the Hb concentration and RBC count increased from baseline to 11.46±2.08 g/dl ($p<0.01$) and 3.56±0.79x1012/l ($p<0.01$), respectively. An Hb increase >2.0 g/dl was observed in 30 patients (68.2%), while a non-response was observed in 14 ones (31.8%). The patients with positive response significantly increased Hb from 9.01±1.26 to 12.41±1.11 g/dl ($p<0.001$) and RBC count increased from 2.78±0.41x1012/l to 3.78±0.51x1012/l ($p<0.001$). In this group the initial dose of rHuEPO were reduced to 20.00 IU at 5 patients (16.7%) because of fast increasing their Hb (more than 2.0 g/dl during 4 weeks) to prevent arterial hypertension. In group non response patients there were not significant difference of Hb concentration and RBC count (from 9.05±1.06 to 9.20±1.91 g/dl ($p>0.2$) and increased from 2.79±0.47x1012/l to 2.88±0.88x1012/l ($p>0.2$), respectively). Besides out of ten patients four ones (40%) had been continued to receive red blood transfusions. FACT-An demonstrated that rHuEPO-therapy in group patients with positive response reduced such symptoms as: feeling fatigue, weakness all over, having trouble starting things because of tiredness, depression, drowsiness, giddiness, headaches, pain in thorax and dyspnea. Conclusions. The study has shown that rHuEPO is effective at increasing Hb concentration, count of RBC and improving QoL in anemic patients with Multiple Myeloma.

1306

THE KAPPA/LAMBDA RATIO IN GROUP OF BENCE JONES MYELOMA PATIENTS

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The quantitation of the monoclonal immunoglobulins (Ig) and its fragments are used for the monitoring of Bence Jones myeloma (BJM) course and effect of therapy introduced. Bence Jones protein in urine (Ig free-light-chains (FLC)) is characteristic of light-chain multiple myeloma (LCMM). Relatively new laboratory tests in serum for the quantitation of kappa (κ) and lambda (λ) FLC and the calculation of the FLC ratio represents additional parameters of malignant plasmocityc production. The aim of FLC examination was to evaluate significance of FLC and kappa/lambda ratio (k/λ ratio) as a prognostic factor for progression, remission and survival in BJM patients. The concentrations of Ig and FLC were measured by immunonephelometric method on a "SIEMENS" DADE BN II analyser. The concentrations of Ig and FLC were measured by immunonephelometric method on a "SIEMENS" DADE BN II analyser. In this examination 37 BJM patients were investigate during the period of last 7 years. Reference interval for k/l ratio is (0.26-1.65). According to the ISS Model of stratification risk of disease progression, values < 0,03 ili > 32 represents high relative risk (RR), < 0,26 ili > 1,65 represents low-intermediate RR and < 0,125 ili > 8 represents high-intermediate RR of disease progression. Results showed that in BJM group 19/37 (51.4%) patients had high RR; 7/37 (18.9%) had low-intermediate RR, while 8/37 (21.6%) patients had high-intermediate RR. Also, 3/37 (8.1%) patients had normal k/l ratio, low RR and good prognosis. Nine patients was died during the period of 24 - 36 months. About 28% patiens which have lowered FLC values more than 50% under therapy, achieved the disease remission in group with BJ myeloma patients. Abnormal FLC ratio in group of BJM patients, could be an independent risk factor of progression and a worse disease prognosis. Serum assays could replace Bence Jones protein urine tests for patients with LCMM.

1307

ESTABLISHMENT OF A REFERENCE RANGE FOR FREE LIGHT CHAINS IN ELDERLY POPULATION

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Background. The quantitative assay of free light chains (FLCs) is a recently introduced commercial test reported to be sensitive and specific for detecting FLC diseases such as primary systemic amyloidosis (AL), light chain deposition disease (LCDD), nonsecretory multiple myeloma (NSMM), and light chain multiple myeloma. Aims. We evaluated the reference range of this test in the elderly population. Methods. We measured the concentration of κ and λ FLCs in the plasma samples of 884 elderly subjects aged over 65 years. Among the 884 subjects, those who had abnormal features (monoclonal peak in immunofixation, hypo or hypergammaglobulinemia, acute phase pattern C-reactive protein (CRP) of >1.0 mg/dL, and renal dysfunction Modification of Diet in Renal Disease (MDRD) glomerular filtration rate (GFR) of <60 mL/min/1.73 m2) were excluded. Levels of FLC were determined using the FLC assay (Freelite; The Binding Site, Birmingham, United Kingdom) performed on a Siemens Dade Behring BN II Nephelometer (Deerfield, IL). The assay consists of 2 separate measurements: one to detect free κ (normal range, 3.3-19.4 mg/L) and the other to detect free λ (normal range, 5.7-26.3 mg/L) light chains. The assay reports the FLC k/λ ratio (normal range, 0.26-1.65), and an abnormal FLC result was defined as an abnormal FLC k/λ ratio. Results Of the 409 subjects included in our study, 206 (50.4%) were male and 203 (49.6%) were female. Their median age was 75 years (range, 65-96). The 95% reference ranges for k FLC, λ FLC, and k/λ FLC ratio were 10.0-49.4 mg/L, 13.7-59.5 mg/L, and 0.54-1.32, respectively. *Conclusions*. Reference ranges were extended in elderly subjects compared with those established previously. This study suggests that the reference range of FLCs need to be established in the elderly population.

1308

EXPERIENCE WITH THE COMBINATION OF BORTEZOMIB, CYCLOPHOSPHAMIDE AND DEXAMETHASONE IN NEWLY AND RELAPSED MULTIPLE MYELOMA AND PLASMA CELL LEUKEMIA PATIENTS

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Background. Novel sequential combination therapy for induction may improve the quality of response and therefore prolong survival in newly diagnosed multiple myeloma (MM) patients. This concept should be allocated in refractory / relapse multiple myeloma patients. Material and Methods: We present our experience with the combination of Bortezomib (1.3 mg/m2 D1, D4, D8, D11), Cyclophosphamide (600 mg/m2 D1, D8) and Dexamethasone (40 mg D1, D4, D8, and D11) for 21-days cycles in newly and relapsed multiple myeloma and plasma cell leukemia patients. From February 2009 until February 2011, we treated 18 patients (12 in first line). On the front line in less than 70 years old as induction therapy prior to stem-cell transplantation, have treated 8 patients (1 plasma cell leukemia included) and over 70 years, two patients with plasma cell leukemia. 7 relapsed patients had received at least one line of prior chemotherapy (1-4), and 2 patients had been transplanted previously. The number of cycles (BCD) administered ranged between 1-8 (mean 4.3). 5 patients received only one cycle, 4 died of disease progression (1 plasma cell leukemia) and in newly diagnosed patients stopped treatment for pneumonia (Candida plus H1N1 virus) and he admitted in intensive care unit because he did need mechanic ventilation support. *Results*. 11 patients are evaluable for response; Overall response rate was 100%, including 9.1% CR, and 98.9% partial response. 6 patients treated in first line had undergone stem-cell transplantation. Table 1. Toxicities were predictable and manageable; the most-commonly reported grade 3/4 toxicity was neuropathy (37.5%).The limiting toxicity was peripheral neuropathy, present in 3 patients, necessitating dose reduction of Bortezomib in one step in 2 patients, and in other patient was reduced two steps. 4 patients (50%) developed neutropenia grade 3-4, and required to use G-CSF. Infectious complications that required hospital admission in 4 patients (1 ZHV spread, 1 bacteriemia Staf., 2 pneumonias).

Table 1. MM patients: BCD treatment.

Conclusions. 1) The BCD combination gets quality and fast respond. 2) hematological toxicity, is easily manageable, and the incidence of infection complications is higher in refractory/relapsed patients and with associated comorbidity. 3) In refractory/relapse patients, respond rate is obtained as first-line patients. Patients retreated with bortezomid had good results, with an increased of neuropathy.

1309

PROGNOSTIC VALUE OF ELEVATED BNP AND NT-PROBNP LEVELS IN ELDERLY PATIENTS WITH MULTIPLE MYELOMA AND CONCOMITANT CHRONIC HEART FAILURE

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Background. Multiple myeloma (MM) is an incurable plasma cell disorder that comprises 10% of hematologic tumors. Incidence increases greatly with age: the median age at diagnosis is 70 years, with 65% of patients older than 65 years. The geriatric patients often have concomitant heart disease and in addition they are forced to receive cardiotoxic chemotherapy for MM. These circumstances as a whole certainly worsen the prognosis of life. *Aims*. To estimate the prognostic importance of

BNP and NT-proBNP markers in patients with MM and accompanying chronic heart failure (CHF). Material and methods: 45 patients are included in the analysis (m-15, f-30) with relapses or refractory MM for which were satisfied following conditions: (1) ECOG ≤ 2; (2) Anemia with Hb < 8.0 g/dL; (3) proved CHF; (4) basic therapy for CHF (inhibitor APF ± diuretic) was spent not less than within last 2 weeks and (5) the chemotherapy concerning MM was assumed. The patients with heart failure with New York Heart Association (NYHA) IV and/or the constant form of atrial fibrillation and/or heart diseases and/or a heavy arterial pathology didn't include in this study. CHF was diagnosed on basis of standard criteria and the present of the left ventricular enlargement or systolic functional impairment by echocardiography, according to the European Society of Cardiology guidelines. The levels of NT-proBNP and a BNP-fragment in blood serum have been defined at the moment of enroll in study by ELISA. The mathematical definition of threshold values of concentration of markers was spent by means of construction of ROC-curves and Kaplan-Mayer analysis. Results: The median of age of patients at the moment of enroll in study has made 66 (42-83 range) years. 3 (7%) patients had IIA stage on Salmon-Durie and 22 (49%) - IIIA and 20 (44%) - IIIB. 17 (38%) patients had no clinical signs of CHF. 16 (35%) patients have CHF with NYHA I and 9 (20%) - II and 3 (7%) - III. 28 (62%) patients have received salvage regimens of chemotherapy with bortezomib and 15 (33%) - with alkylating agents and 2 (5%) - high doses of dexamethasone. The objective response was documented for 26 (58%) patients including complete response (CR) or very good partial response (VGPR) - 7 (16%). 33 (73%) patients have alive at a median of follow up 11 months. The analysis of NT-proBNP levels has revealed correlation between the degree heart failure and a disease outcome ($p < 0.05$). Threshold concerning a failure of disease value of concentration NT-proBNP (sensitivity - 82%, specificity - 62%) has identified as 0.93 ng/ml. For a BNP-fragment of authentic distinctions in the conditions of the limited sample has not received. *Conclusions*. The elevated serum NT-proBNP levels > 0.93 ng/ml is identified as the poor prognostic factor for patients this MM and accompanying CHF.

1310

LENALIDOMIDE PLUS DONOR LYMPHOCYTES INFUSION (DLI) AFTER ALLOGENEIC STEM-CELL TRANSPLANTATION (ALLO-SCT) WITH REDUCED-INTENSITY CONDITIONING IN PATIENTS WITH HIGH RISK MULTIPLE MYELOMA

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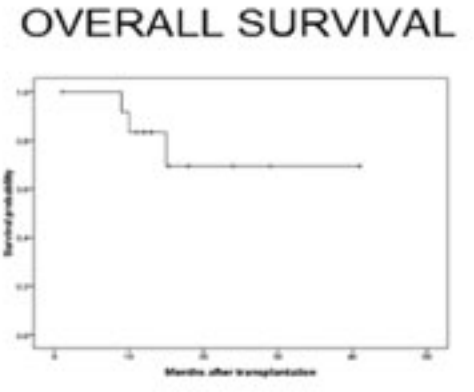
Relapse remains the main problem after allogeneic stem cell transplantation (Allo-SCT) in high risk Multiple Myeloma (MM) patients. The aim of our prospective study is to evaluate the anti-myeloma effect of lenalidomide followed of donor lymphocyte infusion (DLI) as adoptive immunotherapy after transplantation. *Patients and methods*. Twelve patients with refractory and high risk myeloma were analysed. Median age at transplantation was 56 years (46-64); 6 patients (50%) received lenalidomide before Allo-SCT. All patients received a RIC including Fludarabine 30 mg/m² 5 days, ATG 2,5 mg/kg for 2 days and Busilvex 3.2 mg/kg/day (3 days in 6 patients and 2 days in 6 patients). All but one received peripheral blood stem cells (PBSC). Donor was HLA id in 6 patients and matched unrelated in 6 patients. Patients were treated by lenalidomide if myeloma was progressive or residual disease was observed starting from day +100 and if no GVHD signs were evident. Dose was between 10 and 25 mg/day. DLI were administered after at least 2 cycles of lenalidomide. *Results*. The median time between Allo-SCT and lenalidomide was 10 months (3-38). The median initial dose of lenalidomide was 15 mg (10-25). The patients received a median of 6 cycles (1-10). 9 patients (60%) received an escalating dose of DLI; $1x10^7$/kg of CD3+ cells for the first DLI and 1 x 108/Kg of CD3+cells for the second DLI. One patient with GVHD and two patients with progressive disease after lenalidomide did not receive DLI. The toxicity related to lenalidomide was haematological grade II in 4 patients (33%) and grade I in 3 patients (25%); 7 patients (58%) had moderate asthenia. one patient developed a reversible renal insufficiency after 10 cycles of lenalidomide, none of our patients developed thrombo-embolism under treatment. At the last follow up, 9 patients are alive and all of them are under ongoing treatment. Four patients achieved complete remission (CR) and five patients partial remission at last evaluation. The 1 and 2 years probability of the progression-free survival (PFS) was 75% and 50% and overall survival (OS) was 83% and 69% respectively. The median OS was not reached and the median PFS was 23 months. Conclusions: These data show that lenalidomide has an acceptable toxicity profile is well tolerated after Allo-SCT. Combination with DLI should be further evaluated in a larger cohort of patients.



1311

THALIDOMIDE-INDUCED SENSORY NEUROPATY IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Chemotherapy-induced sensory neuropathies differ in clinical picture. There is predominance of paresthesiae in some of them while in others pain or deep sensation failure dominates. *Aim.* Clinical and electrophysiological assessment of peripheral sensory nerves in patients with multiple myeloma (m.m.) treated with thalidomide. Special attention was directed to function of subtypes of sensory fibres which convey different modalities of sensation. *Material and methods*. Twenty seven m.m. patients and 30 controls were examined. Neurological examination together with allocation to different groups acc. to sNCI-CTC scale were performed. Standard sensory conduction velocity was measured in ulnar and sural nerves. Quantitative Sensory Testing (QST) was used to determine thermal detection thresholds. *Results*. All patients informed about subjective positive sensory symptoms and sensory deficit of symmetrical, distal pattern was found. Electroneurography revealed axonal and demyelinating abnormalities with dominance of axonal injury. Warm and heat-pain detection thresholds were elevated, while threshold for skin cooling was decreased both in palm and foot in m.m. patients in comparison with controls. There were no differences in the thresholds for cold-pain detection between examined groups. *Conclusions*. Thalidomide-induced sensory neuropathy can appear shortly after the introduction of treatment. Patients with longer duration of treatment or with higher cumulative dose present higher degree of neuropathy acc. to the sCNI-CTC scale. Sensory deficit in thalidomide' neuropathy is associated with dysfunction in A delta and C caliber primary afferent fibres.

1312

CORRELATION OF SERUM LIGHT CHAIN LEVELS AND ISH LIGHT CHAINS STAINING IN TREPHINE BIOPSIES IN MULTIPLE MYELOMA PATIENTS: SOUTH AUSTRALIAN EXPERIENCE

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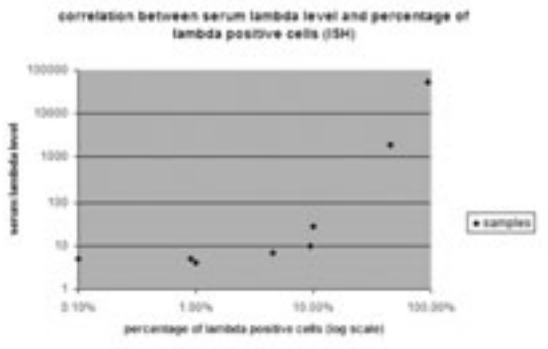
Background. The detection and quantification of monoclonal plasma cells in bone marrow biopsy is essential in the diagnosis of plasma cell neoplasms and in assessment of treatment response. Controversies exist relating to a standard for conventional immunohistochemistry staining (IHC) in the detection of Ig light chains, as interpretation is operator dependent and limited by heavy background staining. In situ hybridization (ISH) is an established technique in the detection of Ig light chain

mRNA, utilizing specific probes to demonstrate monoclonality in myeloma and B cell lymphomas.



Aims. This single centre retrospective review assesses the efficacy of this technique (ISH) by correlating with serum light chain levels. *Methods*. A random cohort of 20% of bone marrow biopsies performed between November 2009 and January 2011 for multiple myeloma was included in this review. The kappa and lambda positive cells in the marrows were quantified as a ratio of the percentage of kappa/lambda positive cells to the percentage of CD138 positive *cells.Results and conclusions*. The mean age of our patients was 65 years (range 38-85yrs) and 48% were female. The majority of patients had IgG kappa subtype (50%). A statistically significant correlation was demonstrated between serum lambda level and lambda positive cells in the trephine biopsy ($R=0.9069$, $p<0.0001$). There was no significant correlation between serum paraprotein level and kappa or lambda positive cells in trephine biopsy. Furthermore, six patients (15% - total 38 patients) were noted to have significant disease by CD138 and kappa/lambda staining despite having low plasma cell count (less than 10% by smear morphology and immunophenotyping). In conclusion, our study highlighted the greater sensitivity of ISH staining in comparison to conventional morphological criteria, with statistically significant correlation with serum free light chain levels and is therefore a useful tool in the diagnosis and follow up of myeloma patients. Further studies are needed to better clarify its role.

1313

MULTIPLE MYELOMA & OBESITY: A META-ANALYSIS

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Background. Excess body weight, expressed as increased body mass index (BMI, weight/height2), is associated with the risk of some common adult cancers. The prevalence of overweight, defined as a BMI of 25-29.9 kg/m2, and obesity, BMI ≥30 kg/m2, has been rapidly increasing during recent decades. The results of the first meta-analysis by Larsson et al. indicate that excess body weight may be a risk factor for multiple myeloma (MM), but controversy remain in more recent studies. *Aims*. To highlight the current evidence on the association between MM and obesity. *Methods*. We systematically searched Medline and Embase (from their commencements to February 2011) for studies on the association between obesity (as defined by WHO) and MM incidence or mortality as outcome, published in English as full reports, indicating relative risks (RR) with 95% confidence intervals on the association of MM with BMI. From each study, we collected information about publication data, study design, characteristic of the study population, sample size, exposure assessment, and potential bias. Summary RR estimates were calculated using a random-effects model. Statistical heterogeneity was assessed with the Cochrane Q test. The magnitude of the heterogeneity was evaluated with the I2 statistic. All statistical analysis were performed with Stata 11.0. Results. 4 case-control (with a total of 1,166 cases and 8,247 controls) and 19 cohort studies (with a total of 15,231 cases) were included in the meta-analysis. The risk of MM was statistically significantly higher among obese individuals compared with those who had normal weight: in cohort studies, overall RR 1.17 (1.08-1.26), men RR 1.17 (1.01-1.33) and women 1.20 (1.10-1.30); in case-control studies, overall RR 1.48 (1.28-1.69), men RR 1.47 (1.17-1.76) and women 1.50 (1.21-1.79). *Conclusions*. The results provide further evidence on the role of obesity as a potential risk factor for MM. From a public health perspective, in view of the global expansion of obesity prevalence, it is warranted to measure the impact of obesity on MM incidence.

1314

SKEWED RATIOS OF HEAVY CHAINS IGM-KAPPA /IGM-LAMBDA CHARACTERIZE REMISSION DEPTH BETTER AS IMMUNOFIXATION: APPRAISAL OF A NEW IMMUNOASSAY IN IGM PARAPROTEIN POSITIVE LYMPHOPROLIFERATIVE DISEASE

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Hevylite™, a novel immunoassay panel is designed for analysis of immunoglobulin heavy chain/light chain pairs. These assays can identify, separately, the different light chain types of each immunoglobulin class i.e. IgGκ IgGλ, IgAκ IgAλ, IgMκ, IgMλ. The molecules are then measured in pairs e.g. IgGκ/IgGλ to produce ratios of monoclonal immunoglobulin/background polyclonal immunoglobulin concentrations. Hevylite ratios (HLR) may serve as a parameter for plasma cell dyscrasia induced immunoparesis and serve as a new marker for validating remission depth and relapse probabilities. The lastly introduced IgMκ/IgMλ pair of assays was tested in patients supposed to be in remission or responding to specific treatments during routine surveillance visits for IgM parapotein secreting diseases [MALT NHL, IgM MGUS, Waldenstroems macroglobulinaemia (MW), IgM Myeloma (IgM MM) and AL-Amyloidosis] in comparison to serum measurements of total immunoglobulins, free light chain ratios (FLR) and immunofixation. Results were correlated to the patients further clinical course in the framework of informed consent to the local clinical registry. *Results*. 20 pts. with IgM MGUS (6 pts.), MW (6 pts.), IgM MM (3 pts.), MALT NHL (2 pts.), Amyloidosis (1 pt.) and previously suspected insecure IgM paraproteins (2 pts.) were analyzed. In comparison to standard electrophoresis (EP), immunofixation (IFT) and freelight ratios (FLR), HLR proved to be more sensitive detecting residual disease in 9 cases compared to EP (3), IFT (5) and FLR (3). 2 of the pts. exposing positivity in HLR only, later showed rapid clinical deterioration. *Conclusions*. As this case series illustrates HLR may serve as a sensitive new diagnostic tool for rational treatment allocation, especially with respect to maintenance and consolidation strategies. An update comprising data on IgA and IgG excreting diseases, mainly focused on myeloma, will be presented, as well as the outline of a cooperative European project to further validate this putative biomarker will be introduced. In the future HLR should be validated against techniques using sCR, flow-CR and molCR. Furthermore the effect of proven immunoparesis on complication rates respective to infections should be evaluated.

	No.	POSITIVE IN EP	POSITIVE IN IFT	POSITIVE IN HLR	POSITIVE IN FLR
IgM MGUS	6	1	1	2	0
MALT NHL	2	0	0	1	0
WM	6	0	2	4	1
Exclusion	2	0	0	0	0
Amyloidosis	1	0	0	0	0
IgM MM	3	2	2	2	2
	20	3	5	9	3

1315

IMMUNOGLOBULIN OLIGOCLONAL BANDS AND ISOTYPE SWITCHING AFTER BONE MARROW TRANSPLANTATION IN PATIENTS WITH ù MYELOMA

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Background: Appearance of new monoclonal or oligoclonal immunoglobulin bands was reported to be common in myeloma patients after receiving bone marrow transplantation (BMT). *Aims*: We aimed to study this phenomenon in a cohort of Chinese patients who had received autologous or allogeneic BMT as part of the treatment of myeloma. Methods: The clinical records and laboratory results of 41 patients over a 6-year period (2005-2010) were reviewed retrospectively. *Results:* The distribution of the original monoclonal immunoglobulin isotypes was IgA/L (n=7), IgA/K (n=3), IgG/L (n=3), IgG/K (n=13), IgD/L (n=4), Free L (n=5), Free K (n=5), hyposecretory (n=1). After receiving BMT, 23 patients (56%) develop new monoclonal or oligoclonal immunoglobulin bands detected by serum/urine protein electrophoresis and immunofixation. Of the remaining 18 patients, 4 were followed up for only 4 months or less and new immunoglobulin bands might develop subsequently. The isotypes of the new bands found were IgG/L (n=4), IgG/K (n=12), IgM/L (n=1), Free L (n=2), oligoclonal with no light chain restriction (n=4). Of those who were followed up for at least 23 months (n=20), 8 survived and 3 succumbed if new immunoglobulin bands were found. Although not statistically significant (p>0.05 by the Fisher Exact Probability Test) this survival rate was better than those who did not have new immunoglobulin bands (3 survived, 6 died). Of those who were followed up for less than 23 months (n=21), the survival rates were similar whether new immunoglobulin bands developed or not developed (10 survived 2 died and 9 survived 0 died respectively). *Conclusions:* This study demonstrates new monoclonal or oligoclonal immunoglobulin bands frequently develop after patients with myeloma receive BMT. Although not significant statistically the long term survival of patients who have new immunoglobulin bands appears more favourable than those who do not have new immunoglobulin bands. A larger cohort is needed to confirm these findings.

1316

PREVIOUSLY NOT DESCRIBED ASSOCIATION OF SKIN CRYOGLOBULINEMIA TO A SYSTEMIC CAPILLARY LEAK SYNDROME (SCLS) WITH MULTIPLE MYELOMA

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We submit the case of a 37 years-old woman diagnosed at the age of 29 of "Systemic Capillary Leak Syndrome"(SCLS),(Clarkson Syndrome). In April 2002 the patient had suffered an episode of hypovolemic shock, hemoconcentration, rhabdomyolysis, massive edema and acute renal failure. In May 2002 a IgG lambda monoclonal peak appeared in serum with 3% of bone marrow plasma cells (MGUS). With the diagnosis of SCLS the patient started prophylaxis with theophylline and terbutaline. Despite these drugs, the patient suffered two new episodes (2nd: October 2002; 3rd: May 2003;). A 4th attack in December 2004 was treated with a low dose of prednisone. A fifth episode in April 2005. The bone marrow study showed 0, 5% of polyclonal plasma cells. Peripheral blood flow cytometry showed CD25 coexpression in 50% of the CD4+ cells. She started treatment with Dexamethasone: 40mg per day, during 4 days every two weeks, for 3 months. She followed maintenance in July 2005 (Dexamethasone 40mg per day, four days each month) finishing in November 2007. In December 2007 a sixth episode occurred: Dexamethasone was discontinued and Verapamil was started. Two new episodes happened in December 2008 (7th) and in April 2009 (8th). In October 2010 the patient associated a symptomatic cryoglobulinemia (skin) to a multiple myeloma diagnosis (serum monoclonal component IgG Lambda of 3.01 g/dl. 11% of plasma cells with abnormal cytogenetics: t (4; 14) and del RB, a left iliac lytic lesion with negative spine MR and PET scan). The following diseases were ruled out: deficiency of C1 esterase inhibitor, autoimmune diseases, muscle disease and toxic exposure. After expert clinical advice the patient began CBD treatment (Cyclophosphamide i.v. 300 mg/m^2 days 1,8,15 and 22 ; Bortezomib 1.9 mg days 1,4,8 and 11; Dexamethasone 40 mg days 1,4,8 and 11) in December 16, 2010. After the first cycle, a 9th episode occurred in January 2011 that was managed (as all before) in the intensive care unit. Afterwards she has received two more cycles without no more episodes of the disease. Discussion. SCLS is a serious illness. The most important thing is an accurate diagnosis. The three criteria to be fulfilled are hypotension (usually with edema), an increase in hematocrit, and a decrease in albumin, accompanied often (75%) by a monoclonal gammopathy. The disease is sometimes confused with Acquired C1 esterase inhibitor deficiency. C1 esterase inhibitor levels both functional and antigenic and C3 and C4 levels should be normal but complement levels may be low. The treatment is prophylactic and consists of a methylxanthine combined with terbutaline. 80% of SCLS patients have MGUS. The risk of progression to myeloma is 1%/yr with an incidence similar in SCLS and in MGUS. In our case SCLS was associated with an MGUS which progressed to symptomatic MM during the disease evolution. Our case is unique because the association of SCLS with MM and cryoglobulinemia was not previously described. Our plan is to perform an autologous stem cell transplantation (ASCT) after chemotherapy that has never been done before to a SCLS patient.

1317

HOW LARGE A DIFFERENCE BETWEEN SERIAL MEASUREMENTS OF FREE LIGHT CHAINS IS NEEDED TO INDICATE A RESPONSE OR PROGRESSION IN PLASMA CELL DISORDERS?

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Background: since the commercially available immunoassay Serum FreeLite® Free Kappa & Free Lambda assay (the Binding Site Ltd, UK), for the measurement of free light chains (FLC) in serum was introduced, the use has been increasing along with the growing documentation for the utility of the assay. However, we lack information on the standards of analytical precision and to define the difference between serial results required for significance. *Aims*: to determine the biological variation of FLC when using the assay, thereby being able to set the desirable performance standards for the imprecision of the assay. Furthermore, we assessed the current imprecision of the analysis in use, measured on the Dade Behring BNII®, to determine whether it can meet the desirable standards. We also assessed the critical difference for two results to be significantly different ($p \leq 0.05$). Methods: FLC were measured in serum of 7 healthy persons (mean age 41 (21-60) years, 2 males and 5 females), 6 patients with multiple myeloma (mean age 63.2 (44-73) years, 3 males and 3 females) and 5 patients with monoclonal gammopathy of unknown significance (mean age 66.2 (62-70) years, 1 male and 4 females). From each patient we collected 8 serum samples, 5 of these taken every morning (between 8 and 9 am) for 5 days (day-to-day variation), and 3 samples during day one (between 12-13 pm, 16-17 pm and 20-21 pm) (24 hour variation). The serum samples were collected in PET serum tubes with gel. Samples were centrifuged, pipetted and stored at -20°C. All 8 samples from each patient were analysed in the same run, on the same instrument and by the same experienced technician. FLC were also measured in 17 serum samples with κ values from 15-11.000 mg/L and 15 serum samples with λ values from 14-516 mg/L to determine the imprecision. The critical difference is then calculated using the following formula: 2.77 (CVa2 + CVw2)½. *Results*. The intra-individual variation, CVw for κ and λ was found to be 6.8% and 7.4%. The inter-individual variation, CVb for κ and λ is 21.6% and 29.8%. The imprecision of the FLC assay, the coefficient of variation for the analysis, CVa, is 8.3% for κ and 5.1% for λ. The critical difference between two serial results to be significantly different is then calculated to be 29.7% for κ and 24.9% for λ. *Summary/conclusions*. We found a minimal and identical intra-individual biological variation for both healthy individuals and patients with monoclonal plasma cell disorders. The current FLC assay cannot meet the desirable laboratory performance standards, or analytical goals, for the imprecision of analytical methods derived from data on biological variation. The FLC analysis is showing great individuality, and the best reference for patients is their own former results. We calculated the critical difference between two serial results to be 29.7% and 24.9% for κ and λ, respectively, to be assessable as evidence of progression or response in plasma cell disorders.

1318

TRANSFORMATION OF MGUS INTO LIGHT CHAIN DEPOSITION DISEASE WITH A FAVORABLE RESPONSE TO BORTEZOMIB TREATMENT

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Background. Recent literature has described only rarely the transformation of MGUS into light chain deposition disease with successful treatment using bortezomib. *Methods and results.* Case description: A 24-year old male has been followed for 10 years for MGUS IgG lambda. During the examination for weight loss (15 kg) and dyspnea we found considerable anemia (Hb 86 g/L), increased levels of creatinine (143umol/L), serum M-protein 11,6 g/L, increase of λ serum free light chains (FLC) to 542.2 mg/l (κ/λ index 0.043), supression of normal immunoglobulin IgM (0.22 g/L), NT pro BNP 2813, slight focal increase of monoclonal plasma cells with expression of FLC -lambda, microscopic hematuria (294.0), proteinuria 2.1 g/24h (B-J-λ). Ultrasonography revealed kidney enlargement with hyperechogenity of the parenchyme, histology of the kidneys demonstrated linear FLC lambda deposits in the basal membrane of the tubules forming the diagnosis of LCDD type lambda. Spiral HR-CT and FDG-PET/CT displayed GGO of lower lung lobes and multiple lung parenchymal consolidations with left-sided pleural effusion. After 4 cycles of bortezomib and dexamethasone we could observe substantial improvement of overall condition, disappearance of dyspnea with pulmonary finding regression, normalization of blood count (Hb 132), decrease of M-protein (2,6g/L), FLC λ (25.8, kappa/lambda index 0.445), proteinuria (0.31 g/24h), creatinine (114 umol/l), decrease of beta2-microglobulin (2.8-1.6 mg/l). *Conclusions*. We conclude that MGUS patients need a permanent follow-up that might enable to record the transformation into another monoclonal gammopathy, in this case a LCDD with successful bortezomib and dexamethasone treatment as an induction for autologous stem cell transplantation.

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PARTIAL 'LIGHT-CHAIN ESCAPE' PHENOMENON & CYTOGENETIC CHANGES - TWO CASE STUDIES

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Background. The 'light-chain escape' (LCE) phenomenon refers to the condition when, in the relapse/progression stage of multiple myeloma (MM), the secretion of monoclonal light chain becomes dominant to the detriment of the decreasing or only slightly increasing level of monoclonal immunoglobulin (MIG). This phenomenon is often observed in patients with the IgA isotype, after the completed chemotherapy involving the administration of latest biological agents. *Aims*. The communication aims at presenting 2 cases of detailed observation of LCE connected with cytogenetic change in the tumor clone. *Results*. Case 1: A 58-year-old patient with MM IgA-κ IIIA, ISS II (MIG 50 g/l, κ 129 mg/l), (46,XY[23]; FISH: 3 copies of IgH, 1, 7; 5 copies of 1q21; polysomy of 9,17), who achieved very good partial remission (MIG 1,47, kappa 20) after induction treatment with CTD and the subsequent autologous transplantation of stem cells (HD-T/ASCT). The following early progression was characterized by partial LCE (MIG 4,8, kappa 1763) and changed cytogenetics (46XY[17]/76-88[8]; FISH: 2 copies of IgH, t(8;14); 3 copies of 1; 6-7 copies of 1q21; polysomy 7, 9, 11, 17). The progression was associated with extramedullar proliferation and resistance to chemotherapy involving the administration of bortezomib as well as lenalidomid. Case 2: A 65-year-old patient with MM IgA-λ, IIA, ISS II (MIG 42, lambda 373), (46XX[9]; FISH: del RB1, t(4;14)) achieved stringent complete remission (sCR) after induction treatment with CTD and the subsequent HD-T/ASCT. After 15 months, focal relapse with extramedullar proliferation was proven in the pelvis area, while sCR was achieved after local radiotherapy and chemotherapy with VCD again. After next 4 months, systemic relapse of the disease was proven in the form of partial LCE (MIG 3,5, lambda 884) associated with karyotype evolution (43-45,XX,-2,-4,-15,+1-3 mar[cp9]; FISH: del RB1, t(4;14), del TP53. The relapse was connected with resistance to further chemotherapy, including the administration of lenalidomid. *Summary*. The above mentioned observation confirms that the presence of LCE and the unfavorable karyotype evolution in the disease progression/relapse tend to be related to the subsequent adverse course of the disease and resistance to therapy. Determining the serum levels of free light chains and repeated cytogenetic examinations allow to recognize early relapse/progression, particularly in case of LCE, and to identify patients with adverse prognosis requiring an early intensive chemotherapy.

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1320

THE IMPACT OF VELCADE TREATMENT ON SURVIVAL OF RELAPSED MYELOMA PATIENTS – A SINGLE INSTITUTION EXPERIENCE

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Background. According to SUMMIT trial, Velcade salvage for repeated relapsed myeloma patients showed the overall response to have 28%. A randomized phase III multicenter, APEX trial, compared velcade to high dose dexamethasone in 669 patients with relapsed/refractory myeloma patients revealed superior median overall survival (29.8 vs 23.7 months) for velcade. Methods Between August 2005 and October 2010, nineteen relapsed myeloma patients underwent salvage velcade treatment at our institute and we compared the earlier 40 myeloma patients not received velcade. The endpoint is the overall survival in these patients with or without the use of velcade. Results. The median ages of velcade group and no-velcade group are 60.2 vs 61.7 (range 41~79 vs 39~80), respectively. The stage III disease percentages are 63.2% vs 55.0%. The median prior lines of treatment before velcade are 1.84. The median overall survivals of velcade group and no-velcade group are 60 vs 25 months (5-year survivals are 50.2% vs 15.5%). Conclusions -In our historical comparison between velcade and no-velcade groups of patients, velcade surely could prolong the overall survival of relapsed myeloma patients.

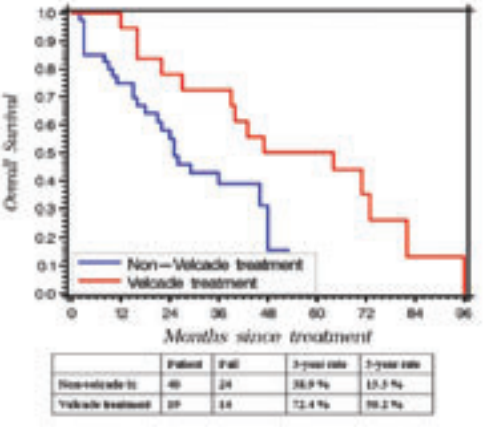

Figure 1. Overall survival of patients with/without velcade.

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MR-PROADM AS MARKER OF VASCULAR DAMAGE REVERSIBILITY IN AL AMYLOIDOSIS PATIENTS

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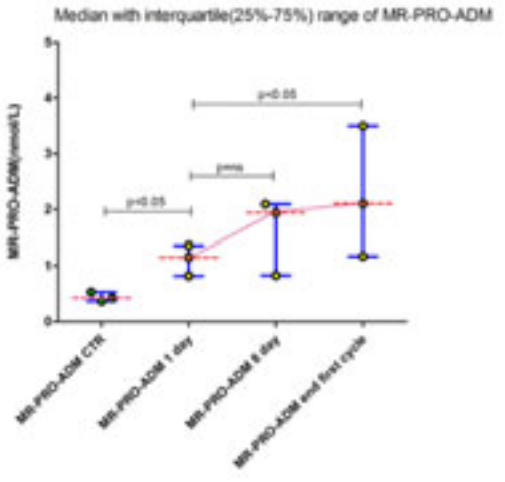
Background. Light-chain AL amyloidosis is the most common form of systemic amyloidosis and is associated with an underlying plasma cell dyscrasia. Circulation dysfunction is very frequent in AL amyloidosis patients. Serum midregional fragment of pro-adrenomedullin peptide (MR-proADM) and free light chain (s-FLC) κ and λ levels were investigated in order to evaluate circulation impairment in systemic AL amy-

loidosis. MR-proADM has been described as an useful marker for heart, respiratory and circulating dysfunction assessment and its role as a prognostic factor we evaluated in patients with systemic diseases. Aims and Methods. MR-proADM and s-FLC levels were detected in 7 patients (median age 63.1 yrs) with systemic AL λ amyloidosis at exordium observed to our Unit. Ten age-matched healthy control individuals were selected. According to age and disease risk stratification six patients were treated with upfront oral MelDex association (Melphalan 9 mg/sm, Dexamethasone 20mg day 1-4 q28); one subject started first line therapy with BorDex association (Bortezomib 1.3 mg/sm, Dexamethasone 20 mg day 1,4,8,11 q21). Three samples of peripheral blood were performed (treatment day 1, day 8 and 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 Mann-Whitney U test, paired t test, Kruskal-Wallis one-way analysis of variance (Dunn's Method versus Control Group), Spearman rank correlation and Linear Regression were performed. P values ≤ 0.05 were considered statistically significant. *Results*. s-FLC λ values were significantly decreased during treatment ($p<0.05$). s-FLC κ/λ ratio and MR-proADM level were both increased at the end of first course of therapy ($p=0.002$ and $p<0.05$ respectively). On day 1 a positive correlation between s-FLC λ and MR-proADM level was observed ($r2=0.82$, $p=0.03$). *Conclusions*. The reduction of s-FLC levels observed in patients with systemic AL amyloidosis is indicative of hematological response to treatment. On the other hand, the progressive increase of MR-proADM serum level could represent a warning for possible vascular damage even if haematological response has been achieved. Therefore MR-proADM could be considered an additional useful biomarker in the evaluation of systemic AL amyloidosis



1322

PERCUTANEOUS VERTEBROPLASTY IN PATIENTS WITH SPINAL MYELOMA LESIONS

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Background. About 80-90% of patients with multiple myeloma will develop skeletal-related complications, including diffuse osteopenia, focal lytic lesions, pathological fractures and bone pain. The therapeutic intervention for spinal myeloma lesions (SML) is based on analgesic medications, biphosphonates, radiation therapy, and in some cases, percutaneous vertebroplasty (PV) and balloon kyphoplasty. PV consists in the injection of polymethylmethacrylate into the damaged vertebral body via a percutaneous approach under image guidance. While in patients with non myelomatous osteoporotic vertebral fractures, 2 randomized trials have shown no beneficial effect of PV compared with placebo or a simulated procedure without cement, no specific data in controlled trials are available for myeloma patients. *Aims*. To evaluate the efficacy and safety of PV in patients with SML. *Methods*. Patients with SML not responsive to medical treatment were eligible. A computed tomography and/or magnetic resonance were performed, previously to the procedure. The presence of pathological fractures was evaluated by a rheumatologist and/or radiologist specialist and performed by an interventionist radiologist with local anesthesia and light sedation. In some cases, more than one vertebra was treated in the same procedure. Pain response was evaluated by a qualitative scale at 24 hours, 1 and 6 months after PV. *Results*. Nineteen PV were performed in 15 patients (12 females and 3 males). Thirty-eight vertebras were treated (maximum 4 vertebras in the same procedure), being the most frequent localization L3. Medium age was 74.8 years (range, 39 to 88 years). The evaluation of pain at 24 hours, 1 and 6 months after PV, showed improvement in 79%, 47% and 37% of cases, respectively. Notably, most patients reported pain in other skeletal localization caused by disease progression, but unrelated to treated vertebra. The incidence of cement leakage was 47%. Four out of 15 patients developed severe complications: 1 psoas hematoma without hemoglobin decrease in the first 24 hours after PV with good outcome, 1 death by respiratory failure of unknown etiology 11 days after PV and 2 pulmonary embolism (the first one died in the first 24 hours after the third PV because of a cement pulmonary embolism; the second one was hospitalized one week after PV and treated successfully with heparin). *Conclusions*. PV is an easy technique for SML not responsive to medical treatment that results in immediate pain relief in 79% of patients. Severe clinical complications secondary to cement leakage can be observed in 26% (4 out of 15) of patients, with some being life-threatening. These results suggest that PV can be useful in acute SML treatment to improve pain related but further studies with more patients and more follow-up should be undertaken to confirm the efficacy and the incidence of adverse effects.

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INTERNATIONAL STAGING SYSTEM PREDICTS PROGNOSIS OF CHINESE PATIENTS WITH MULTIPLE MYELOMA ACROSS DIFFERENT CALENDAR PERIODS WITH APPLICATION OF NOVEL AGENTS

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Background and Aims. The International Staging System (ISS) has been proposed since 2005 but the applicability of Chinese patients with multiple myeloma (MM) is not known, especially for those who had received treatments with novel agents. Methods. MM Patients who were newly diagnosed in Taipei Veterans General Hospital were enrolled between 1996 and 2007. Data of clinical features, laboratory tests and outcome at last follow up were collected. *Results*. Total 389 MM patients were enrolled, with median age of 71 years. Seventy-one percents of patients were male and more than 70% were older than 65 years. At diagnosis, patients had disease at Durie-Salmon (DS) stage III and ISS stage III were 72.7% and 56.2%, respectively; and those with serum creatinine Cr ☒2.0 mg/dL at diagnosis was noted in 34%. Comparing with those diagnosed in the first calendar period 1996-2001, patients of the second calendar period 2002-2007 were older and more have had received novel agents, especially for thalidomide.

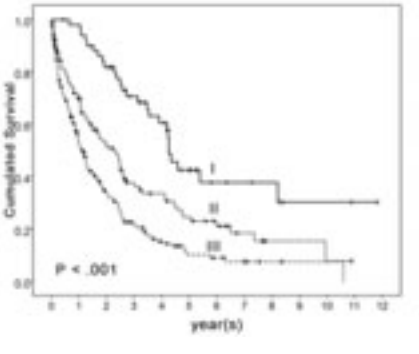

Figure 1. Survival curve of 351 MM patients by ISS.

The median overall survival was 20.5 months, with a significant increase in the second calendar period (15.3 and 28.2 months, respectively; P=0.002). In the Cox proportion model using those factors at diagnosis (except for DS and ISS themselves, and calendar period, those factors including elevated serum β2 microglobulin at diagnosis (>3.5), old age (> 65 years), and impaired renal function were independently associated with a poor survival. For the whole period, the ISS is better than DS in predicting the prognosis. The prognosis of MM patients in stage I and II of the second calendar period is significantly better than those of first period; however, the difference is not significant for stage III.

Conclusions. The findings of our study is the first to show the applicability of ISS in Chinese patients with MM, especially for those who have had received thalidomide.

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EFFECTIVENESS AND SAFETY OF LENALIDOMIDE IN PATIENTS DIAGNOSED OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA WITH EXTRAMEDULLARY DISEASE

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Background. Secondary extramedullary plasmacytoma (EP) is an aggressive form of plasma cell disorders characterized by tumour masses of malignant plasma cells outside of the bone marrow in patients diagnosed of multiple myeloma (MM). Different therapeutical approaches have been reported with poor outcomes. Our study reports the role of lenalidomide for EP in daily clinical practice. We analyzed the clinical data of 18 Spanish patients (pts) with refractory or relapsed multiple myeloma with EP treated with lenalidomide outside of clinical trials in 12 GEM/PETHEMA centers between October 2007 and July 2010. The treatment consisted on lenalidomide ranging from 10 to 25 mg, given on days 1-21 of a 28-days cycles, combined with dexamethasone. The response rate was evaluated according to the international criteria and the response of EP by measuring size changes by physical examination and/or radiological imaging. The median observation time was 12 months (3 - 24). Safety data were evaluated according to the National Cancer Institute Common Toxicity Criteria for Adverse Events v.3. *Results*. Eighteen unselected patients (eight females, median age 68 years) were analyzed. A median of 3 previous lines of therapy (1 - 6) were given, including autologous stem cell transplantation (4/18), and novel agents such as bortezomib (18/18) and thalidomide (3/18). Median number of lenalidomide cycles administered was 7 (3 - 21) with a maximum response after a median of 3 cycles (2 - 10). The overall response rate (ORR) ORR of MM was 61.1%, (complete response (CR)CR 16.6%, very good partial response (VGPR)VGPR 22.2%). EP disappeared in 8/18; EP size decreased in 3/18. The progression free survival (PFS) and overall survival (OS) PFS and OS were 9.8 and 14.6 months, respectively. To date, 3/11 responding patients relapsed, and 10/11 patients are alive. Lenalidomide toxicity was predominantly hematologic (8/18) and; the incidence of venous thrombotic events was low (1/18). *Conclusions*. Our results suggest lenalidomide could be an effective and manageable drug for patients with advanced myeloma with EP. A randomised trial is needed to assess the role of lenalidomide compared with other treatment options for secondary EP.

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SURVIVAL ACCORDING TO END-ORGAN DAMAGE PATTERNS IN SYMPTOMATIC MULTIPLE MYELOMA

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Aims. This retrospective analysis was performed to evaluate the differences of survival according to end-organ damage (CRAB) patterns in symptomatic multiple myeloma patients. *Methods*. Between September 1995 and December 2009, 400 consecutive patients of symptomatic multiple myeloma were treated in the Asan Medical Center, Seoul, Korea. We retrospectively analyzed the results of these patients. The Cox proportional hazards model was used to assess prognostic significance of end-organ damage (CRAB) patterns adjusted for age, sex, performance status, autologous stem cell transplantation (ASCT), international staging system (ISS) stage and the use of novel agents. *Results*. The median overall survival was 30.0 months (95% CI 24.4-35.6) with a median follow-up period of 23.6 months (range, 0.2 - 184.8). The median age was 61 years (range 20-85). Male patients consisted of 55% (222/400). Calcium level increase (C, serum calcium >11.5 mg/dL) was present in 10% (40/389), renal insufficiency (R, serum creatinine > 2mg/dL) in 24.5% (98/390), anemia (A, hemoglobin 2 g/dL below the lower limit of normal or hemoglobin <10 g/dL) in 66% (264/392), and bone lesions (B, lytic lesions or osteoporosis with compression fractures) in 82% (328/395). ASCT was performed in 37.8% of patients (151/400) and 36.5% received novel agents at some time during treatment. In univariate analysis, ROTI patterns of R (C-R+A-B-), A (C-R-A+B-), RA (C-R+A+B-), RB (C-R+A-B+), AB (C-R-A+B+) and RAB (C-R+A+B) were associated with shorter overall survival (OS) (p<0.01). In multivariate analysis, only R, RA and RB patterns were prognostic for OS with marginal statistical significance (p=0.073, 0.082 and 0.090, respectively). However, R and RA patterns were significantly associated with poor prognosis in those who did not undergo ASCT (Hazard ratio 1.507, p=0.038; HR 1.694, p=0.016, respectively, Table 1).

Table1. Cox proportional hazards model for CRAB patterns in patients who did not undergo ASCT

Variable	Hazard Ratio	95% CI	P value
C	1.123	0.658-1.917	0.671
R	1.507	1.024-2.218	0.038
A	1.391	0.960-2.016	0.081
B	1.164	0.728-1.860	0.526
RA	1.694	1.101-2.606	0.016
RB	1.410	0.947-2.098	0.090
RAB	1.461	0.935-2.281	0.096

*Adjusted for age, sex, performance status, stage by international staging system and the use of novel agents at some time during treatment
*Other patterns are not shown here.

Conclusions. Patterns of end-organ damage may be prognostic in symptomatic multiple myeloma patients. Renal insufficiency in particular seemed to correlate with poor prognosis and it was more evident in those who did not undergo ASCT. In contrast to renal insufficiency, hypercalcemia or bone lesions were not related to OS.

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EMERGENCE OF OLIGOCLONAL BANDING IN PATIENTS WITH MULTIPLE MYELOMA AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background. The emergence of oligoclonal banding in immunofixation of patients with Multiple Myeloma (MM) after autologous stem cell transplantation (ASCT) is a well-recognized feature of humoral response. This oligoclonal pattern appears to reflect an indirect sign of immune reconstitution and a robust humoral response with an uncertain clinical meaning. *Aims*. To address the clinical meaning of the oligoclonal banding presence in MM patients after ASCT. Prevalence of oligoclonal humoral response (OHR), progression-free survival (PFS) and time to relapse (TTR) were considered as outcomes. Methods: MM patients who underwent ASCT in our Hospital, from 2000 to 2009, were retrospectively analyzed. PFS and TTR were assessed using the International Myeloma Working Group criteria. An oligoclonal humoral response was defined by a serum immunofixation (IFE) different either in heavy and/or light chain component from the original monoclonal protein resulting in multiple banding providing an typical oligoclonal serum IFE pattern. Serum analysis for immunoglobulins and other biochemical markers was performed. Results were expressed as median (range). Results: Sixty five patients (31 female, median age 56yr, median follow-up 12 months) were studied. The 3-year survival was 58.5% (95% CI, 51.9-65.1%) and 36 patients had relapsed. Thirty-five (53.8%) developed an oligoclonal humoral response after ASCT, 3 episodes of OHR was observed in 2 patients and 2 episodes of OHR in 7. Oligoclonal

humoral response was observed for 7 (1 - 37) months and in some cases it had persisted beyond relapse (5 patients). This response occurred with a 4 months increase in patient's survival. Interestingly, relapse occurred simultaneously with oligoclonal banding disappearance in 4 patients. Conclusion: Our results are consistent with the hypothesis that disappearance of oligoclonal pattern is a hallmark of an early relapse and may be used as a surrogate marker of response in patients with MM after ASCT.

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AN AUDIT OF REVLIMID (LENALIDOMIDE) USAGE, MONITORING AND TOXICITY IN MYELOMA IN A SINGLE UK HEMATOLOGY CENTRE

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Background. Myeloma has a median overall survival of 3-5 years from diagnosis. New drug therapies, such as Revlimid are now available with results from clinical trials (MM-009/MM-010) indicating a significant improvement in PFS and OS compared to conventional therapy. Following NICE guidance in June 2009, Revlimid has been used in relapsed myeloma patients after two or more lines of previous therapy. Regional East Midland (EM) Guidelines have also been written. Data on effectiveness and toxicity of Revlimid is limited outside of clinical trials and this study looks at usage at Nottingham University Hospitals (NUH) NHS trust, a large UK Haematology centre, which has treated the highest number of relapsed myeloma patients with Revlimid in the UK to date. Aims. To audit the use, response rates and toxicity of Revlimid in NUH against NICE guidance and EM guidelines. Methods. All patients initiated on Revlimid prior to September 2010 at NUH were audited using criteria defined by NICE(TA171) and EM guidelines. Case notes and online records were used to obtain information. Audit criteria included the indication for initiation of therapy, monitoring of response and continuation of therapy, frequency and management of grade III and IV haematological and non haematological toxicities (as per NIH CTC), initial dosing and modification, management of disease progression and all cause mortality. *Results*. The records of 78 patients, 37 female and 41 male, were audited. No females were of child-bearing potential. Median age of patients starting Revlimid was 70.5 years (range 42-87). All patients received Revlimid within its licensed indication. 76/78(97%) received it within NICE criteria. Median number of previous therapies was 3 (range 1-10) with 44/78 (56.4%) patients having had previous ASCT. Median number of cycles of Revlimid received was 7 (range 1-20). 47/78 (60.3%) patients received at least 6 cycles of treatment with 19/78 (24.3%) still on treatment after 12 cycles. 31/78 (39.7%) stopped prior to 6 cycles; 11 due to progressive disease and 14 due to toxicity. Dose was modified in 39/78 (50%) patients; 16 reduced due to declining renal function and 19 reduced due to haematological toxicity. Maximal response was achieved at a median of 3 cycles (range 1-10) and was CR in 8/78(10.3%), VGPR in 6/78 (7.7%) ,PR in 36/78 (46.2%) and SD in 19 (24.4%). 32/78 (42.3%) suffered grade III or IV haematological toxicity; 25 anaemia, 18 thrombocytopenia and 14 neutropenia. 31/78 (39.7%) suffered non haematological toxicity; including 6 with venous thromboembolism and 6 with worsening neuropathy. 47/78(60.3%) had a hospital admission during Revlimid therapy, most commonly with infection (23/47). 28/78 (36%) patients died: 19(67.9%) due to progressive disease and 7(25%) to Revlimid related toxicity including neutropenic sepsis and acute kidney injury. Summary/Conclusions. This single centre audit supports existing evidence that Revlimid is an effective treatment for relapsed myeloma, having maximal effect early in the treatment course. Its toxicity profile should not be underestimated, and its use requires careful monitoring and review.

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LENALIDOMIDE IN COMBINATION WITH LIPOSOMAL DOXORUBICIN AND LOW DOSE DEXAMETHASONE (RDD) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS

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Background. Despite advances in multiple myeloma (MM) therapy, the optimal treatment for relapsed/refractory MM patients has not been determined. Lenalidomide is approved, as single agent or in combination with dexamethazone, for treatment of myeloma relapsed patients. Previous trials have highlighted the activity of Liposomal Doxorubicin in this setting of patients. *Aims*. The aim of the study is to evaluate the efficacy and the safety of Lenalidomide in combination with Liposomal Doxorubicin and low dose dexamethasone (RDd) in relapsed/refractory multiple myeloma patients. *Methods*. Between June 2008 and November 2010, 17 relapsed/refractory myeloma patients were enrolled. Fifteen patients (9 male and 6 female; median age 72 years with range 62-83) are evaluable for response and toxicity. During treatment induction phase, patients received oral Lenalidomide 25 mg/die, on days 1 through 21, intravenous liposomal doxorubicin 30mg/mq on day 1 of the cycle, and dexamethazone 20 mg/die, on days 1, 8, 15, 22 of a 28 days cycle for 6 cycles. Subcutaneous low-molecular weight heparin has been used for thromboprophylaxis. Transfusion support, neutrophil and erythropoietic growth factors were allowed. The patients in complete and partial response (IMWG criteria) after induction phase, received 3 other cycles of RDd as consolidation. All responsive patients received low maintenance dose of Lenalidomide 10 mg/die on days 1 through 21 every 35 days until progression or unacceptable toxicity. If not reached partial response at least, the patients got out of study. Results: At the enrollment, 10 patients (67%) previously received two line therapies and 6 of these (40%) had been relapsed after autologous Stem Cell Transplantation. Six patients (40%) completed the induction and consolidation phase and are still in maintenance treatment; 5 patients (33%) completed only induction phase. Four patients are ongoing and not evaluable for response. At the end of the induction phase 8/11 patients were in VGPR (IMWG criteria), 1 in stable disease and 2 not response. All patients who finished 9 cycles are currently in VGPR (6 patients). With a median follow up of 15 months (6-18 months), 9 patients obtained VGPR, 1 SD, 2 died (1 for progression and 1 for transplant related mortality) and 3 patients are not evaluable. About toxicity, the most common adverse events were: neutropenia II/III grade in 9 patients (60%), anaemia and thrombocytopenia II/IV grade in 4 patients (26%). Thirteen patients (87%) needed G-CSF, 4 patients (26%) needed blood and platelet transfusion. Extra-hematological toxicity was: neuropathy I/II grade in 6 patients (40%), 2 case of FUO (fever of undetermined origin). One patient had deep venous thrombosis treated with therapeutic dose of subcutaneous low-molecular weight heparin. *Conclusions*. This preliminary experience suggests that Lenalidomide in combination with Liposomal Doxorubicin, and low dose of dexamethasone (RDd) seems to be efficacy and safety in relapsed/refractory myeloma patients.

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SEQUENTIAL THERAPY WITH VALD, BORTEZOMIB AND CYCLOPHOSPHAMIDE AND HIGH DOSE MELPHALAN AS INDUCTION THERAPY FOR MULTIPLE MYELOMA, FOLLOWED BY MAINTENANCE THERAPY WITH BORTEZOMIB AND INTERFERON

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Background. High-dose Melphalan and autologous stem cell transplantation (ASCT) is the standard therapy for Multiple Myeloma (MM) patients younger than 65 years even allogeneic Hemopoietic Stem Cells (HSC) donor is available. In multivariate analysis the Complete Remission (CR) before high-dose therapy is a significant independent variable for Overall Survival (OS) and Time To Progression (TTP). The advantages of a maintenance therapy and association with new drugs in a multi-step therapy is still under investigation. *Aims*. We tested the efficacy and toxicity of sequential therapy with Vincristine, Pegylated Liposomal Doxorubicin and Dexamethasone (VALD) followed by Bortezomib and Cyclophosphamide to obtain a CR before high dose of Melphalan and ASCT. The role of association Bortezomib and α-2-Interferon (α-2-IFN) as maintenance therapy was also investigated. Methods From September 2006 to June 2010 we treated 19 consecutive pts (6 M, 13 F) with new diagnosed symptomatic MM. Median age was 53 years (34-64). Thirteen pts had measurable M-protein: 8 presented IgG type and 5 IgA type. Two pts had non secretory and 4 micromolecular disease. International Staging System (ISS) score was 1 in 15 pts, 2 in 3 pts and 3 in 1 pt. All pts presented bone marrow plasmocytosis > 30% and 8/19 pts an extensive bone disease. Patients received every 21 days VALD regimen (V 2 mg, AL 40 mg/m2 at day 1 and Dexamethasone 40 mg day 1-4) for 3 cycles and 3 courses of Bortezomib (1.3 mg /m2 on days 1,4,8,11) followed by Cyclophosphamide 4g/m2 and G-CSF for HCS harvesting. On average 45 days later pts underwent to ASCT after infusion of Melphalan 200 mg/ m2 as conditioning regimen. The maintenance therapy with Bortezomib (1.3 mg/m2 every 21 days) was asso-

ciated to α-2-IFN (1,5 megaU/twice a week) for two years. α-2-IFN alone was administered at the same dosage until disease progression. Results All patients achieved Partial Remission (PR) after VALD scheme, 6/19 pts and 8/19 pts obtained a CR and a Very Good Partial Remission (VGPR) respectively after Bortezomib therapy with Overall Response Rate 73.7%. Three pts with a PR and 1 pt in early relapse after Bortezomib were excluded from the study. After Cyclophosphamide fourteen patients mobilized HCS and they harvested the CD34+ preset target (10x106/kg). Toxicity consisted in: 15 % WHO grade I-II neutropenia and 20% WHO grade I-II peripheral neuropathy. Two pts died for pneumonia, the first one after 2nd VALD cycle and the second one during autologous transplantation (pts showed ISS score 2 and 3, respectively). All 13 pts submitted to ASCT obtained a CR and 11 of them started maintenance therapy. After 33 months (range 7-46) of median follow-up 7/11 pts (63.6%) are in continuous CR. Conclusion The sequential therapy with VALD, Bortezomib and Cyclophosphamide, followed by high dose of Melphalan and maintenance therapy with Bortezomib-α-2-IFN is effective and low toxic as up-front therapy in MM and could be experienced in a larger number of patients.

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SIMILAR TOXICITY AND LOWER EFFICACY OF ALTERNATING WITH RESPECT TO CONTINUOS LOW-DOSE THALIDOMIDE FOR RELAPSED OR REFRACTORY MULTIPLE MYELOMA PATIENTS

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Background. Thalidomide is still a valid therapy for relapsed/refractory multiple myeloma (MM) patients. Prolonged exposure represents a risk factor for peripheral neuropathy (PN) occurrence. Aims. This study explores the feasibility of an alternating with respect to the standard continuous low-dose thalidomide scheme. Methods. Twenty-three MM pts relapsed refractory after at least one previous line of therapy were consecutively enrolled in a randomized multicenter two-arm trial. The study was performed according to the Declaration of Helsinki and approved by the ethics committee of each participating institution. Patients with baseline grade ? 2 NCI PN were not eligible. Pts received Thalidomide 100 mg on days 1-28 of each 28 day cycle in arm A (continuous treatment) and Thalidomide 100 mg on days 1-21 of each 28-day cycle in Arm B (alternating treatment). Oral Dexamethasone (20 mg on days 1-4; 14-18 of each cycle) was added to both treatment arms for the first six cycles then stopped if registering at least a partial response (PR). Thalidomide was given in both arms until progression or significant toxicity occurred. Additional neurologic assessment was performed with clinical neuropathy scores and nerve conduction studies at baseline, every three months for the first 2 years and then every six months until neurotoxicity resolution or end of follow-up for any cause. Results. Ten patients were randomized in arm A, thirteen in arm B. Patients in both arms had similar baseline characteristics. Twenty pts (86%) were in relapse after one previous chemotherapy. One patient had baseline grade 1 PN. The median follow up was 42 months (range 3-49 months). Twenty-two out of 23 pts were evaluable for response (there was one early-death for myocardial infarction). ORR was 77% (CR+VGPR 18%, PR 59%). Median time to best response: 5,7 months. OS from therapy starting was 44 months with 10 pts alive (43%). Sixteen pts progressed (73%), median PFS was 24.8 months. Arm A correlated to better responses and outcome. All patients in arm A were responsive (100%) with respect to 58% in arm B (p=0,03). In arm A there were 1 CR (10%), 2 VGPR (20%), 7 PR (70%), in arm B there were 1 CR (8%), 6 PR (50%), 5 NR (42%). Median PFS was 42 months in Arm A vs 7 months in B (p=0,02). Median OS was not jet reached in arm A compared to 24 months in Arm B. Seventeen pts (74%) developed PN: grade 3 PN was registered in 2 pts (12%), grade 2 PN in 7 pts (39%), grade 1 PN in 8 pts (49%). Median time to PN occurrence was 7,5 months. PN after thalidomide discontinuation was unchanged in 14 pts (82%) and slightly improved in 3 pts (18%). Incidence of PN (90% in arm A vs 62% in arm B, p=0,15) and time to PN occurrence (7,7 in arm A vs 5,6 months in arm B, p=0.3) were not statistically different in the two arms. Conclusions. Continuous low-dose thalidomide seems to give better results with a similar neurological toxicity to the alternating schedule.

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FIVE YEAR EXPERIENCE OF BORTEZOMIB FOR RELAPSED MYELOMA IN A REGIONAL CANCER NETWORK - THE ADDITION OF CYCLOPHOSPHAMIDE TO BORTEZOMIB IMPROVES RESPONSE RATE BUT NOT TIME TO NEXT TREATMENT

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Background. The novel agent bortezomib has become a standard of care for relapsed myeloma within the UK. This followed National Institute of Clinical Excellence (NICE) guidance in 2007. However it is not certain whether bortezomib's effect is enhanced when given in combination with cyclophosphamide. *Aims*. We aimed to retrospectively determine the effect of bortezomib at relapse when given within a regional cancer network. At clinician's discretion, a cohort of patients also received cyclophosphamide in combination with bortezomib-steroid. In addition to determining response rate and time to next treatment, we also sought to establish the frequency of drug related side effects which limited treatment delivery. Methods. We audited the outcome of 118 myeloma patients identified chronologically who received treatment with bortezomib at first or subsequent relapse in the Peninsula Cancer Network, UK, between April 2005 and July 2010. Hospital records, chemotherapy prescriptions, and pathology systems were retrospectively analysed to provide audit data. Results. Median age at diagnosis was 61.6 (range 41.2-88.4), paraprotein class IgG (51%), IgA (30%), IgD (1%), light chain disease (18%). The median time from diagnosis to first receiving chemotherapy was 0.4 months (range 0-110.4). 39% of patients (n=46) received a melphalan autograft after initial chemotherapy. The median time from diagnosis to receiving bortezomib was 31.3 months (range 1.5-143.2). 68% received bortezomib at first relapse, 17% at second relapse, and 15% at third or subsequent relapse. 65.3% (n=77) received bortezomib alone or in combination with prednisolone or dexamethasone. 34.7% (n=41) received cyclophosphamide in addition to bortezomib-steroid at clinician discretion. Both groups received a median of 4 bortezomib containing treatment cycles (range 1-8). The overall response rate (ORR=CR+PR) was 56.1% (n=24) in patients who received cyclophosphamide-bortezomib-steroid compared to 39% (n=29) in the non-cyclophosphamide cohort (p=0.03) - CR 12.2%, PR 43.9% versus CR 10.4% and PR 31.2% in the cyclophosphamide vs no cyclophosphamide group respectively. Fewer patients receiving bortezomib with cyclophosphamide (2.4% versus 11.7%) ceased treatment because of loss of initial treatment response, although this did not reach statistical significance (p=0.16). There was no statistically significant difference in the median time to next treatment: 11.1 months for the bortezomib-steroid group, compared to 9.9 months for those receiving additional cyclophosphamide (p=0.502, Mantel-Cox test). 33.9% of all patients stopped treatment prematurely due to side-effects, mainly peripheral neuropathy (18.6% of all patients), indicating the difficulty in tolerating the regime, even if a response is occurring. The rates of treatment limiting neuropathy were similar in those receiving cyclophosphamide (17.1%) compared to bortezomib alone (20.8%). Six patients (5%) died whilst receiving treatment. *Summary/Conclusions*. In this retrospective audit, although the addition of cyclophosphamide to bortezomib produced a higher ORR, it did not result in an increase in the time to next treatment. Arguably the strategy of choosing myeloma treatments by best ORR also needs to consider the duration of the response. This study also demonstrates that more than one-third of patients needed to stop bortezomib therapy prematurely due to side effects, mainly neuropathy - highlighting the difficulties of bortezomib delivery in a *real world* clinical setting.

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COMBINATION OF BORTEZOMIB, MELPHALAN AND PREDNISONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background. Because of the incurable nature of multiple myeloma (MM), patients will inevitably experience relapse after initial therapy and require further treatment. The latest studies show that bortezomib plus melphalan-prednisone (VMP) is an effective therapy as first-line treatment in patients with MM ineligible for stem cell transplantation. Aim: To assess the efficacy and safety of VMP regimen in patients with MM previously treated with ≥ 1 lines of treatment. Patients and methods: 32 patients with relapsed/refractory MM received nine 6-week cycles of VMP: bortezomib (1.3mg/m^2 days 1,4,8,11,22, 25, 29, 32, cycles 1-4; and days 1, 8, 22, 29, cycles 5-9) plus melphalan (9mg/m2) and prednisone (60 mg/m^2) days 1 to 4, cycles 1-9. Response rates were measured using IMWG criteria and toxicities were assessed by CTCAE, v3.0. Results: Median age was 70 years (range 42-83) and included 15 male (47%). Disease characteristics at the time of diagnosis were: ISS I 39%, II 26%, III 35%, ECOG ≥ 3 in 17% and 21% of patients presented impaired renal function. Median number of prior lines therapies were 2 (range1-6).Previous schedules received were: conventional poliquimiotherapy 45%, alquilant agents 45%, bortezomib 48%, IMIDs-containing regimens 33% and ASCT 12%. With a median follow-up of 6 months (1-35) response rates could be evaluated in 20 patients (63%) after a median of 3.5 cycles (1-9) received. Overall response rate was 70% (20% CR, 5% nCR, 15% VGPR and 30% PR). OS was 87.5%. Most frequent grade ≥3 AEs included: anemia 16%, neutropenia 16%, thrombocytopenia 16%, peripheral neuropathy 16% and gastrointestinal symptoms 9%. 59% of patients required dose reduction of bortezomib, 18% and 9% of melphalan and prednisone, respectively. Ten patients (31%) discontinued treatment, 4 because adverse events. Summary: Our results confirms the tolerability and good responses possible with VMP schedule in previously treated MM patients and could be considered as alternative therapy in relapsed/refractory MM patients.

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THE EFFICACY AND SAFETY OF BORTEZOMIB,MELPLALAN PLUS PREDNISONA IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS NO ELIGIBLE FOR TRANSPLANTATION

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Background. Bortezomib plus melphalan and prednisone (VMP) is significantly better than melphalan plus prednisone alone for elderly patients with untreated multiple myeloma (MM).Our study reports the efficacy and safety of VMP schedule as first-line treatment in MM patients not eligible for high-dose therapy, in daily clinical practice. *Patients and Methods*. Between July 2007 and November 2010,we analyzed 62 patients treated with nine 6-week cycles of VMP: bortezomib (1.3 mg/m2 days 1, 4, 8, 11, 22, 25, 29 and 32, cycles 1-4 and days 1, 8, 22 and 29, cycles 5-9), with melphalan (9mg/m2) and prednisone (60mg/m2) days 1-4, cycles 1-9. Response was evaluated using the EBMT criteria. Toxicity was graded according to the NCI CTCAE v3.0. Results: Median age was 71 years (range 59-90) with 25 males (40%). After a median follow-up of 9.5 months (1-24), response was assessed in 48 patients (77%). Median cycles received was 4.25 (range 1-9). Overall response rate was 83 % (31% CR, 52% PR) with 2% MR, 8% SD and 6% progression. The incidence of grade 3-4 adverse events was: neutropenia 12%, anemia 7%, thrombocytopenia 7% and 10% gastrointestinal symptoms. Peripheral neuropathy was reported in 45% of patients, 13% grade 3-4. Seven (11%) patients had VZV infection. 39% required dose adjustment of bortezomib, 24% of melphalan and 7% of prednisone. Treatment was discontinued in 22 patients (36%), due to toxicity in 14. Overall survival was 87%. Conclusions: VMP is an effective and well tolerated treatment option for patients with newly diagnosed MM who are not candidates for intensive therapy. Our results are similar to those described in previous studies and requiring a longer follow-up for confirmation.

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ATYPICAL CASE OF POEMS SYNDORME WITH A GOOD RESPONSE TO LENALIDOMIDE - DEXAMETHAZONE

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Poems syndrome is a rare disease characterized by peripheral neuropathy, skin lesions, organomegaly, endocrinopathy and monoclonal gammopathy. No effective treatment is available. We describe here a case of an atypical presentation of POEMS syndrome with response to lenalidomide. A 29 years old man was referred to our department due to anemia. Clinical examination revealed pale skin and maxilar lymph nodes in both maxilas, splenomegaly and ascites. Laboratory exams revealed an anemia, hypochromic microcytic, increase ESR, low iron levels and low TIBC levels with normal levels of ferritin and hypothyroidism. Lymph node biopsy revealed Castlleman's disease. The patient was treated with erythropoietin and methylprednizolone. Two months later peripheral neuropathy of left upper arm was occurred. The patient was admitted to the university hospital for evaluation. Detailed evaluation revealed, monoclonal gammopathy, and skin rash, while splenomegaly persisted despite the treatment with steroids. The patient was initially treated with 6 cycles of rituximab without any improvement. The patient was subsequent treated with Lenalidomide (25 mgr/2nd day) and Dexamathazone 40 mgr every week. A significant response was occurred after 2 months of treatment. Correction of anemia with normalization of red cell parameters, disappearance of monoclonal gammopathy, resolve of skin rash and decrease of splenomegaly was occurred . The patient continue the treatment and after 6 months of treatment is in a very good clinical situation. Conclusions : Poems syndrome is a rare disease with no specific treatment. Increased levels of VEGF and neoangiogenesis has been proposed as the pathogenetic mechanism. Different kinds of treatments (steroids, melphalan, monoclonal antibodies (rituximab)) have been used without success. Lenalidomide plus dexamethazone may be an potential treatment acting against VEGF, as we can propose, according to the results observed to our patient.

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THE OPG/RANKL SYSTEM IN MYELOMA BONE DISEASE

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Aims. The aim of this study was to asses the implications of serum levels of OPG (osteoprotegerin) and sRANKL (soluble receptor activator of nuclear factor-κappa B ligand) in the process of bone remodeling in myeloma bone disease. Methods. The study was performed one group of patients with myeloma bone disease (n=9) compared with a control group (n=8, healthy patients). The serum levels of the bone markers were quantified by using ELISA (enzyme-linked immunosorbent assay) method. Results: In group with myeloma bone disease the serum levels of OPG were 32.55±2.65pg/ml ($p<0.001$), those of sRANKL were 47.53±4.25pg/ml ($p<0.001$), and OPG/sRANKL ratio were 0.684±0.061 ($p<0.002$), in comparing the control group: OPG were 42.15±3.19pg/ml, those sRANKL were 35.18±2.35pg/ml, and OPG/sRANKL ratio has the average of 1.198±0.134. Conclusions: The serum levels of OPG in myeloma bone disease decreased, secondary to the stimulation of osteoblastic apoptosis. These high levels of sRANKL certify the activation of osteoclasts with secondary increased bone resorption in myeloma bone disease. In this study we demonstrated a significant reduction of OPG/sRANKL ratio in group with myeloma bone disease, favoring osteolisis appearance, in comparison with the control group.

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BORTEZOMIB, ADRIAMYCIN AND DEXAMETHASONE IS SUPERIOR TO VAD-TYPE CHEMOTHERAPY: THE USE OF A NOVEL STATISTICAL METHODOLOGY IN THE PAD IRELAND TRIAL

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Background. Bortezomib with adriamycin and dexamethasone (PAD) is a highly effective induction combination with response rates of up to 95% (Oakervee et al, BJH 2005; 129:755-62). In this study patients who had received VAD/VAD-like regimen acted as their own controls and were given further treatment using the PAD regimen and compared using appropriate statistical methods. *Aims*. To test the efficacy of bortezomib, adriamycin and dexamethasone combination therapy utilising a novel statistical analysis incorporating the patients own initial response to VAD as an internal control. *Methods*. This was a Phase 2 study with 3 cohorts of 23 patients. Cohort 1 was patients treated with VAD and auto transplanted, cohort 2 similar patients but not transplanted and cohort 3 was patients refractory to VAD who proceeded directly to PAD without any intervening chemotherapy. The paraprotein level at the start of each type of treatment (VAD or PAD) was used for estimation of response. *Results*. Using EBMT criteria with addition of VGPR, 7 patients in cohort 1 achieved CR after PAD compared to one patient achieving CR after VAD. Using the exact McNemar significant probability comparison of all responses gave p=0.0078 and using the Wilcoxon Signed Rank Test to compare reductions in paraprotein (or Bence-Jones protein) p=0.093 was achieved. A combined analysis of group 1 and 2 together gave similar results. In cohort 3 similar comparisons gave an exact McNemar significance of p=0.0005 and the Wilcoxon Signed Rank test p=0.002 in favour of the PAD therapy. Data on overall survival and toxicities will be presented. *Conclusions*. PAD was demonstrated to be significantly superior to VAD particularly in the refractory group. This type of study is an alternative to large phase 3 studies.

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RELATION BETWEEN SERUM FREE LIGHT CHAIN RATIO (FLC) AND SERUM MONOCLONAL PROTEIN CONCENTRATION IN PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background. Serum FLC ratio has been identified as an independent risk factor for progression of monoclonal gammopathy of undetermined significance (MGUS) as reported in one retrospective study published by Rajkumar (Rajkumar et al., Blood 2005), where serum free light chains (FLC) ratio was abnormal in 33% of MGUS. *Aims*. To describe the frequency of abnormal serum free light chains measurement in a Canarian cohort of patients with MGUS and to correlate the results with the classic biological characteristics. *Methods*. Data from 22 patients with MGUS were analysed. FLC were measured during follow up consultation between January 2001 and December 2010. Demographic and laboratory data were compared using chi square tests for nominal variables and Kruskal-Wallis test for ordinal variables Results: Patients were 10 men and 12 women. Median age was 66 years (27 - 91). Isotype was: IgG in 16 patients (72%), IgA in 3 (14%), IgM in 2 (9%) and Light Chain in 1 (5%). Median level of monoclonal protein was 986 mg/dL (190-4850). Free-kappa light chain values ranged from 102 mg/dL to 847 mg/dL (median, 342 mg/dL), and free-lambda light chain ranged from 16 mg/dL to 563 mg/dL (168 mg/dL). Ten patients (45%) had elevated levels of k or l FLC. The median FLC ratio was 5.703 (r: 0.24-54.2). An abnormal FLC ratio was detected in 13 (57%) patients. The monoclonal protein was of the same isotype as the FLC in all patients. Patients with an abnormal ratio had higher monoclonal component concentration (median, 1306 mg/dL) than patients with normal FLC ratio (481 mg/dL) (p=0.011). There were no differences in the two groups about sex, age, hemogram, creatininemia, calcemia. According to the three factors risk models defined by Rajkumar (FLC ratio, size and type of monoclonal protein), 6 patients (27%) were classified in low risk group, 9 patients (41%) in low intermediate risk group, and 7 (32%) in high intermediate risk group. No patient was identified as belonging to high risk group. *Conclusions*. Our data report in this cohort that approximately a half of patients with MGUS have a normal serum FLC ratio and that approximately 70% of patients could be classified as low or low intermediate risk, with patients with an abnormal k/l ratio are associated with higher monoclonal component concentration Further prospective studies are necessary to confirm the predictive value of serum FLC ratio

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RETROSPECTIVE STUDY OF MONOCLONAL GAMMOPATHIES DETECTED IN THE CLINICAL LABORATORY IN A CANARIAN ISLAND: 9-YEAR SERIES

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Introduction: We studied the incidence, classification and isotype distribution of monoclonal gammopathies detected between 2001 and 2010 in the clinical laboratory of Hospital "Nuestra Señora de los Reyes", El Hierro Island, Canarias, with a population of approximately 9500 inhabitants. Aims: This retrospective analysis is to analyse the incidence of monoclonal gammopathies in our clinical population. Methods: Database results on routinely ordered serum protein electrophoresis (SPE), between 01/01 and 12/10, were analysed, looking for identification of pathological results. Age-specific prevalence rates for El Hierro island data were calculated by dividing the number of participants with MGUS in each age stratum by the number of participants in that stratum The age-adjusted incidences were standardized with respect to the WHO World Standard Population Distribution, based on the world average population between 2000 and 2025. The clinical diagnosis was recorded from the patient case history. Results: We examined the results of 2449 SPE registered during this period. M-protein with either SPE or IFE as well as sFLC results were detected in a total of 29/609 >18 years-old patients; all of them had been diagnosed within this period, representing a 2,76% prevalence in our population. The mean age-adjusted incidence of monoclonal gammopathy was 7,56 per 100,000 inhabitants/year, ranging from 2,31/100,000 in 2001 to 9,25/100,000 in 2009. The median patient age at diagnosis was 68 years (range 27-91 years), with males accounting for 41% of all cases of monoclonal gammopathy. A 76% of the patients were clinically defined as presenting monoclonal gammopathy of undetermined significance, and 24% presented multiple myeloma. The most frequent M-protein isotype was IgG (668%), followed by IgA (17%) and BJ protein (10%). Conclusion: Many of our community hospital patients are over 50 years of age and present with non-specific symptoms potentially related to disorders associated with an abnormal EEF requiring laboratory evaluation and in which the clinical haematological analysis should play an important role in the diagnosis of MGUS.

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COEXISTANCE OF MULTIPLE MYELOMA (MM) AND MYELOPROLIFERATIVE, LYMPHOPROLIFERATIVE OR AUTOIMMUNE DISORDERS; A SINGLE CENTER EXPERIENCE

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Background. The simultaneous occurrence of multiple myeloma (MM) and myeloproliferative (MPD), lymphoproliferative (LPD) or autoimmune disorders (AD) is extremely rare. *Aims*. To report the characteristics of patients presenting coexisting MM and MPD, LPD or AA and to discuss the possible biologic mechanisms involved. Methods: Medical files from 297 MM patients diagnosed and followed in our section from 1999 to present were reviewed. *Results*. 15 patients that presented another MPD, LPD or AD together with multiple myeloma, were identified. Among them, 9 were females and 6 were males. Their median age was 55 years (range 25-70). 5 of the studied females had presented the other disease (one chronic myeloid leukaemia [CML], one chronic lymphocytic leukaemia [CLL], one Hodgkin lymphoma [HL], one follicular lymphoma [FL], one essential thrombocytosis [ET], one systemic lupus erythematosus [SLE]) prior to MM; their MM was asymptomatic, of IgG type and preceded by a documented IgG-MGUS in 5 of them; the 6th patient had light chain (LC) MM accidentally diagnosed with the onset of acute renal failure. The remaining 3 females (all symptomatic and of IgG type) presented the second disease (two idiopathic thrombocytopenic purpura [ITP], one Erythroleukemia) after MM; the patient with

Erythroleukemia was in CR and had previously received VAD and lenalidomide. 4 out of 5 males patients had symptomatic MM. In one ET and gastric MALT lymphoma preceded IgA-MM and in another myelofibrosis also preceded IgA-MM. In the other patients both disorders presented concomitantly (one vasculitis and IgA-MM, one polycythemia Vera [PV], one autoimmune haemolytic anaemia [AAA] and LC MM, one acquired FX and VIII deficiency and IgA-MM). *Discussion/Conclusions.* The frequency of concomitant MM and MPD, LPD or AD was equally distributed (5% for each subgroup). In the majority of females patients a documented MGUS coexisted with an MPD or LPD and preceded MM onset. In MM patients presenting AAA, ITP or acquired FX and VIII deficiency, the paraprotein may have act as autoantibody. In the other patients with a preceding or simultaneous MPD or LPD, a molecular abnormality could have been the triggering factor for the appearance of the second in turn disease.

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THE RELEVANCE OF THE INTERNATIONAL STAGING SYSTEM IN MULTIPLE MYELOMA IN THE ERA OF NEW TREATMENT MODALITIES

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Background. The International Staging System (ISS) was established and validated, providing consistent risk distinction of multiple myeloma (MM) patients (pts). The aim of study was to analyze the relevance of ISS scor in the first relapse of MM patients treated with thalidomide or bortezomib based regimens. Patients and methods. The study included 53 MM pts (27 male/ 26 female, mean age 56 yrs, range 38-81) in the first relapse of myeloma. IgG myeloma was diagnosed in 38pts, IgA in 7pts, IgD in 1pts, light chains in 6pts and non-secretory in 1pts. According to the clinical stage (CS, Durie-Salmon), patients were distributed as follows: II 14pts; III 39pts. Regarding ISS score, the group included: ISS1 12pts; ISS2 14pts; ISS3 27pts. Renal impairment was present in 6pts. Thalidomide based regimens (Thal-Dex 13pts; CTD 7pts; TCED 5pts) were applied in the group of 25pts (mean no.6 cycles, range 2-8cycles), while 28 pts were treated with bortezomib based therapy (Vel-Dex 26pts; CVD 1pts; MPV 1pts, mean no.6 cycles, range 2-8 cycles). Results: In the group of pts treated with thalidomide based regimens, positive treatment response (CR+VGPR+PR+MR, EBMT criteria) was achieved in 18/25pts. According to the ISS score, there was no significant difference (Fisher test, p=0,378) in the treatment response between high-risk pts (ISS3: 13/25 pts) and myeloma pts of low- and intermediate risk (ISS1+ISS2: 12/25pts). High-risk pts had significantly shorter duration of progression-free interval (mean: ISS3 3,38m vs. ISS1+ISS2 26,8m, Mann-Whitney test p=0,021). Median overall survival for this group of pts was 81m (range 14-100m). In terms of the overall survival, no difference (Log-rank test p=0,175) was found between pts with ISS3 (median 62m, range 18-120m) and pts with ISS1 and ISS2 (median 48m, range 14-114m) treated with thalidomide based regimens. In the group of pts treated with bortezomib based therapy, treatment response (CR+VGPR+PR+MR, EBMT criteria) was achieved in 25/28pts. Still, there was no difference (Fisher test, p=0,5) in the treatment response between pts with ISS3 (13/28pts) and pts of low- and intermediate risk (ISS1+ISS2: 12/28pts). No difference was found in duration of the progression-free interval (mean: ISS3 15,17m vs. ISS1+ISS2 15,38m, Mann-Whitney test p=0,641). Median overall survival for pts treated with bortezomib based therapy was 72m (range 18-120m). Patients with ISS1 and ISS2 had significantly longer overall survival (Log-rank test, p=0,019) comparing to pts with ISS3 (ISS1+ISS2: median 84m, range 42-120m vs. ISS3: median 60m, range 18-96m). Conclusion: The ISS score, as a surrogate marker of MM activity, is of relevance for the relapsed myeloma patients in terms of "tailored-treatment"approach in the era of new treatment modalities. According to the ISS score, the notified influence of thalidomide- and bortezomib-based treatment on duration of progression-free interval and overall survival of relapsed myeloma patients indicates these agents as promising constituents of induction and maintenance therapy.

1341

BONE DENSITY CORRELATES WITH MICRO-CT TRABECULAR VOLUME CHANGES IN ROUTINE DIAGNOSTIC BONE MARROW SAMPLES IN MULTIPLE MYELOMA (MM) PATIENTS AFTER BORTEZOMIB EXPOSURE

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Bone disease is present at diagnosis in almost all patients with MM and decreased bone mineral density is also frequently observed. Available clinical data indicates that bortezomib has a positive impact on bone health through a bone anabolic effect. We prospectively analyzed sequential bone densities from 11 patients with MM (2 smoldering and 9 relapsed cases), treated with single agent bortezomib without use of biphisphonates. Dexascan was obtained at baseline and after treatment with a median time of bortezomib exposure of 6 months ranging from 2 to 12 months. We compared T-score changes at lumbar spine and at femoral neck with micro-ct analysis of bone marrow biopsies obtained at the time points of the radiological studies. With a median age of 63 years, 9 males and 2 females were enrolled. At baseline mean Lumbar Spine and femoral neck T-scores were -1, 08 and -1, 9 respectively. After bortezomib exposure the mean Lumbar Spine T-score was -0, 9 and the Femoral Neck T-score was -1, 5 with mean positive changes in lumbar T-score of 0.3 and at femoral neck of 0.57.Five patient's slides from routine diagnostic core biopsy samples were digitally scanned with the Aperio XT system (Vista CA USA). The entire hematopoietic area and bony trabeculae were identified using the genie pattern recognition algorithm and each compartment was quantified .The calculated trabecular area was then divided by total surface area to obtain trabecular volume (TV). The change in mean lumbar spine bone density (0.38), and the change in mean femoral neck bone density (1.1) were statistically significant (p=0.03, p=0.04 respectively) .At the same time the change in mean trabecular volume (23.8) was also found statistically significant (p=0.02 paired t-test). This pilot study has confirmed that patients treated with single agent bortezomib experience a significant increment in bone mineral density at lumbar spine and at femoral neck even after two months of therapy and for the first time we were able to report parallel statistical changes in TV by micro-ct analysis of routine diagnostic bone marrow biopsies.

1342

ALLELIC BURDEN OF JAK2 V617F MUTATION AND CLINICAL OUTCOMES IN MYELOPROLIFERATIVE NEOPLASMS

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Background. Myeloproliferative neoplasms (MPNs) are clonal haematopoietic stem cell neoplasms characterized by increased proliferation of the erythroid, megakaryocytic, and myeloid lineages. The discovery of JAK2 V617F mutation in 2005 had significantly advanced our understanding of the BCR-ABL negative MPNs. JAK2 V617F mutation is a point mutation in the pseudokinase domain of the Janus kinase 2 gene resulting in the substitution of valine for phenylalanine. To date, it is still unclear how a single mutation can give rise to the different clinical phenotypes seen in MPNs. It has been postulated that additional genetic factors such as the degree of allelic burden may be contributing to the difference in the clinical phenotypes. Aims. The main aim of this study was to investigate the relationship between the allelic burden of JAK2 V617F mutation and clinical outcomes in patients with polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF). The clinical outcomes of these patients were studied according to common haematological parameters and clinical characteristics. Methods. A retrospective study was performed on patients who were diagnosed with PV, ET and PMF. Demographic data and peripheral blood samples were collected for molecular analysis of the JAK2 V617F gene mutation. In addition, full blood count, erythropoietin and ferritin levels were measured. RNA was extracted from the peripheral blood samples and amplified using reverse transcription-polymerase chain reaction (RT-PCR) and direct DNA sequencing techniques. Clinical outcomes of the patients were critically evaluated. Results. 48 patients were recruited into the study: 27 PV, 19 ET and 2 PMF. Although RNA was successfully extracted from all blood samples, only 46 out of 48 gene sequencing chromatograms were eventually analysed as the remaining 2 patients with PV had very high background interference rendering them uninterpretable. JAK2 V617F mutation was found in 31 out of 46 patients (67.4%). Out of the 25 patients with PV, 22 patients (88%) were found to have the mutation, 14 (63.6%) were homozygous and 8 (36.4%) were heterozygous. Out of the 19 patients with ET, 7 patients (36.8%) were found to have the mutation, all of whom were heterozygous. Both patients with PMF had the mutation, 1 homozygous and 1 heterozygous. At diagnosis, homozygous PV patients were noted to have significantly higher leucocyte counts ($p<0.05$) and lower platelet

counts (p<0.05) as compared to their heterozygous and wild-type counterparts. On the other hand, heterozygous ET patients had significantly elevated (p<0.05) haematocrit levels as compared to their wild-type counterparts. JAK2 V617F positive patients had more incidence of splenomegaly and thrombosis although this was not directly related to the degree of allelic burden. However, homozygous patients with PV demonstrated a higher incidence of constitutional symptoms and required more treatment. Conclusions. Although the incidence of JAK2 V617F mutation in MPNs is similar to previous studies, there is a higher incidence of homozygosity in patients with PV in Malaysia which is associated with significantly higher leucocyte counts and lower platelet counts, higher incidence of constitutional symptoms and more requirement for treatment. Further research is required to elucidate this further.

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JAK2 MUTATIONAL SCREENING: HIGH RESOLUTION MELTING CURVE ANALYSIS OR SEQUENCING?

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Background. The V617F mutation in the JAK2 gene is found in 95% of the patients with Polycythemia Vera (PV) and about half the patients with Myelofibrosis (MF) and Essential Thrombocythemia (ET). Commonly used methods for the detection of the mutation includes allele specific PCR, ARMS and sequencing. More recently however, high resolution melting curve analysis is being used as a screening tool as it is *Aims*.The aim of the study was to use high resolution melting curve analysis to screen exon 14 of the JAK2 gene in a cohort of patients with myeloproliferative neoplasms using copy DNA as template. The presence of the mutation in both the myeloid and lymphoid lineage was also investigated. Methods: A cohort of 15 patients diagnosed with several MPN's according to WHO 2008 criteria was selected. Blood was collected and the two cell lineages were separated using density gradient centrifugation and magnetic bead selection. RNA was extracted from each cell type, converted to cDNA and used as template for high resolution melting curve analysis and sequencing. Results: The V617F mutation was identified in ten of the fifteen patients and the mutation was found in both cell types for each patient. Sequencing identified the mutation to be negative in five patients, heterozygous in nine of the patients and homozygous in one PV patient. The melting curve data did not correlate with these findings. *Conclusions*. In the study the V617F mutation was the only identified mutation in exon 14. The mutation was found in 83.3% of the PV patients, 60% of the ET patients and 40% of MF patients when sequencing was performed which correlates well with the current prevalence data of the mutation in literature. High resolution melting curves analysis was unsuccessful to determine the V617F mutation accurately.

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THE MONITORING OF AUTOIMMUNE PROCESS IN PATIENTS WITH ACUTE AND CHRONIC MYELOID LEUKEMIA

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The inadequate reaction of the immune system on autoantigens develops due to the breakdown of the mechanisms supporting immunologic tolerance, and can be observed in blood diseases. It has been established that in patients with acute myeloid leukemia, before treatment, that the maintenance of T-regulative CD 4+CD25+ -lymphocyties in peripheral blood has lowered an average of 3.5 times in comparison with control group (p<0.05), whereas levels of autoantibodies in double-stranded deoxyribonucleic acid (DNA) of class M (anti-dsDNA-IgM) and class G (anti-dsDNA-IgG) in blood serum has raised an average of 2.3 and 2.2 times in comparison with control parameters (p<0.05). Between the number of T-regulative lymphocyties and levels of anti-dsDNA-IgM and anti-dsDNA-IgG a strong return correlation interrelation has been found. K.Spirman's coefficient of rank correlation (r) was equal to -0.92 and -0.81 accordingly. In patients with chronic myeloid leukemia in acceleration phase before beginning treatment the number of T-regulative lymphocyties in the peripheral blood has lowered by 6.1 times (p<0,01), and levels of anti-dsDNA-IgM and anti-dsDNA-IgG in serum have raised an average of 3.5 times (p<0.05) and 5.3 times (p<0.01) compared to control parameters. These patients also revealed a strong return correlation between the quantity of T-regulative lymphocyties and levels of anti-dsDNA-IgM (r= -0.83) as well as anti-dsDNA-IgG (r=-0.92). Therefore, one of the probable factors promoting the breakdown of immunologic tolerance at acute myeloid leukemia and chronic myeloid leukemia, can be the significant reduction of the subpopulation of T-regulative lymphocyties.

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EFFECTOR AND IMMUNOREGULATORY CELL IN POLYCYTHEMIA VERA

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Background. Polycythemia vera (PV) is a myeloproliferative disorder of slow development, which may allow the development of an immune response. It is characterized in most cases (95%) by the presence of a mutation in the JAK2 gene, which constitutive activation leads to the proliferation of myeloid cells, especially red blood cells. Aims. We have focused our work on natural killer (NK) cells in since, on the one hand, NK cells have antitumor and antiviral properties, while, on the other hand, abnormalities of these cells, including a decrease in cytotoxic activity, have been described in the majority of haemopathies. Thus, we hypothesized that, in PV, NK cells share phenotypic or functional abnormalities that allow immune escape of the malignant clone. Methods after free and informed consent 16 patients before any cytotoxic treatment (15 of them sharing the the mutated JAK2 gene) and 25 healthy controls (matched for age and sex ratio) were studied for NK phenotype and function, together with analysis of the variois immune cell subpopulations. Results. While an equivalebt number of lymphocytes were numbered in control subjects and in patients with polycythemia vera, we observed in the latter an increased number of NK cells. Nonetheless, these NK cells displayed poor cytolytic activity, despite normal perforin and granzyme expression. Phenotypic analysis revelaed an increased expression od inhibitory receptors. Conclusions. We now have to explain the impaired cytolytic activities of NK cells by two main research axix; first, we will analyse the transcriptomic profile of normal versus polycythemia vera patients NK cells. Then, we will focus our attention on the transcriptional regulation of inhibitory and activating receptors in normal versus polycythemia vera patients NK.

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VITAMIN D 24-HYDROXYLAZE (CYP24A1) GENE IS UPREGULATED IN JAK2V617F POSITIVE ET AND PMF

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Background: Vitamin D is myeloid cell-differentiating and proliferation controlling molecule. One of the metabolizing enzyme for vitamin D synthesis and degradation is the mitochondrial enzyme D-24-hydroxylaze (CYP24A1) that maps to 20q13.2, below the telomeric end of common deleted region in MPN. Aim: We studied the expression of CYP24A1 in a cohort of MPN patients and associated this to their JAK2 V617F mutation status and hematological data. Methods: Fifty newly diagnosed, untreated patients with MPN (12 PV, 26 ET and 12 PMF) were taken into the study after obtaining informed consent. RNA was isolated from unfractionated bone marrow MNCs and after RT tested for expression of CYP24A1 (AB Hs00989014 Taqman Assay) in AB7300 Real-time PCR analyzer.

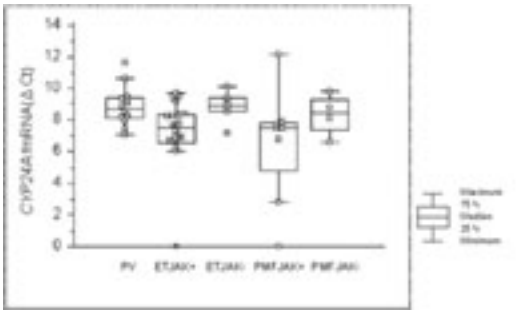

Figure 1. Post-hoc analysis of CYP24A1 expression

Data of relative expression of CYP24A1 were correlated to JAK2 mutation status and their core hematological parameters. Results: For the entire MPN group CYP 24A1 was not differently expressed concerning

the presence of JAK2V617F mutation: median ΔCT =7,823 for JAK2+ vs. 8,77 for JAK2- (p=0,10). However, when ET-JAK2+(67%ET) and PMF-JAK2+(66%PMF) were compared to their JAK2-mutation negative counterparts the expression was significantly higher in JAK2+ cases (7,48 vs 8,92; and 7,44 vs 8,42 respectively p< 0,05 (Figure 1). There was no correlation of CYP24A1 expression with patients' age, blood counts or ALP score. Conclusion: CYP24A1 gene for metabolizing the 1,25(OH)2D3, active form of vitamin D is upregulated in JAK2 mutated ET and PMF. Due to the important biological role of vitamin D in myeloid cell diferentiation and proliferation, this observation deserves further studies.

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JAK2 V617F MUTATION AND JAK2 46/1 HAPLOTYPE ANALYSIS IN A GROUP OF PATIENTS WITH ISCHEMIC STROKE. A PRELIMINARY REPORT

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Background. The JAK2 V617F mutation, the most common marker of the myeloproliferative neoplasms (MPN), has been reported to occur also in some patients with thrombotic events, including ischemic stroke, without any sign of an overt MPN. The JAK2 46/1 haplotype is a predisposing factor for the JAK2 V617F-positive MPN. *Aims*. To evaluate the frequency of the JAK2 V617F mutation in a group of patients with ischemic stroke, and the possible contribution of the JAK2 46/1 haplotype to the occurrence of the ischemic stroke. Methods. Ninety-five patients with ischemic stroke, without any overt MPN, and 150 individuals without ischemic stroke or MPN, were included in the study. The JAK2 V617F mutation was assessed in all the patients by a semiquantitative tetra-primer PCR assay. The rs10974944 (C/G) SNP, in which the G allele tags the JAK2 46/1 haplotype, was analyzed in all the patients and controls by a PCR-RFLP assay. *Results*. Only one patient (1%) was found to harbour the JAK2 V617F mutation; his mutant allele burden was less than 5%. The GG/CG genotypes of the JAK2 rs10974944 SNP had a similar distribution both in patients and controls (41% versus 45.3%, p-value >0.05). *Summary/conclusions*. Our results indicate that the JAK2 V617F is a rather rare finding in patients with ischemic stroke, without overt MPN. Also, the V617F-predisposing JAK2 46/1 haplotype, does not seem to have a significant contribution to the occurrence of the ischemic stroke.

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COMPARISSON OF TWO REAL TIME PCR METHODS FOR JAK2-V617F ALLELIC CHARGE DETERMINATION

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Background. In 2005, JAK2V617F mutation was detected in the vast majority of patients with PV and in nearly 50% of patients diagnosed of ET and PMF. Nowadays, several efforts have been made in order to standardize JAK2 allelic charge quantification, which has been considered to be useful in monitoring bone marrow transplanted patients or treated with alpha interferon or new JAK2 inhibitors. *Aims*. To compare two determination methods for allelic charge of JAK2-V617F. MATERIAL AND METHODS: 47 DNA samples of patients with a JAK2V617F positive myeloproliferative disorder were studied by two quantitative methods: 1) The standard one (Ipsogen Kit) was performed according to manufacturer instructions. It uses specific primers for the mutated allele and for JAK2 wild-type, normalizing the number of copies with plasmid calibration curves for the mutated and wild-type alleles. 2) The second one is also based on the use of specific primers for the mutated allele and for the total JAK2. We used as calibration curves DNA dilutions from HEL cellular line in K562 for the mutated allele and DNA dilutions from a healthy donor for the total JAK2. In both cases LightCycler platform was employed for the PCR and the ratio of mutated JAK2/total JAK2 was calculated as well. Results were analyzed by statistical methods. RESULTS: 1) Statistical analysis by concurrent test shows a correlation coefficient of 0,9421 (95% CI of 0,9033-0,9656). 2) Using Passing Bablok method, the linearity test, which is used to determine how data are adjusted to a line, shows lack of significant linearity deviation (p>0,10). However, the line that correlates both procedures is above the line of equality, which might indicate that our method slightly underestimates the quantity given by standard method, being the bias greater in medium and high values, what is confirmed by Bland & Altman analysis. 3) Using T-test to compare the means of both methods, we determined that the mean of our method is lower in an average of -3,61 (95% CI -5,5557 to -1,6486) with a p=0,0006. This was confirmed by U-Mann Whitney test (p<0,0001). *Conclusions*. 1) The coefficient of concurrent between both methods is quite high. 2) There exists a bias of 3,6 units lower in our method, being greater in higher values, which indicates that the bias is not constant, but proportional to those values. 3) Both methods are useful for MRD study, as it is within the lowest values where the concordance is higher.

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CLINICAL AND BIOLOGICAL CHARACTERISTICS ACCORDING TO THE BURDEN OF JAK2V617F MUTATED ALLELE IN BCR-ABL NEGATIVE MPNS

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Background. The association of JAK2V617F mutation with BCR-ABL negative myeloproliferative neoplasms (MPNs) has been one of the most seminal medical discoveries in recent years. Although it is a controversial issue, some groups have shown that the amount of V617F allele correlates with a more pronounced myeloproliferative phenotype favoring a higher hemoglobin level and leukocyte count, higher risk of pruritus, splenomegaly and thrombosis and more probability of transformation either to myelofibrosis or to acute myeloid leukemia. *Aims*. The aim of this study was to analyze the prognostic relevance of JAK2V617F mutational status and the allele burden of mutated cases in serial newly diagnosed patients with MPNs who had not received previous treatment. Methods A total of 128 consecutive patients were included (median age 65; 59 males) with BCR-ABL negative classic MPNs (90 ET, 20 PV, 18 PMF) fulfilling the 2008 World Health Organization criteria. This study was conducted in accordance with the Declaration of Helsinki. Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA mini kit (Qiagen). All samples were coded and assayed blindly in duplicate for the JAK2V617F mutation. To detect the presence or absence of JAK2V617F mutation an Allele-Specific PCR using TaqMan allelic discrimination was used. This assay is based on the simultaneous use of 2 specific TaqMan probes and the measurement of the respective fluorescence of the 2 alleles (FAM for V617F and VIC for wild-type) to differentiate the amplification of each allele. The JAK2 MutaQuant assay (Ipsogen, Luminy Biotech) was used to detect the JAK2V617F quantity in genomic DNA by real-time detection of fluorescent signals using double-dye hyrolysis oligonucleotide probes with calibration standards at 4 different concentrations, according to the manufacturer's protocol. Laboratory parameters (red blood cell indexes, leukocyte and platelet counts) and clinical data (constitutional symptoms, complications and progression) were collected. ResultsA total of 81 (63%) patients were JAK2V617F positive (57.8% ET, 95% PV and 55.5% PMF). The median value of V617F allele burden was 23.93% (range, 1.26%-95.02%). Neither a JAK2V617F mutated status nor the quantity of burden of mutated allele was correlated with the presence of constitutional symptoms or complications (thrombosis, hemorrhage, transformation or others). Patients harboring a JAK2V617F mutation had significantly higher hemoglobin level (mean (SD) 144 (36)g/L vs. 129 (17)g/L, p<0.001), leukocyte count (mean (SD) 11.4 (7.9)x10^9/L vs. 9.9 (9)x10^9/L, p=0.021) and lower platelet count (mean 650 (373)x10^9/L vs. 780 (463)x10^9/L, p=0.018). By considering the quantity of the JAK2V617F allele burden, a statistically significant correlation with leukocytosis (p=0.027, Rho Spearman positive coefficient of 0.252) was observed. Conversely, wild-type patients had a higher probability of progression (15%), than those harboring the mutation (4%), p=0.032. Summary In newly diagnosed MPNs the presence of JAK2V617F showed statistically significant association with a more pronounced myeloproliferative phenotype favoring higher hemoglobin lev-

el and leukocyte count. Nevertheless the platelet count was lower in mutated cases. When considering the quantity of the JAK2V617F allele burden, statistically significance was only confirmed for leukocytosis. The risk of progression was significantly higher in wild-type patients.

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SOMATIC MPL MUTATIONS ARE NOT FOUND IN A LARGE COHORT OF PEDIATRIC PATIENTS WITH SPORADIC ESSENTIAL THROMBOCYTHEMIA

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More than 50% of adult but only about 25% of pediatric patients with Essential Thrombocythemia (ET) carry JAK2V617F mutation. Furthermore, 1 to 9% of adult ET patients carry mutations in the thrombopoietin receptor, (MPL) gene, leading to ligand-independent activation. The known somatic mutations of this gene are MPLW515L, MPLW515K, MPLW515A while germinal MPLS505N mutation was discovered in familial thrombocytosis. ET rarely occurs in pediatric patients and few is known about MPL mutations in sporadic pediatric ET. To study the prevalence of MPL mutations in children with sporadic ET. This study includes 53 ET pediatric patients (29 females and 24 males, age range 0.6-18 years) who have been diagnosed with ET according to WHO criteria. In all cases platelet count continuously exceeding $600x10^9$/l in the absence of known cause of reactive/secondary thrombocytosis. Five patients (9,4%) resulted JAK2V617F-positive and were excluded from further analysis. We investigated the remaining 48 patients for MPL mutations with direct sequencing of granulocytes DNA. As controls, we searched MPL mutations in 46 ET JAK2V617F-negative adult patients with ET.We found only one germinal mutation (MPLS505N) in a girl coming from Rome area; her thrombocytotic mother and her grandfather, who had normal platelet count, carry the same mutation. This family seems to belong to the same cluster described by other authors (*Teofili et al, Haematologica 2009*). We didn't identify any MPL somatic mutation in pediatric population but MPLW515L in 4 (9%) adult patients. Our data confirm that pediatric thrombocytoses are heterogeneous diseases. In this rare set of patients there is a very low probability of carrying JAK2 mutation; MPL mutations are even rarer. The biological basis in pediatric ET seems different than in adults.

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THE OLFACTOMEDIN-4 GENE IS HIGHLY EXPRESSED IN PRIMARY MYELOFIBROSIS

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Introduction. Olfactomedin 4 (OLFM4) is a member of the olfactomedin-related glycoprotein family, which is specifically expressed in neutrophils and gastrointestinal tract. The findings of highly expressed OLFM4 in immature neutrophils in the bone marrow and markedly downregulated in neutrophils in peripheral blood might suggest OLFM4 to be involved in trafficking of neutrophils from bone marrow into peripheral blood. Being involved in cell cycle regulation, cell adhesion and inflammation we focused upon OLFM4 as a candidate gene to be involved in these processes in PMF and related neoplasms. *Patients and Methods.* Gene expression microarray studies have been performed on whole blood from control subjects (n = 21) and patients with ET (n =19), PV (n = 41), and PMF (n = 9). Gene expression profiles were generated using Affymetrix HG-U133 2.0 Plus microarrays recognizing 54,675 probe sets (38,500 genes). Total RNA was purified from whole-blood and amplified to biotin-labeled aRNA and hybridized to microarray chips. Results: 20.439, 25.307, 17.417, and 25.421 probe sets were identified to be differentially expressed between controls and patients with ET, PV, PMF, and CPMNs as a whole, respectively (false discovery rate (FDR) adjusted p values < 0.05). Amongst the 50 most up-regulated genes in ET, PV and PMF, the OLFM4 gene and several other genes encoding constituents of neutrophil granules (MMP8, DEFA4, ELA2, CRISP3, CTSG, AZU1, MPO, BPI, PRTN3, LTF) were significantly and uniquely deregulated in PMF patients, not being found among the Top-50 in ET and PV. Among these genes, the MMP8 gene displayed by far the highest upregulation (fold change (FC) 22.5 and FDR adjusted p value $6.9x10^{-7}$) followed by DEFA4 (FC 12 and FDR adjusted p value $9.4x10^{-7}$), ELA2 (FC 12 and FDR adjusted p value $9.5x10^{-7}$) and OLFM4 (FC 11 and FDR adjusted p value $8.4x10^{-5}$). Except for MPO, CRISP3 and BPI all the above genes showed no significant changes in either ET or PV as compared to controls. *Discussion and Conclusion.* Using transcriptional profiling of whole blood, we have for the first time identified a highly significant and unique upregulation of the OLFM4 gene in patients with PMF. This gene may be involved in abnormal trafficking of immature myeloid cells into the circulation with egress of CD34 + cells from the bone marrow to extramedullary sites in spleen and liver. In support of this contention was the concomitant highly upregulated MMP8 and ELA2 genes, encoding enzymes which are considered to account for altered adhesion of myeloid progenitors to bone marrow niches in myelofibrosis. The highly upregulated OLFM4 may also contribute to clonal expansion both by enhancing myeloproliferation and myeloaccumulation consequent to decreased apoptosis. In conclusion, we have shown that OLFM4 together with other constituents of neutrophil granules are highly expressed in patients with PMF but not in ET and PV patients.

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MOLECULAR MARKERS IN MYELOPROLIFERATIVE NEOPLASMS THE NEED OF A STUDY ALGORITHM

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Introduction. The molecular markers (JAK2 and MPL mutations) have been proven to have an enormous value not only for the understanding of the MPN's etiology and pathogeny but also for diagnosis. According to recent data, the JAK2 mutations are identified in 90-95% of the Polycythemia Vera (PV) and in 50-55% of Essential Thrombocythemia (ET) and Primary Myelofibrosis (pMF). Objectives. Identification of JAK2 and MPL mutations in a group of 271 patients suspected of MPN. Material and Methods.Observational, retrospective and descriptive study in a group of patients suspected of MPN, studied in the Hematology Laboratory of Centro Hospitalar de Coimbra between the 1st January 2008 and 31st December 2010. JAK2 V617F mutations were studied by ASO PCR and JAK2 exon12 and MPL (exon 10) mutations were studied by SSCP and sequencing. All data collected were statistically worked using SPSS® v 17.0. Results. During the period analyzed, we studied 271 samples from patients suspected of MPN (175 from our out-patient clinic - Group 1, and 96 sent from other hospitals - Group 2). Group 1: Median age 64 years, M:F ratio 81:94; 77/175 (44%) studied for polyglobuly, 98/175 (56%) for thrombocytosis; polyglobuly group: - 67,2% JAK2V617F positive; 2,6% with exon12 mutations - Median full blood count (FBC) values: - (JAK2 positive) Hemoglobin(Hb) 18,5g/dL, hematocrit (Ht) 56,3%, leukocyte (Leuk) 11,2 103/L and platelets 532 103/L;- (JAK2 negative) Hb 17,2g/dL, Ht 51,4%, leuk 6,4 103/L and platelets 211 103/L. Thrombocytosis group - 54% JAK2 V617F positive; 2,8% with MPL (exon 10) mutations. Group 2. In this group, as we only received blood samples (BS) for the JAK2 V617F screening, we could only analyze the information that was sent along with the samples. Median age 66y, M:F ratio 60:36; 46 (47,92%) studied for polyglobuly, 50 (52,08%) for thrombocytosis; Polyglobuly group - 26,09% JAK2 V617F positive; - Median FBC values: Hb 17,2g/dL, Ht 52,4%, leuk 7,07 103/L and platelets 228 103/L. Thrombocytosis group - 44% JAK2 V617F positive Conclusions. In group 1 - patients observed in our department - we diagnosed: 54 PVs, (51 JAK2 V617F positive, 2 exon12 positive and 1 PV JAK2 negative), corresponding to 98,15% JAK2 positive PVs; 72 ET (61,11% JAK2 V617F positive and 2,78% MPL exon 10 positive); 10 pMF (70,0% JAK2 V617F positive). Among all the MPN diagnosed we were able to identify a molecular marker in 75,71% of the patients. In Group 2, BS sent to our lab, curiously the median FBC values were similar to the JAK2 negative polyglobulies from group 1. Even though we lacked clinical information in group 2, when we compared the percentage of JAK2 V617F positivity in both thrombocytosis groups (1 and 2), the results were similar (54,08% Vs 44%). When comparing the polyglobuly groups, we found a very different positivity of JAK2 V617F: 67,23% group1 Vs 26,09% group 2. This last data emphasizes the importance of a study algorithm for polyglobulies which are, undoubtedly, a diagnostic challenge.

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STUDY OF EFFICACY RECOMBINANT HUMAN ERYTHROPOIETIN IN PRIMARY MYELOFIBROSIS PATIENTS WITH ANEMIA

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Background. Primary Myelofibrosis (PMF) is Phyladelphia-negative myeloproliferative neoplasm with different clinical presentation including anemia, splenomegaly, leukocytosis or leukopenia, thrombocytosis or thrombocytopenia and constitutional symptoms. Anemia is frequent symptom and may get worse prognosis and survival of PMF patients. To correct anemia are used red blood cell (RBC) transfusions. However long period RBC transfusions may cause iron overload and reduce survival. Aims. To study the efficacy of Recombinant Human Erythropoietin (EPO) in PMF patients with anemia: reducing RBC transfusion-dependency, increasing hemoglobin (Hb) concentration and anemic symptoms. *Methods.* This study includes 26 PMF patients diagnosed between 1998-2009. Diagnosis of PMF was based on WHO criteria for Primary Myelofibrosis. The median age of patients was 70 years (range 50-84). All patients had anemia with initial Hb concentration 5.3-9.9 g/dL. RBC transfusion-dependency was defined as the persistence of RBC transfusion administrated to correct anemia with Hb concentration <8,0 g/dL. EPO-therapy was administrated to correct anemia for patients with Hb concentration <10,0 g/dL. EPO was injected subcutaneously on 10.000IU 3 times a week (30.000IU a week). The effectiveness of EPO was estimated on Hb concentration, red blood cells count and hematocrit (Ht) concentration. The target Hb level was 12,0 g/dl and planned duration of treatment with EPO within 24 weeks. Positive response was considered as 1) transfusion-independency in patients before needed RBC transfusions or 2) increase Hb level <2,0 g/dL. All patients received constantly antitumor therapy (Hydroxyurea, Interferon alpha, small doses Ara-C). The patients with iron, vitamin B12 deficiency and so severe hemolysis (bilirubin >30 µmol/L) were excluded in this investigational study. *Results.* Mean baseline Hb concentration was 8.09±1.49 g/dL (5.3-9.9 g/dL), RBC count - 2.55±0.59x10^{12}/L (1.35-3.31x10^{12}/L) and Ht - 24.4±5.9% (15.2-36.8%). 13 patients (Hb <8.0 g/dL) were depended on RBC transfusions and they needed of 2-9 unite of RBC every 1-3 months. Others (n=13) were transfusion-independent patients (Hb was 8.0-9.9 g/dL). The period of EPO-therapy was from 6 to 24 weeks (mean 11.1±6.2 weeks). During the study period out of 13 patients 7 ones kept on transfusion-dependency and their Hb level was depended on RBC transfusions. In whole group of PMF patients with anemia (n=26) positive response was observed in 12 patients (46.2%) and their Hb concentration, RBC count and Ht significantly ($p<0.05$) increased from baseline to 11.54±1.56 g/dL (10.3-14.6 g/dL), 3.38±0.77x10^{12}/L (2.59-7.70x10^{12}/L) and 35.3±4.8% (30-43%), respectively. Besides most patients with positive response reduced such symptoms as feeling fatigue, weakness all over, having trouble starting things because of tiredness, depression, drowsiness, giddiness, headaches, pain in thorax. However 11 positive response patients needed in administration of EPO-therapy in 2-4 months because of anemia relapsed (Hb concentration decreased <10,0 g/dL) but such way of persistent treatment could prevent RBC transfusions. We observe no one case of thrombosis during the period >6 months. The count of platelets before and after EPO-treatment was not significant difference ($p>0.1$): 372.5±248.7x10^{9}/L and 355.0±285.4x10^{9}/L, respectively. *Summary.* EPO-therapy is effective treatment of reducing RBC transfusion-dependency, increase Hb concentration and decrease anemic symptoms in PMF patients.

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ABNORMAL PRODUCTION OF INTERLEUKIN (IL)-6 IN ESSENTIAL THROMBOCYTHEMIA

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The essential thrombocythemia (ET) is a myeloid neoplasm characterized by platelet-endothelial activation and a inflammatory component responsible for myeloproliferation. Therefore, we evaluated platelets, platelet factor 4 (PF4) and tissue factor pathway inhibitor (TFPI), tissue factor (TF) and von Willebrand factor (vWF), as indicators of platelet-endothelial activation, inteleukin-6 (IL-6) as inflammatory marker and white blood cell count (WBC) and lactate dehydrogenase (LDH), as myeloproliferative indexes. We recruited 25 patients with ET (10 males and 15 females, mean age 58 years) who fulfilled WHO criteria. Their mean duration of disease was 8 years (range, 5-21 years). All patients were on aspirin. PF4, TFPI, TF, IL-6 and vWF were measured by ELISA and immunoturbidimetric assay, respectively. Platelets and WBC were measured by automated analyser. LDH was determined by enzymatic method. All patients had thrombocytosis (961±235x10^{9}/L), high PF4 (153±62 IU/ml vs 6±2 IU/ml) ($p<.0001$), TFPI (142±66 ng/ml vs 91±11 ng/ml(($p<.0001$), TF (214±266 pg/ml vs 5±3 pg/ml) ($p<.0001$), low vWF (25±9.6% vs 81±18%) ($p<.0001$), high IL-6 (68±53 pg/ml vs 8.3±2.6 pg/ml) ($p<.0001$), leukocytosis (9.2±18x10^{9}/L vs 5.5±11x10^{9}/L) ($p<.0001$) and elevated LDH (461±190 UI/L vs 154±20 UI/L) ($p<.0001$). No correlation there was between IL-6 and platelets and PF4, and TFPI, and TF, and vWF. A positive correlation was found between IL-6 and WBC and LDH ($p=0.037$ and $p=0.001$, respectively). These data suggest that IL-6 may be a clonal marker promoting myeloproliferation.

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INCREASED PLATELET AND LEUKOCYTE ACTIVATION AS CONTRIBUTING MECHANISMS FOR THROMBOSIS IN ESSENTIAL THROMBOCYTHEMIA AND CORRELATION WITH JAK2 MUTATION STATUS

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Background and Aims:Leukocytes contribute to the pathogenesis of thrombosis in Essential thrombocythemia (ET) through recently discovered mechanisms of activation and interaction with platelets and endothelial cells. The aim of the present study was to ascertain the role of platelet and leukocyte activation in the thrombosis of ET. Methods: The activation status of platelets and leukocytes was assessed by flow cytometry studies in 74 patients with ET (34 with previous and 40 without history of thrombosis) and in a group of age-and sex-matched healthy individuals. The assessment included platelet P-selectin expression (measured both at baseline and after stimulation with ADP trombin, arachnoidic acid- (AA),and collagen, platelet-neutrophil and platelet-monocyte complexes, determination of CD11b in the platelet-neutrophils and monocytes. Results: As compared with controls, ET patiens had significantly higher values of baseline P-selectin expression,as well as higher platelet-neutrophil, platelet- Mo complexes and neutrophil CD11b expression. Platelet P-selectin, monocyte CD11b was significantly higher in ET patients with a history of thrombosis than in patiens without thrombosis. Patients with the JAK2V517Fmutation or thrombosis showed higher baseline and AA-induced platelet P- selectin expression than did those without thrombosis Conclusions.These results would support a role for platelet and monocyte activation in the thrombosis of ET, in these patiens, the presence of the JAK2V617F mutation is associated with higher platelet activation.

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NOVEL MULTIPLEX BEAD-BASED ASSAY WITH LNA-MODIFIED PROBES FOR DETECTION OF MPL EXON 10 MUTATIONS

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Background: MPL exon 10 mutations were the second class of mutations shown to be associated with the pathogenesis of Philadelphia chromosome - negative myeloproliferative neoplasms (MPNs). Various molecular approaches have been applied, yet universally accepted method is still lacking. Aims: We aimed at development and validation of a novel bead-based liquid assay for multiplexed detection of the following MPL mutations: W515L/K/A/R. *Results:* We developed a novel multiplex bead-based liquid assay for detection of the most frequent MPL exon 10 mutations using Locked nucleic acids (LNA)-modified oligonucleotide probes. Testing on both artificial plasmid constructs and on clinical samples revealed that the method was comparable in terms of specificity to direct sequencing and had a much higher sensitivity of 1% mutant alleles. *Conclusions:* This method could be successfully implemented in the diagnostic work-up for MPNs. Furthermore, the system allows further multiplexing for single-tube identification of different mutations associated with MPNs.

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STUDY REGARDING THE ASSOCIATION BETWEEN MULTIPLE PRIMARY CANCERS THAT INCLUDES A MYELOPROLIFERATIVE DISORDER AND THE METABOLIC SYNDROME

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Background. It is known that cancer patients have a 20% higher risk of new primary cancer compared with the general population. Genetic instability, family predisposition, chemotherapy, radiation, environmental factors, smoking, alcohol consumption, infections, etc predispose to the appearance of a 2nd cancer. *Aims*. We aimed to study the prevalence of the metabolic syndrome and its components in patients with multiple primary cancers that include a myeloproliferative disorder comparing with those with only myeloproliferative disorders. Methods: Of the 668 patients with hematological malignancies who were registered in the Department of Hematology of the Emergency County Hospital Sibiu during January 2006 - January 2011 we selected the 239 with myeloproliferative Disorders, and among them were selected all 20 multiple primary cancers (group A), which were compared with a group of 37 consecutive patients with myeloproliferative disorders which were in our evidence in January 2011 (group B). We made a comparative analysis of the components of metabolic syndrome and hypercholesterolemia in the two groups. The results were statistically analyzed. Results: The average age in group A patients was 59.26+/-14.25 years, and those in group B - 60.11+/-16.18 years. Distribution by gender: group A - 14 men and 6 women, group B - 14 men and 23 women. The average time to onset of the 2nd cancer was 2.85+/-2.96 years. Among patients with myeloproliferative disorders 8.37% had multiple primary cancers: 6 - acute myeloid leukemia, 4 - lung cancer, 4 - nonHodgkin malignant lymphoma, 2 - skin carcinoma, 2 - breast cancer and one - multiple myeloma, colon cancer, essential thrombocythemia, primary myelofibrosis, fibrosarcoma or mediastinal tumor. Four patients had triple neoplasia. Patients in group A had more frequently obesity (50% vs. 16.22%) (p=0.007), diabetes (35% vs. 10.81%) (p=0.027) and hypertriglyceridemia (35% vs. 21.62%) (p>0.05). Those in group B were more often hypertensive (51.35% vs. 30%) (p>0.05). 25% of patients in group A and only 8.11% in group B had metabolic syndrome. The average of the metabolic syndrome components was higher in patients in group A compared with those of B (1.28+/-1.24 to 1.0+/-1.0) (p=0.027). Conclusions: A significant proportion of patients with myeloproliferative disorders has one or two primary cancers. They often have metabolic syndrome. Patients with multiple primary cancers that include a myeloproliferative disorder have obesity, diabetes mellitus, and more components of metabolic syndrome in a higher proportion, compared to the patients with only a myeloproliferative disorder. Studies are needed to investigate the mechanisms that explain the relationship between metabolic syndrome and multiple primary malignancies.

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ESSENTIAL THROMBOCYTHEMIA AND FAILURE OF FIRST LINE TREATMENT: WHAT IS THE OPTIMUM METHOD FOR INITIATING ANAGRELIDE? THE FOX (FRANCE OBSERVATOIRE XAGRID) STUDY

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Background. Essential thrombocythemia (ET) is an acquired myeloproliferative syndrome that has little effect on the survival of patients, but may be complicated by severe thrombo hemorrhagic events. The therapeutic strategy is guided by assessing the risk of complications in each individual patient. In Europe, patients receive hydroxyurea (HU) as the first-line therapy. However, HU is ineffective or poorly tolerated in some patients and, in these cases, anagrelide is the recommended second-line treatment. Currently, there are no published recommendations or guidelines for switching a patient to anagrelide treatment, and methods for initiating anagrelide treatment vary according to the usual practice of individual prescribers. For example, immediate cessation of HU treatment or a gradual reduction of the dose, with or without a parallel escalation of the anagrelide dose, can be used. Aims. The objective of the current FOX (France Observatoire Xagrid) study is to appraise clinical practice in France when switching patients from HU to anagrelide treatment. The aims of the study are to determine whether the switching strategy may influence the tolerability and the efficacy of anagrelide in the second-line setting, or the care of the patients. The FOX study also offers the opportunity to identify the most efficient initiation protocol for anagrelide. Patients and Methods. FOX is a multicenter, prospective, observational study assessing adult patients with ET who switch to anagrelide following failure of, or intolerance to, HU. Patients will be assessed prospectively over a 6-month period following the introduction of anagrelide treatment. During the assessment period, all relevant data available from the patients' medical records will be compiled. No clinical examinations or procedures will be required during the study. The target recruitment is 180 patients from 60 participating centers, with the aim of obtaining 160 evaluable cases. Each investigator will enroll each consecutive patient fulfilling the inclusion criteria. Results. The study analysis will compare the number of patients maintaining treatment with anagrelide after 6 months, including details of the initiation schedules, using a Fisher exact-test. The study is ongoing and final results are expected in June 2012. The study is sponsored by Shire Pharmaceutical Development Ltd. *Summary/conclusions*. ET is a chronic condition with limited treatment options that requires long-term therapeutic management. Where there is intolerance or lack of efficacy with the first line treatment, HU, anagrelide is indicated as second-line therapy. The method of initiating treatment switch to anagrelide is not universally agreed but may have an impact on its efficacy and tolerability. This issue is currently being assessed in the FOX study in order to determine the optimal procedure for initiating treatment with anagrelide.

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LEUKEMIC TRANSFORMATION OF THE PHILADELPHIA (BCR/ABL)-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background. The BCR/ABL (Ph)-negative myeloproliferative neoplasms (Ph-negative MPN) embody polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). These are pluripotent hematopoietic stem cell disorders and which have several clinical, laboratory and histological features in common. The discovery of JAK2-V617F and JAK2 exon 12 mutations and, more recently, mutations in MPL, TET2, CBL, and IKZF1 alleles has advanced the molecular taxonomy and our understanding of pathobiology of the Ph-negative MPN. The incidence of leukemic conversion of the Ph-negative MPN has an incidence of 2-30%. Little is known about the factors and mechanisms that lead to and bring on acute leukemic transformation in the Ph-negative MPN. *Aims*. A prospective single-centre study of immunophenotypic and cytogenetic characteristics as well as the clinical course and therapy outcomes in consecutive patients with acute leukemia supervening on the Ph-negative MPN. *Patients and Methods*. Twenty patients (pts) were studied (med. age, 61, range 34-73 years). Thirteen pts had PMF, four ET, and three PV. The type of acute leukemia was confirmed using flow cytometric methods and Immunohistochemistry. Cytogenetic analyses were performed in all patients at diagnosis of a primary disease and at the time of acute transformation of MPN. Results: The median duration of a chronic phase of MPN was 45.4 months (range 10.5-300). One pt developed acute myeloblastic leukemia (AML) M-1 (by FAB Group Classification), fifteen pts AML M-2, two pts AML M-4, and one pt AML M-7. One pt acquired the BCR/ABL-negative B-cell acute lymphoblastic leukemia. The karyotype evolution was evidenced in 7/20 pts. A 48,XY,+8,+16 was observed in two pts, del(20) in two pts, and i(17)(q10); der(20) and multiple kariotypic abnormalities in one patient each. Five out of eight JAK2-V617F-positive pts lost the signal of this mutation in the leukemic phase. Ten pts received intensive induction chemotherapy. Another ten were treated by palliative chemotherapy or support only. Two pts are presently alive and in complete remission (CR) for 59 and 50 months each. Two pts survived 47 and 42 months in CR and died in relapse. One patient in partial remission survived an average of 16.5 months. Four pts with stable leukemia lived 6, 16.5, 19, and 48.5 months. The remaining patients died within nine months of acutization. The pts on intensive chemotherapy lived distinctly (p=0.0470) longer and mostly had a preceding PMF (p=0.0479). *Conclusions*. Acute leukemia complicating the Ph-negative MPN merits further studies on larger cohorts of pts. This is warranted by the present study showing that long survival could be attained by a considerable proportion of younger (<60) patients without comorbidities.

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HIGHLY SENSITIVE QPCR INCREASES THE RATE OF DETECTION OF JAK2-V617F IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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Background. Approximately 95% of patients with polycythaemia vera (PV) and 50% of patients with essential thrombocythaemia (ET) or primary myelofibrosis (PMF) harbour the JAK2-V617F mutation with smaller numbers exhibiting either MPL or JAK2 Exon 12 mutations. The presence of JAK2-V617F has become a major diagnostic criterion for these disorders, emphasising the need for accurate testing. Recent data from external quality assessment schemes involving large numbers of European laboratories suggest marked variation in performance of assays measuring JAK2-V617F allele load, yielding highly divergent results. Targeted therapy using JAK2 inhibitors within clinical trials, and other modalities such as transplantation, may require accurate molecular monitoring. Interestingly, patients testing negative for JAK2-V617F may respond to targeted therapy and are not distinguishable from those with mutant JAK2. This is consistent with the finding of activated JAK signalling, but could also suggest that they may harbour the mutation at a lower level than presently detectable. *Aims*. To reassess a cohort of patients with myeloproliferative neoplasms (MPN), negative for JAK2-V617F by standard allele-specific PCR (AS-PCR), using highly sensitive real-time quantitative PCR (qPCR). *Methods*. We identified patients from our hospital laboratory records who had tested negative for JAK2-V617F mutation by AS-PCR between 01.08.2005 and 01.08.2010. AS-PCR was performed using a common reverse primer and two forward primers. The first forward primer was specific for the mutant allele, with an intentional mismatch at the third nucleotide from the 3' end, giving a 203-bp product. The second primer amplified mutant and wild type alleles to give a 364-bp product, acting as an internal control. During the study period, 824 samples had been tested for JAK2-V617F mutation, of which 569 tests were reported as negative. Of patients testing negative, 84 met the WHO criteria for ET, 23 were diagnosed as PMF, 6 as PV, 4 as myeloproliferative/myelodysplastic overlap (MPN/MDS) and 11 as MPN, unclassifiable (MPN-U). Peripheral blood samples were analysed using a qPCR assay which has been shown in QC rounds conducted within the European LeukemiaNet to detect a 0.1% level of JAK2-V617F. V617F was quantified relative to wild-type JAK2, and to albumin, used as an independent control gene. *Results*. 47 samples were tested; 32 ET, 12 MF, 1 PV, 1 MPN/MDS and 1 with MPN-U. In 46 cases, the albumin gene was amplified with a mean cycle threshold (CT) of 22 cycles (range=20-25) but no V617F was detected. One patient, with PMF, had 5.2% JAK2-V617F relative to wild-type JAK 2 and albumin. This patient has since undergone a reduced intensity conditioning allogeneic stem cell transplant. At reassessment four months post transplant, with full donor chimaerism, the V617F level had fallen to 0.2%. *Summary/conclusions*. In this small cohort of 47 patients, previously diagnosed as having JAK2 negative MPN, one was found to have a significant V617F mutant burden relative to wild type. There was a significant reduction in mutant load at reassessment four months post transplant. This finding supports qPCR as a highly sensitive method to detect JAK2-V617F and its potential role in monitoring residual disease during treatment.

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MYELOPROLIFERATIVE NEOPLASMS, STEM CELL MOBILIZATION AND AUTOLOGOUS TRANSPLANTATION

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Background. Myeloproliferative neoplasms (MPN) are chronic disorders that evolved for many years but they can be complicated by thrombotic complications or haematological transformations as myelofibrosis or acute leukaemia. Actual management of MPN diminishes thrombotic complications but no treatment can cure the disease. The discovery of JAK2 mutation has led to the development of JAK2 tyrosine kinase inhibitors which may affect positively the disease and benefit many patients. Allografting is reserved for patients with haematological evolution as myelofibrosis. Autologous transplant is not a standard procedure in myeloproliferative disorders, apart for few cases reported in the palliative treatment of myelofibrosis. Recently increased incidence of lymphoid haematological malignancies has been reported in patients with MPN. Stem cell collection and autografting should be used in this setting in patients with both haematological malignancies. Aims - We report two cases of successful stem cell mobilization and autologous transplantation for plasma cell malignancies with concomitant myeloproliferative neoplasms, the first with multiple plasmacytoma and essential thrombocythemia and the second with multiple myeloma and primitive polycythemia. Methods - In our two cases, the myeloproliferative neoplasm was diagnosed several years before plasma cell malignancies (eleven and ten years respectively). Both patients were treated with hydroxyurea for ten and nine years before stem cell collection. Symptomatic plasma cell malignancies was then diagnosed in both patients and treated with bortezomib dexamethasone cycles. Stem-cell mobilization was realized after granulocyte colony-stimulating factor and allowing 7 x 106 and 5 x 106 CD34+ cells/kg, respectively. Conditioning chemotherapy before autografting was melphalan 200 mg/m2. No complications were noted during all these procedures. Platelet and granulocyte recovery after autografting are respectively 19 and 16 days for the first patient and 14 and 12 days for the second. Results - Our two cases show the feasibility of stem cell mobilization and autologous transplantation for haematological malignancy with concomitant myeloproliferative neoplasms. Although disease evolution of myeloproliferative neoplasms has reached the first decade for our two patients, stem cell mobilization seems to be possible. Moreover, cytoreductive treatment as hydroxyurea and possible fibroses seem do not influences stem cell mobilization. However, autografting does not seem to influence the course of MPN as the platelet count returned above normal range six months after autografting for our first patient. Conclusions - Stem cell mobilization and autologous transplantation are feasible for patients with haematological malignancy and concomitant myeloproliferative neoplasms, even after decades of history. It was an important point because an increased risk of lymphoid neoplasms has been described for patients with MPN compared with general population.

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MYELOPROLIFERATIVE DISEASES AS A CAUSE OF THROMBOSIS

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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 data from literature, thrombotic complications occur in about one third of patients with PV. The main cause of morbidity and mortality in ET are bleeding and thromboses. About 40% of patients showed a tendency to thromboses, and 60% to bleeding. Thrombotic complications are, as a rule, arterial thromboses, although venous thromboses appear as well, particularly in the deep veins of lower extremities. The most common sites of arterial thrombosis are cerebrovascular, peripheral, vascular and coronary circulation. Thromboses are common in splenic, hepatic, portal and mesenteric blood vessels. The aim of the paper is to show the extent to which the thrombotic 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. The methods of clinical examinations, endoscopies, exosonographies and computer-assisted tomographies have been used. In our research, presence of thrombotic complications was recorded in almost 20% of all subjects with MPD. In the group with CML about 5%, in the group with PV about 30%, and in the group with IMF about 7,6%, in the group with ET about 63%, and in the group with MPS about 42% of patients had one or more thrombotic complications. The highest percentage of thrombotic complications is within the group of subjects with ET, that is statistically more significant compared to PV (PV ($p<0,05$), IMF and CML ($p<0,001$). We proved that prevalence of thrombosis is in a statistical dependence on the type of MPD. Mechanism of thrombophilic state in patients with MPD is not entirely clear. Uncorrected polycythemia with elevated values of hematocrit and increased blood viscosity, increases the tendency to thrombosizing. Impaired platelet function within MPD could also be significant for the development of thrombotic and hemorrhagic complications as well.Some authors have described the disturbances in the fibrinolytic system and the function of natural coagulation inhibitors (AT III and protein C),

which further contribute to the tendency of thrombosizing in patients with MPD. One of the explanations for increased risk of thrombosis in patients with ET is that the total amount of thrombin generated on the surface of platelets in those patients, has been significantly higher than with the control group or in patients with reactive thrombocytosis. Thrombotic complications often accompany chronic myeloproliferative diseases. They are usually present in patients with ET and also in patients with PV and MPS, and much less frequently in patients with CML and IMF, which is in accordance with data from literature.

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ACTUAL MANAGEMENT OF PH NEGATIVE MYELOPROLIFERATIVE MALIGNANCIES - THE EXPERIENCE OF AN EST EUROPEAN CLINIC

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Background. The description of the JAK2V617F mutation in classical Philadelphia (Ph)-negative MPN provide us a rational target for novel innovative treatment strategies but currently, clinical studies testing various JAK2-inhibitors in PV, ET as well as in primary and secondary myelofibrosis (MF) are under way. Interestingly, first data indicate that despite marked clinical activity in terms of spleen size reduction and improvement of constitutional symptoms, these inhibitors might not sufficiently reduce disease burden. So we tried to compare eficacy of well established treatment strategies, such as inhibition of thrombocyte aggregation by low dose aspirin, cytotoxics (e.g. hydroxyurea, anagrelidum), immuno- and stroma-modifying therapy with interferon, tyrosine kinase inhibitors and, in selected cases, allogeneic stem cell transplantation and to make a correlation between the response at these therapeutical alternatives and the mutational status. Methods and results: We investigated a lot of 430 patients diagnosed in our clinic between 2004 and 2010; the study population was female in procent of 58%, ages ranged from 22 to 72 years (median age 52). We tried to establish the indications on our study group for cytoreductive therapy in which the key targets were to reduce thrombohemorrhagic complications, relieve disease-related symptoms and minimize the risk of transformation to secondary myeloid malignancy such as myelodysplasia, leukemia, and secondary myelofibrosis. We correlate the rate of disease progression with the mutational status and investigated the role of interferon treatement in JAK2 V617F positive cases. We tried to establish if there is a significant molecular response in these subset of patients. In our study 87% of patients treated wih IFN had a hematologic response, and from these 71% were complete responses (CRs). Conclusions: We reported that in our study group the patients with myeloproliferatives disorders treated with rIFN-2b had lower JAK2V617F allele burdens compared with a control group that included patients treated with phlebotomy, hydroxyurea, or anagrelide, or who remained untreated. May IFN induce high levels of clinical and hematologic remission and in some patients, a molecular CR?

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PREVALENCE OF THE JAK2 V617F MUTATION IN CROATIAN PATIENTS WITH CLASSIC PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background. The classic Ph-negative myeloproliferative neoplasms (MPNs) are clonal disorders of multipotent haematopoietic progenitors. The Janus-associated Kinase-2 mutation JAK2 V617F in such neoplasms has been described as a frequent genetic event in majority of these patients. Aims: To investigate the status of the JAK2-V617F mutation in patients with classic Ph-negative MPNs treated in Clinical Hospital Center Rijeka and to compare it with hemoglobin and hematocrit level, white blood cells and platelet count, splenomegaly, leukocyte alkaline phosphatase score and clinical features. Methods: DNA was isolated from peripheral blood granulocytes in 115 patients: 41 with polycythemia vera (PV), 43 with essential thrombocythemia (ET), 9 with primary myelofibrosis (PMF), 10 with myeloproliferative neoplasm-unclassifiable (MPN-u), 4 with secondary erithrocytosis and 5 patients with secondary thrombocytosis. The JAK2-V617 mutation was determined using allele specific PCR. Results: The JAK2-V617F mutation was found in 71/106 (66.98%) patients with MPNs or in 36/41 with PV (87.80%), 25/43 with ET (58.14%), 5/9 with PMF (55.56%) and 5/13 MPNs-unclassified (38.46%) disorders. The JAK2-V617 mutation was absent in patients with secondary erythrocytosis and seconrary thrombocytosis. There were significant differences in hemoglobin and hematocrit level, leukocyte alkaline phosphatase score and white blood cells at diagnosis in JAK2-V617F non mutated versus mutated patients with MPNs. Vascular events were present in 23/106 (21.7%) patients with MPNs or more specifically in 13/41 (31.7%) with PV, 8/43 (18.6%) with ET, 1/9 (11.1%) with PMF and 1/13 (7.7%) with MPN-u. The majority of those patients with one or more vascular events were JAK2-V617F positive. When analyzed within each entity than it could be emphasized that one or more vascular events, or recurrent thrombosis was detected in 13/41 (31.7%), 7/41 (17.1%) cases, respectively with PV, or in 10/13 with JAK2-V617F positive status. In patients with ET, compare to PV, vascular events (8/43) were less present (18.6%). Though, as noticed for PV, most of those patients (6/8) were associated with JAK2-V617F mutation. In patients with PMF only 1 vascular event was confirmed (patient was JAK2V617F negative), as well as in patients with MPN-u (patient was JAK2 positive). *Conclusions*. The JAK2-V617F mutation was frequently detected in our patients with MPNs in accordance with literature data, and therefore should be incorporated in the diagnostic evaluation of patients with suspected MPNs. Further analysis should focus on contribution of the JAK2-V617F mutation in the clinical phenotype of patients with distinct subgroups of MPNs.

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SPLACHNIC VEIN THROMBOSIS AND MYELOPROLIFERATIVE SYNDROMES. THE ROLE OF JAK2V617F MUTATION

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Background. Myeloproliferative diseases(MPDs) are shown to have an increased risk of thrombotic complications such as splachnic vein thrombosis(SVT).Mutations on JAK2 pathway are thought to play key role on such thrombotic complications. *Aims*. The focus of the current work is to evaluate the risk of SVT in MPDs patients and its colleration with the mutation JAK2V617F. *Methods.* Patients with non-cirrhotic, non-cancer related SVT and with clinical or laborating findings suggesting MPD were assessed for the presence of JAK2V617F mutation. We suspected that normal or light increased platelet count might mask MPDs (portal hypertension-hypersplenism, occult bleeding).Assessment for hematological pro-coagulant conditions included factor V Leiden, antithrombin III, protein C, protein S, homocysteine, MTHFR mutation, prothrombin gene mutation PT20210A, anticardiolipin antibodies and lupus anticoagulant. Paroxysmal nocturnal hemoglobinuria was screened using standard flow cytometry techniques. Patients with known history of pylephlebitis were excluded. SVT was confirmed with computerized tomography and abdominal doppler ultrasound. SVT was characterized as chronic if there was evidence of intra-abdominal venous collaterals, carvenous transformation of the portal vein, or signs of portal hypertension. 4.Results:In the study 14 patients were included. The median age at the time of diagnosis was 50.71 years (range, 21-78) and 57% were male. All patients had chronic SVT, 64% had PVT and the rest were diagnosed with BCs. Every patient underwent bone marrow biopsy: polycythemia vera(PV) 4 patients, essential thrombocytosis(ET) 7 patients, primary myelofibrosis(PMF) 3 patients. JAK2V617F was analyzed in 12/14 patients and was positive in 100%. Inherited thrombophilia was not found. Acquired thrombophilia was mentioned in two patients. A woman with Budd-Chiari syndrome(BCs) who was provided oral contraceptive pills, and a man with portal vein thrombosis(PVT) postsplenectomy. Patients with BCs had mean age 43.2 years(range, 35-56) and 60% were female. Three were diagnosed with PV, 1 ET and 1 PMF. One patient died after 17 years and one was scheluded for liver transplantation after 6 years. The other three patients had no signs of ascites or portal hypertension in a six-year follow up. Patients with PVT had mean age 54.8 years(range, 21-78) and 67% were male. Six were diagnosed with ET, 2 PMF and 1 PV. On admission 5 patients had esophageal/gastric varices whereas 89% patients had splenomegaly. Five patients had also evidence of superior mesenteric vein thrombosis. Nobody died. All of the patients have signs of portal hypertension. Mean time of follow up is 1.8 years(range, 0.2-6). All patients were managed with routine anticoagulation therapy from diagnosis. Three patients had indications for decompressive procedures such as TIPS, all in the group of BCs. 5.Summary/Conclusions:SVT is frequent presenting complication of undiagnosed MPDs.In patients with SVT, portal hypertension is a virtually constant feature. The resulting hypersplenism and hemodilution decrease the accuracy of blood cell counts for MPD diagnosis. The

atypical peripheral blood picture in the setting of SVT has led to a variety of denominations such as *latent* MPDs. In our study, all patients with MPD and SVT were positive for the mutation JAK2V617F.The presence of this mutation may predict a more aggressive phenotype with an increased risk of thrombosis.

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ESSENTIAL THROMBOCYTHEMIA: CORRELATION BETWEEN JAK2 ALLELE BURDEN AND CLINICAL OUTCOME

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Essential thrombocythemia (ET) is a myeloproliferative disorder characterized by persistently elevated platelet count (>450x10^9/l, 2008 WHO classification) with normal red cell mass. Thrombosis, acute myeloid leukemia or myelofibrosis are its main complications. Janus kinase 2 mutation (JAK2V617F) appears involved in the molecular etiology of disease development. Its identification markedly simplified the approach to clinical diagnosis and provided molecular targets for the development of biologically targeted drugs. Recent studies reported an association between JAK2V617F and thrombosis in patients with myeloproliferative disorders, but it remains unknown whether inherited thrombophilia has an impact as an additive risk factor in mutated subjects. Therapeutic strategies are hydroxyurea (HU), anagrelide and alpha-interferon (IFN), aimed to reduce platelet count and ASA to control the thrombotic risk. We studied 52 patients with TE aiming to 1. evaluate variation in JAK2 (V617F) mutant allele burden during different treatments (HU, anagrelide, interferon or none); 2. evaluate relationship between allele burden and thrombotic risk; 3. evaluate role of additional thrombophilic risk factors in these patients. We analyzed JAK2 allele burden with MutaScreen kit at the beginning of treatement and after 21 months follow up, and split patients in 6 groups according to % of allele burden: 2-5%, 5-12.5% (heterozygotes), 12.5-31%, 31-50%, 50-78%, 78-100% (homozygotes). Furthermore we searched for thrombophilic risk factors (ATIII, omocysteinemia, factor V Leiden and prothrombin gene mutations, protein C and protein S levels, lupus anticoagulant, antiphospholipid antibodies). Statistical analysis was performed by the Student T test and l(2) test. RR for thrombosis was calculated with a 2 × 2 table (confidence interval of 95%). 24 patients were treated with HU and their allelic burden on average increased of 10.2%. 4 patients were treated with anagrelide with an allelic burden median increase of 4.6%. 2 patients were treated with IFN: in 1 case we found a decrease in allele burden from 50-78% to 12.5-31%, median decrease was 27.1%. 22 patients were not treated and their median increase in allelic burden was 21.3%. We observed 13 thrombotic events: 6 arterial events and 7 venous events. 5/52 patients (9.6%) were homozygous for the mutation and 47/52 (90.4%) heterozygous. Incidence of thrombotic complications was higher in the homozygote group: 2/5 (40%) compared to 11/47 (23.4%) in heterozygotes, with a RR of 1.7. Thrombophilia was recorded in 21/52; of these, 8 patients (8/21, 38%) developed thrombosis. Patients with allele burden > 12.5% and concomitant thrombophilic risk factors showed a higher incidence of thrombotic events (RR =7) compared to patients with allele burden < 12.5% and no additional risk factors. In our study IFN showed to reduce allele burden, according to literature. Subjects treated with HU demonstrated a statistically significant increase in allele burden (p=0.018) during follow up. Data analysis shows an association between allele burden > 12.5% and thrombotic risk, and this association becomes stronger in presence of further thrombotic risk factors. Evaluation of patients' thrombotic risk factors may influence the treatement choice in TE subjects.

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CYCLICAL THROMBOCYTOSIS AND ACQUIRED VWD ARE RELATIVELY COMMMON IN PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background. Cyclical thrombocytosis, acquired von Willebrand disease (vWD), aggressive non-melanoma skin cancers (NMSC) and hydroxyurea-related fever have been reported in Philadelphia-negative myeloproliferative neoplasms (MPNs) but to our knowledge their incidence and clinical consequences has not been clearly defined in a large cohort of patients. *Aims*. To conduct a retrospective analysis of less commonly reported disease and treatment related complications in patients with Philadelphia-negative MPNs. Methods. A retrospective audit was conducted on patients under the care of two haematologists, one at a metropolitan tertiary referral centre and the other at a regional community based practice. *Results*. 188 patients were included in the audit, of which 115 (61%) were female and 73 (39%) were male. The MPN subclassification breakdown was: 104 ET (55%), 62 (33%) PV, 18 (10%) PMF, and 4 (2%) MPN-U. 136 (72%) were JAK2+. The mean age at diagnosis was 61 years (0.4-93 years) with a mean length of follow-up of 7.7 years (0.5-44 years). 90% of patients were treated with hydroxyurea (HU) at some stage during this follow-up, with a mean dose of 707mg/day and an average length of exposure of 4.2 years. 54 patients were treated with non-HU cytoreduction including interferon, anagrelide, busulphan and radioactive phosphorous. 29 patients (15%) had platelet counts that varied by over 100x10^9/L over a 6 week period while on a stable dose of cytoreductive therapy. Platelet cycling occurred in all MPN subgroups (hitherto the literature has documented this in PV only); there was no difference in incidence according to gender or JAK2 positivity. 25 patients (86%) were taking HU at a mean dose of 920mg/day. The mean maximum difference between the lowest and highest platelet count over the 6 weeks was 350 x10^9/L (100 - 1346 x10^9/L). 60 patients (32%) had non-melanoma skin cancers, 29 (48%) of whom had multiple skin cancers. Those with skin cancers had a longer exposure to HU than the overall population (5.7yrs vs 4.2 years). Those with multiple NMSC were predominantly male (66%) and were older (mean age 79years). Acquired vWD occurred in 17 patients (9%), was mostly type 2 and was not associated with any haemorrhagic complications. HU 'allergy' was seen in 12 patients (7% of those taking HU) and predominantly presented as fevers (9 patients) that recurred in all those re-challenged with HU. Of those taking HU, 8 patients (5%) had leg ulcers and 6 patients (4%) experienced mouth ulcers. 77 patients (41%) had a thrombotic event with the majority of these (67%) occurring prior to or at diagnosis. Conclusions. Cyclical thrombocytosis is not infrequent in all Philadelphia-negative MPNs and appears associated with a higher HU dose. Other features that are intrinsic to the disease (such as acquired vWD) or occur as a consequence of therapy (skin cancers and HU-fever) are also not uncommon.

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FIRST CASE OF KIT SER715DEL AND JAK2 VAL617VAL MUTATION IN SYSTEMIC MASTOCYTOSIS WITH ASSOCIATED ESSENTIAL THROMBOCYTHEMIA

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In March 2009 a 22-years-old woman presented to our Institution with persistent high platelet count (>650x10^9/L) and no other peripheral blood alterations. At physical examination splenomegaly was present; headache, erythromelalgia and diarrhea were referred. Blood chemistry was normal and reactive thrombocytosis was excluded. The morphological examination of the bone marrow (BM) aspirate revealed an increase of enlarged megakaryocytes (MK) with emperipolesis phenomena. BM smears showed also spindle shaped mast cells (MC) with oval and eccentric nuclei and focal granule accumulation. Eosinophil forms were increased. Biopsy specimens were hypercellular and showed a marked proliferation of giant MK, preferentially grouped into clusters; in addition to this myeloproliferative pattern, nodules of ovoid or spindle shaped MC were present, surrounded by areas of localized dense network of reticulin fibers. MC identity was confirmed by toluidine-blue and immunohistochemical staining for anti-CD117 (KIT) and CD2. Cytogenetic analysis revealed a normal karyotype. A specific immunophenotypic analysis of MC was not performed at this time. The allele-specific-PCR demonstrated the presence of the JAK2V617F mutation. DNA sequencing did not confirm the G>T substitution on the base 1849 codifying for the Val617Phe mutation, but we detected a G>T substitution on the nucleotide 1851 codifying for a silent Val617Val mutation. FIP1L1-PDGFRA and BCR-ABL transcripts search was negative. DNA sequencing of KIT gene, did not show D816V mutation, but detected a three nucleotides deletion (ACG; bases: 2143-2144-2145), on the exon 15 that bring to a serine 715 deletion. Systemic Mastocytosis (SM) clinical staging showed not skin involvement, normal bone density and a spleen volume of 500 mL by ultrasound; serum tryptase was 17,6 ng/mL. We concluded for SM with Associated clonal Hematological Non-Mast cell lineage Disease (SM-AHNMD), id est Essential Thrombocyhemia. Because of symptoms we began treatment with IFN-α-2a at 3 MU five times a week. The therapy was well tolerated, all

symptoms disappeared and the platelet count was lowered to less than 350 x10^9/L. After four months of therapy, IFN-α dose was tapered to three times a week. The BM aspirate was repeated after eleven months, showed the absence of MC and a MK reduction, while the BM biopsy demonstrated persistence of MC nodules. The specific immunocytofluorimetric assay was negative. The molecular analyses confirmed the Ser715 del on KIT gene but the JAK2V617V silent mutation was undetectable. The tryptase serum level was 20,9 ng/mL. The spleen volume was 290 mL and resulted reduced compared to the baseline. This is the first case of a SM-AHNMD harboring a silent mutation on JAK2 and Ser 715 del on KIT. This KIT deletion has been previously described in GIST and if it is an activating mutation or a gene polymorphism is still matter of debates. The significance of this mutation for myeloproliferative disease is unknown. However this mutation leads to deletion of a polar amino acid placed near Tyr719 which is necessary for KIT PI3K binding and downstreaming; this mutation could induce abnormalities in KIT local folding and signaling and contribute to pathogenesis of both MC and MK disease.

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HYDROXYUREA PLUS ANAGRELIDE COMBINED THERAPY IS MORE SUITABLE THAN SEQUENCE SCHEDULE OF THE TWO DRUGS

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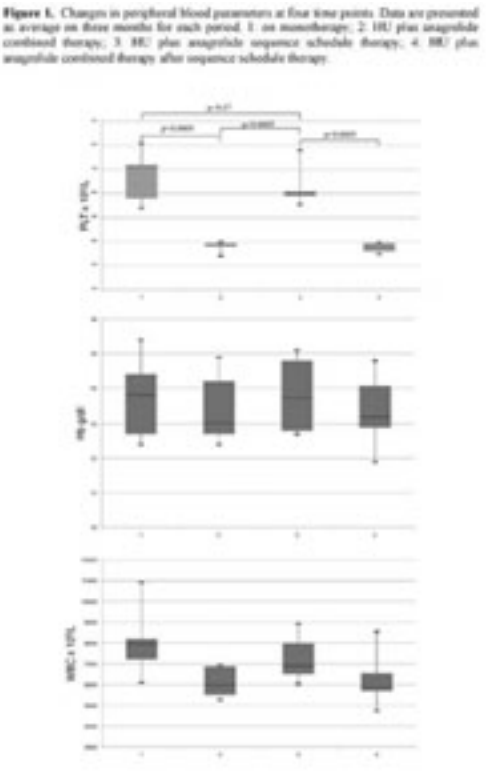
Background. Hydroxyurea (HU) and anagrelide are widely used treatment of Essential Thrombocythemia (ET). Given their different mechanisms of action, they can be used as combined therapy at reduced doses. This association could represent a second-line treatment in patients considered resistant or intolerant to HU, or a therapeutic option to reduce both HU and anagrelide related side-effects. The combined use of these two drugs gives a more balanced control of the hematological parameters, improving the platelet lowering action and better controlling WBC count, known to be correlated with thrombotic risk. We avoid larger doses of either drug because anagrelide is ineffective in lowering WBC count, and higher doses of HU have an unwanted Hb lowering action. Aims. We describe a combined sequence HU plus anagrelide therapy to improve patients' adherence to therapy and quality of life. Patients and methods. Five ET patients received a combined sequence therapy of HU and anagrelide. They were a spontaneously formed cohort of patients treated with others medicaments for comorbidities. They casually requested that the daily numbers of pills be reduced. We used a sequence therapy based on the following schedule: HU at a dose of 1 g daily for 10 days, followed by anagrelide at a dose of 1 mg daily for 10 days for three months. ET diagnosis was based on the 2008 WHO criteria. Patients' mean age was 48.6 years: 3 females and 2 males. Three were JAK2V617F positive. Results. All patients received HU as first line treatment and none achieved complete hematological response (CHR); this resistance was dealt with administering a schedule of HU and anagrelide combined, at a daily dose respectively of 1 g and 1 mg, for a total amount of 4 daily pills. All patients achieved CHR. Mean platelet counts compared to the one while on monotherapy, were 673,3 vs 379,4x10^9/L (p=0,0004). (fig.1.1vs2). Given the patients' need to take other pills we began to schedule a sequence therapy. The side effects reported decreased, but in all patients platelet count increased. Platelet count means were 379,4 vs 626x10^9/L (p=0,0003) respectively on combined and sequence therapy (fig.1.2vs3). Due to the lack of CHR, we interrupted the alternative sequence schedule and returned to the previous combined and continued therapy. Patients achieved CHR after only one month. The mean platelet counts compared with the sequence therapy were 626 vs 374,5x10^9/L (p=0,0002) (fig.1. 3vs4). Conclusions. Our data confirm that HU and anagrelide have a synergic or at least additional effect on platelet count reduction. Indeed, all patients, who took the two drugs simultaneously, achieved rapid CHR, which had never been reached with the previous monotherapy treatment. The sequence therapy, instead, was not as effectively as the combination therapy. Furthermore, patients' mean platelet counts were similar to those during monotherapy (673,3 vs 626x10^9/L; p=0,47), demonstrating the loss of CHR (fig.1.1vs3). Despite the availability of some management recommendation and guidelines on ET treatment, therapy based on the association of HU and anagrelide has not yet been codified.



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CHROMOSOMAL TRANSLOCATION T(15;17)(Q22;Q25) WITH PML-RARA POSITIVITY IN PATIENT WITH PRIMARY MYELOFIBROSIS (PMF)

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Background. Primary myelofibrosis (PMF) is a clonal stem cell disorders characterized by splenomegaly, fibrotic bone marrow, extramedullar hematopoiesis and leukoerythroblastic blood smear with dacryocytes. Approximately, one third of patients with PMF have cytogenetic abnormalities at diagnosis. They are respected as favorable (isolated abnormalities like 13q-, 20q- or +8) but also unfavorable (all other ones). Aims: We are presenting a male patient with PMF and with unusual cytogenetic abnormality, translocation t(15:17)(q22;q25) and JAK-2 V617F mutation. Patient and methods: A 58-year old man was diagnosed with a six months history of abdominal fullness, weakness, weight loss and pain in left upper abdomen. In August 2009, he was referred for further diagnostic work-up to Clinics of Hematology CCS, Belgrade. The diagnosis of PMF was confirmed by bone marrow biopsy and further clonality assays were performed by karyotyping and molecular biology. PCR was applied for detection of JAK-2 V617F mutation and BCR-ABL transcripts. Afterwards, RNA was extracted from the bone marrow sample and RT-PCR has been performed according to BIOMED-1 protocol in order to analyze PML/RARα fusion transcript. Results: Physical examination revealed splenomegaly, +10cm below LCM, or 22 cm on CT scan. Patient had normal hemoglobin, 142 g/L, slight leukocytosis 18,9x10^9/L with mild shift to the left and normal platelet count, 279x10^9/L. Standard blood chemistry tests were within normal range. Core bone marrow biopsy corresponded to PMF (clusters of hyperlobulated megakaryocytes and grade 3 fibrosis). Cytogenetics from bone marrow revealed balanced translocation t(15:17)(q22;q25) in 15 metaphases and 3 metaphases with addition on chromosome 18, add(18)(p11). According to International Prognostic Scoring System patient was low risk. PCR detected bcr1

fusion transcript of PML/RARα. This result was subsequently confirmed by direct sequencing of PML/RARα gene. JAK-2 V617F mutation was also positive. BCR-ABL rearrangement was absent. Patient commenced hidroxyurea 1g/d and after 16 months he reduced spleen to 16 cm (CT) and normalized blood counts, without any signs of acute leukemia during follow-up. Conclusions: The occurrence of PML-RARα and JAK-2 V617F mutation simultaneously in the same patient is exceptionally rare event. Even that our patient had unfavorable cytogenetic profile with JAK-2 mutation indicating adverse predictors for survival, he had a good response with hidroxyurea treatment, and no evolution into promyelocytic leukemia.

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SYSTEMIC MASTOCYTOSIS WITHOUT CKIT D816V MUTATION TREATED WITH DASATINIB; A CASE REPORT

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Background: Systemic mastocytosis (SM), often termed systemic mast cell disease (SMCD), is an hematologic clonal disorder characterized by abnormal growth and accummulation of Mast Cell (MC) in one or more organs. It is now considered a myeloproliferative neoplasm (MPN) in the 2008 revision of the WHO classification of myeloid neoplasms. The clinical symptoms and signs of SM are due to the accumulation of clonally derived mast cells in different organs, including bone marrow, skin, gastrointestinal tract, liver and spleen. In more than 80% of the patients, the cKIT mutation D816V is detectable. Those cases without the cKIT D816V mutation respond to Imatinib not to Dasatinib. We report a case of a SM without the cKIT D816V mutation, that achieved a good response to dasatinib after failing previous imatinib therapy. 2. Case report: A 77 year old man was admitted to our hospital because of astenia, hyporexia and fever during the three previous months. Blood analysis showed anemia, leucocytosis with monocytosis, thrombocytopenia, elevated GGT, alkaline phosphatase and hyperferritinemia. Abdominal ultrasound and thoraco-abdominal CT-scan showed homogeneus hepatomegaly, mild splenomegalia and retroperitoneal lymphadenopaties. Bone marrow biopsy revealed the presence of an excess of mast cells expressing CD2, CD25 and CD117 in flow cytometry testing. Tests for bcr-abl, JAK-2 and cKIT D816V mutations were negative and karyotype was normal. The hepatic biopsy reveled infiltration by MC. With diagnosis of SM, we started treatment with hydroxycarbamide 1gr/day and imatinib 400mg/day. One month later, our patient complained of intense asthenia, dyspnea and malaise. Abdominal ultrasound revealed moderate ascites and bilateral pleural efussion; This was considered a disease progression. We stopped imatinib and started prednisone 1mg/kg/day. Despite this treatment the disease progressed and caused weekly need of platelet and packed red cell transfusion. Finally we added dasatinib 70mg/day to prednisone. Four months later, astenia and dyspnea have improved considerably, as have ascites and patient's status. No new trasfusion have had to be made since the start of dasatinib therapy. As a side effect of the treatment the patient presented left pleural efussion that prompted dasatinib stop and hospital admission. Dasatinib was reintroduced after pleural evacuation and has maintained to date without significant side effects. *Conclusions*. Dasatinib shows promise in the treatment of systemic mastocytosis negative for D816V mutation in cKIT.

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BUSULFAN: STILL A USEFUL THERAPY FOR ESSENTIAL THROMBOCYTHAEMIA (ET) AND POLYCYTHEMIA VERA (PV)

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Background. The chronic myeloproliferative disorders ET and PV are relatively common disorders with higher prevalence in the elderly. These disorders can lead to major morbidity and mortality through both disease progression/transformation and vascular events. A number of agents are available for cytoreduction in these disorders. Busulfan, a non-specific alkylating agent, has been in therapeutic use for over 50 years. Although other agents are often preferred (in part due to concern over leukemogenesis), Busulfan usually means less clinic attendance which makes it particularly useful in the elderly. Aims: To analyse the effectiveness of Busulfan in terms of blood count control and the incidence of thrombotic complications. To assess the incidence of side effects, such as leukaemic transformation and lung fibrosis.Methods: We retrospectively analysed the clinical records of 53 patients who received oral Busulfan for a diagnosis of ET (32) or PV(21) during the period Jan 2006 - Jan 2011. We assessed for vascular events, leukaemic transformation, platelet count / Hct at 0 months, 6 months and 12 months from commencing Busulfan. We recorded the indication for utilising Busulfan as opposed to other cytoreductive agents. Results: 53 patients were analysed (mean age at commencing Busulfan 81 years, range 44-94). Mean cumulative Busulfan dose was 270.5mg (range 18mg - 1106mg), given as intermittent short courses. A total of 2,435 patient-months of Busulfan therapy (mean 45.9 months per patient) were analysed. There was only 1 leukaemic transformation (possibly related to prior therapy). There were no cases of lung fibrosis. There was 1 definite arterial event (TIA) and 1 possible venous event (femoral DVT), thus there was only 1 clear (+ 1 possible) treatment failure in a cumulative 202 patient years of treatment. PV patients had a mean Hct at commencement of Busulfan of 0.492 l/l. At 6 months post commencement of Busulfan, mean Hct was 0.448 l/l, and at 12 months mean Hct was 0.454 l/l. For ET patients mean platelet count at commencement of Busulfan was $871x10^9$/l. At 6 months post commencement of Busulfan, mean platelet count was $478x10^9$/l, and at 12 months mean platelet count was $407x10^9$/l.Of the 33 patients who received Busulfan as a first line cytoreductive agent, logistical ease was recorded as the reason in 32 patients. 20 patients received Busulfan as a second line cytoreductive agent; 5 patients switched to Busulfan due to mouth/skin ulcers with Hydroxycarbamide; 8 patients were switched to Busulfan for greater logistical ease. Summary/Conclusions: Oral Busulfan for both ET and PV is both safe (with a low rate of leukaemagenesis and pulmonary fibrosis) and effective (with a low rate of vascular events), with reasonable control of platelet count or Haematocrit. Busulfan also offers the advantage of being logistically easier to deliver without risk of skin/mouth ulceration.

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THE RISK PROFILE FOR THROMBOTIC EVENTS IN EARLY PREFIBROTIC PMF - LEUKOCYTOSIS IS A RISK FACTOR FOR ARTERIAL THROMBOSIS IN EARLY PREFIBROTIC PMF BUT NOT IN ET

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Background. There is strong evidence indicating that the clear-cut separation of prefibrotic primary myelofibrosis (pPMF) from essential thrombocythemia (ET) by consequent application of the WHO 2008 criteria is reflected in different, well defined clinical pictures and divergent prognoses. All published data on vascular events in ET and primary myelofibrosis (PMF) so far were exclusively based on the outdated criteria of the Polycythemia Vera Study Group. Consequently risk profiles for vascular events within the sub-entities of Bcr-Abl negative myeloproliferative neoplasm ask for re-assessment. We aimed to evaluate whether patients with pPMF have a distinct risk profile for vascular events.Methods: Risk and risk profiles for vascular complications were determined in 87 patients from our database with a valid diagnosis of pPMF according to the WHO 2008 criteria and compared to a cohort of 127 patients diagnosed with WHO-defined ET. Results: Leukocytosis and a JAK2V617F mutated genotype emerged as significant risk factors for arterial thrombosis after diagnosis in pPMF, whereas in WHO-diagnosed ET generic vascular risk factors such as arterial hypertension and diabetes mellitus enhanced the risk for arterial thrombosis. Conclusions: Our results challenge the current knowledge of established and suspected risk factors for thrombosis and certainly need to be confirmed in larger studies. If validated, the finding of a different relevance of certain risk factors within the increasing variety of sub-entities will certainly change the current treatment strategies and help to better allocate patients to the appropriate treatment.

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FIRST RESULTS FROM THE GREEK ESSENTIAL THROMBOCYTOSIS REGISTRY

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In this study we present the first results from the registry of Greek patients with essential thrombocytosis, which has been developed by the study group for myeloproliferative neoplasms of the Greek Society of Hematology. There are 352 patients included in the study. 42% of them are male. Median age at diagnosis was 60 years. An elevated platelet count was a routine test finding in 80.5% of them. Thromboembolic and hemorrhagic events before the onset of treatment were observed in 9.1% and 2.9% respectively. In 21.7% there was a history of cardiovascular disease. Coronary disease was reported in 14.6%. JAK-2 V617F mutation was detected in 55.1% of the 352 patients. In 71% of them no fibrosis in trephine biopsy was detected, whereas fibrosis grade I and II was found in 19% and 9% respectively. 18.8% of them had splenomegaly at diagnosis. Watchful waiting strategy for more than 6 months was initially adopted in 155 patients (44%). First-line therapies were hydroxyurea, anagrelide and interferon in 76.7%, 13.4% and 7% patients respectively. Hudroxyurea was the treatment best tolerated and exhibited the best response rates in comparison to anagrelide and interferon (93.2.% vs 73.9% and 80.6% respectively).Thrombosis or hemorrhage during therapy was observed in 4.5% and 2.1% respectively. Secondary malignancies developed in 6.3% of the patients, 2.7% of them being hematologic. Comparing the presence of JAK-2 mutations with age we observed that patients bearing the JAK-2 mutation tend to be older (64 vs 56 years old, p=0.0002) and to have higher hemoglobin values (14.08 vs 13.32, p=0.0001). White blood cell count and platelet count does not seem to correlate to JAK-2 mutation. No correlation was also observed between JAK-2 mutation presence and fibrosis or splenomegaly. The probability for thrombosis was higher in patients with elevated haemoglobin levels but the difference was not statistically sighnificant.

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VERY LOW DOSE LENALIDOMIDE CAN RESULT IN PROLONGED TRANSFUSION INDEPENDENCE IN PATIENTS WITH MYELOFIBROIS

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Background: Myelofibrosis is frequently associated with red blood cell transfusion dependence resulting in significantly reduced quality of life and complications including iron overload. Lenalidomide has been shown to be effective in some patients with myelofibrosis, but can result in neutropaenia and/or thrombocytopaenia. Aims: To analyse the effects of very low dose lenalidomide on transfusion requirements in patients with myelofibrosis. Methods: We describe 5 patients (aged 55-70 years old) with an established diagnosis of myelofibrosis - five with idiopathic myelofibrosis and one with myelofibrosis secondary to Polycythemia Vera - who were treated with very low dose Lenalidomide. The starting dose was 5mg daily or on alternate days, increasing to a maximum dose of 10mg daily. Three patients also received Erythropoietin. *Results*. Two patients were highly transfusion dependent prior to treatment, requiring two units of red cells every 1-2 weeks. Following two months of lenalidomide treatment their transfusion requirements significantly decreased (1-2 units every 2-3 weeks). One patient (treated with lenalidomide 5mg on alternate days) became transfusion independent 4 months after treatment was commenced. It was possible to discontinue his erythropoietin therapy (haemoglobin 12.9g/dl) and and he is now being treated for transfusion-related iron overload with therapeutic venesection. A third patient treated with both lenalidomide and erythropoietin became transfusion independent a month after lenalidomide was commenced, and continues to maintain a haemoglobin at the level of 10.5-13.9g/dl. Two patients treated with very low dose lenalidomide were anaemic but did not require any red cell transfusion prior to therapy. Both had a significant haematologic response, with an increase in haemoglobin of > 2g/dl after 2 months of treatment. No patients required therapy discontinuation or interruption due to neutropenia or thrombocytopenia. *Conclusions*. Very low doses of lenalidomide (5mg on alternate days) can produce dramatic clinical responses in some patients with myelofibrosis, and sustained transfusion independence can be achieved. Late responses (>4 months after commencing therapy) may occur. Starting lenalidomide at very low doses in patients with myelofibrosis may be an important strategy to avoid therapy interruptions due to cytopenia.

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INCIDENCE AND RISK FACTORS OF SPLANCNIC VENOUS THROMBOSIS (SVT) IN CHRONIC MYELOPROLYPHERATIVE NEOPLASMS (CMNS)

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Several data in literature underline the clinical and pathogenetic role of JAK2 V617F mutation in CMNs. Its role in additional thrombotic risk in these patients is still under discussion. The same mutation has been identified as occult marker in several SVT patients; in particular, 45% of patients with Budd-Chiari syndrome (BCS) and 34% of portal vein thrombosis patients (PVT) have associated CMNs. The main BCS patients without CMNs present an additional congenital or acquired thrombosis risk factor. The aim of our study is to evaluate SVT incidence in CMNs patient followed up in our Istitution and the presence in the same population of other prothrombotic risk factors. Out of the 405 CMNs Ph1 negative patients retrospectively evaluated (jun 2000-jun 2010), 9 (7 females and 2 males) (2,2%) presented SVT. 6 of them had essential thromobocitemia(ET) 2 idiopatic myelofibrosis(IM) and 1 polycitemia vera(PV).Among ET incidence were 3% and 5/6 were femals They have been treated with antiagregant and anticoulant therapy . 6 have been treated with idrossiurea. All of them are JAK2 V617F positive. 7 patients had SVT before CMNs diagnosis, 2 of them had splenectomy. The other 2 CMNs patients have had a SVT diagnosis during routine CMNs follow up. 75% of SVT/CMNs patients had one prothromobtic risk factor, at least (factor V Leiden, Protein C deficience, hyperhomocystinemia) and 50% had 2 or more associated defects. The remaining CMNs patients (396) had a less significant prevalence of prothrombotic risk factors and developed other venous thrombosis: wbc, platelets values and JAK2 V617F mutation correlate with the thrombotic event The 7 patients had SVT before CMNs diagnosis no other major thrombotic event has been occurred during follow-up. Conclusion: Even SVT has a low incidence in CMNs patients, we discuss on the potential benefit of searching for other prothrombotic risk factors in the whole population in order to properly treat with anticoulant therapy.

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MORPHOMETRIC ANGIOGENESIS PARAMETERS FOR INDOLENT AND AGGRESSIVE NON-HODGKIN'S LYMPHOMA

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There is much evidence about importance of angiogenesis in development and progression of solid tumors. The role of angiogenesis, as an indicator of higher malignant potential in non-Hodgkin's lymphoma, is not clear at the moment. Morphometric characteristics of microvessels in lymph node sections in previously untreated patients with small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) and diffuse large B-cell lymphoma (DLBCL) were studied and relationship between angiogenesis and NHL histological malignancy grade was also evaluated. Lymph node biopsies samples of 30 newly diagnosed patients with SLL/CLL (n= 30) and DLBCL (n = 30) were studied. All samples were fixed in 10% buffered formalin solution and embedded in paraffin. Microvessels were visualized by immunohistochemical staining for anti F-8 antibody. In the area showing the most intense vascularization (i.e. the "hot spot"), microvessel density (MVD), total vascular area (TVA), as well as the size related parameters were estimated, by using image analysis program"analysSIS". *Results*. Number and size-related microvessels angiogenic morphometric parameters were statistically higher in group with DLBCL compared with SLL/CLL: MVD ($p=0.002$), TVA ($p<0.0001$), area ($p<0.0001$), perimeter ($p<0.0001$), minor axis length ($p<0.0001$) and major axis length ($p<0.0001$). It is to be noted that positive correlation existed between TVA and MVD in DLBCL and SLL/CLL. *Conclusions*. The present study supports the view that angiogenesis correlate with histological grade of NHL.

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NUCLEAR FACTOR-KB ACTIVATION IN PRIMARY LYMPHOMA OF BONE

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Background. Primary lymphoma of bone (PLB) is a rare type of extranodal diffuse large B-cell lymphoma (DLBCL) with a relatively indolent clinical behaviour and a favourable outcome. Previous reports have demonstrated a subdivision of PLB in germinal center (GC) and non-GC phenotype by immunohistochemistry. Recent scientific interest has focused on elucidating the role of nuclear factor (NF)- κB pathway in lymphomagenesis and its possible value as a therapeutic target. In nodal B-cell non Hodgkin lymphomas, constitutive activation of NF-κB appears to be especially involved in tumour cell survival in the non-GC type of DLBCL, although it is not exclusively restricted to this subtype. *Aims*. We here investigate NF-κB activation in PLB with GC and non-GC phenotype. *Material and methods*. In order to assess involvement of NF-κB activation in PLB, immunohistochemical staining procedures for NF-κB family members p50, p52 and p65 were performed on paraffin-embedded tumour tissues of 50 cases of PLB. Results: Nine cases (16% of our cohort) showed nuclear positivity for p50, and one case showed nuclear co-expression of p52. Positivity for p50 was not restricted to either GC- or non-CG phenotype of the tumour (20% and 25%, respectively), or related to an inferior prognosis or treatment resistance. P65 did not show significant nuclear staining. *Summary and conclusions*. The immunohistochemical nuclear staining for p50 in 16% of the cases suggests constitutive activation of NF-κB via the classical pathway in a minority of PLB patients. In contrast to other extranodal types of DLBCL, there was a lack of nuclear co-localization of p65 staining, which may suggest different pathway activation. The alternative pathway of NF-κB activation does not appear to be involved, as only one case showed significant nuclear staining for p52. Finally, nuclear expression of p50 was not preferentially detected in non-GC type or GC-type PLB, or related to an inferior prognosis.

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DIAGNOSIS OF NON HODGKIN'S LYMPHOMAS (NHL) BY FLOW CYTOMETRY (FC) ON LYMPH NODE CELL SUSPENSIONS AT BLIDA , ALGERIA

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Background. The diagnosis of malignant lymphoma is primarily based on histological examination. The FC is a powerful tool for cell analysis that helps a closer definition of cell proliferation. *Aims*. The aim of our study is to compare the FC analysis of cell suspensions obtained by grinding lymph node biopsy or fine-needle aspiration with histologic diagnosis. *Methods*. The diagnosis of NHL is confirmed by histological study on lymph node biopsy. The biopsy was divided into two fragments: one for pathological studies, the other is crushed and put into a culture medium to obtain a cell suspension; If the biopsy is deferred, a fine-needle aspirate of lymph node is performed. Once the cell suspension obtained, cells are washed with phosphate-buffered saline to remove cytophilic antibodies and then treated by FC ; FC immunophenotyping was performed with antibodies targeting T, B and NK lymphoid populations (CD3, CD2, CD4, CD8, CD5, CD7, CD1a, αβTCR, γδTCR, kappa and lambda light chains, CD19, CD20, CD22, CD10, CD43, CD79a, CD79b, CD23, CD25, CD11c, IgM, CD56, CD34 ; the acquisition was made using four-color multiparameter flow cytometry. Detection of immunoglobulin ligtht- chain-restriction was used for diagnosis of B NHL.Detection of multiple abnormalities in the sames cells and restriction of TCRs were used for diagnosis of T NHL. Results. Cell suspensions from 67 patients were included in this study: they involve 19 women and 48 men with an average age = 41 years (15 -76). A fine-needle aspirate of lymph node is performed in 42 patients (68.7%) with a mean of 13752 cells / µl (800-71 500) and lymph node crush in 21 patients (31.3%), with a mean of 11055 cells/µl (900-64560) ;in 04 cases, number of celles was insufficient (<100 cells / µl) for analysis. Thus out of 63 analyzed cell suspensions, FC showed no monoclonality of B or T in 10 cases for which histology revealed : Hodgkin lymphoma : 02 cases carcinoma: 01case, Tuberculosis : 01 case and a non specific lymphadenitis: 06 cases; in this group, concordance between FC and Histology was 80%. In 10 cases the histology was not available, the CMF showed the monoclonal proliferation of B cells in 7 cases and of T cells in 3 cases. In 43 cases, when biopsy was available, the FC has concluded for: B-cell NHL in 33 cases (76%) and T cell NHL in 10 cases (24%). In the group where the monoclonality was confirmed by FC, when histology was available (43 cases), the correlation between FC and histology was 90.8% ; discordance was related to 4 cases (9.2%) with advantage for FC in 2 cases and histology in 2 cases. Summary. Because the WHO classification of NHL incorporate immunophenotypic criteria, FC helps to better characterize these entities. In our study, FC has been contributory to the diagnosis of NHL, specifying monoclonality and has shown the absence of monoclonality in benign lymphoid hyperplasia, undifferentiated carcinomas and Hodgkin's disease. The CMF is a reliable and quick method for the evaluation of NHL.

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HIGH KI 67 INDEX IS A POOR PROGNOSTIC FACTORS FOR DLBCL TREATED WITH CHOP WITH OR WITHOUT RITUXIMAB

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Background : Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease and patients exhibit a wide range of outcomes. The addition of Rituximab to CHOP chemotherapy (R-CHOP) has led to a marked improvement in survival and has altered the significance of previously recognized prognostic markers. Aim: we performed a retrospective analysis of 111 patients with de novo DLBCL to assess the impact of clinical variables and biologic features on Response Rate (RR), Overall Survival (OS) and Progression Free Survival (PFS). Methods: 58 patients were treated with R-CHOP (post-Rituximab era) and 53 with CHOP (pre Rituximab era). For each case the following clinical data were recorded: patient's age, sex; performance status, B-symptoms; serum lactate dehydrogenase (LDH); serum β2microglobulin (β2µg); bone marrow involvement; bulky disease; extranodal disease; clinical stage and International Prognostic Index (IPI). A maximal tumor burden > 10 cm was defined as bulky disease. Immunohistochemical analyses were performed: Bcl2 expression was scored positive when at least 50% of tumor cells expressed the protein; proliferative index assessed by ki-67 immunostaining was scored using a cutoff of >80%. Results: the variables predictive of RR in CHOP group were B symptoms (P=0.047), age > 60 years (P=0.045), clinical stage (P<0.032), bone marrow involvement (P<0.045), bulky disease (P<0.007), IPI risk group (P<0.001) and bcl2 expression (P<0.002); in the R-CHOP group they were bulky disease (P<0.002); bone marrow involvement (P<0.049), IPI risk group (P<0.011), and Ki67 expression >=80% (P<0.049). At multivariate analysis, in patients treated with CHOP the independent prognostic factors associated with PFS were age, bulky disease, IPI risk group and bcl2 expression; associated with OS were Performance Status, clinical stage, IPI risk group and bone marrow involvement. By contrast, among patients treated with R-CHOP, the variables proving to be independent prognostic factors were bulky disease on PFS, and Ki67 expression >=80% on OS and PFS. Conclusions: Our data show that a high proliferative index could represent possible predictive factor of poor prognosis, which would help to identify a high risk subgroup of newly diagnosed DLBCL. Further large-scale and prospective studies will be required to confirm these results.

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GSTP1 POLYMORPHISM AS A PREDICTOR OF CLINICAL OUTCOME OF THE THERAPY OF NON-HODGKIN'S LYMPHOMA IN ELDERLY PATIENTS

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Background. The worldwide incidence rate of non-Hodgkin lymphoma (NHL) increases on 2-3 % annually. Therapy of NHL was improved over the last two decades, but prognosis for a half of patients still remains poor. Glutathione S-transferase P1 (GSTP1) is a member of the GST enzyme superfamily involved in the metabolism and detoxification of chemotherapeutic agents. The proteins encoded by the different alleles of the gene show different abilities to metabolize carcinogens and anticancer agents. Therefore, genetic polymorphism of GSTP1 could be essential in the determination of the susceptibility to the toxic effects of chemicals, and might also be involved in the tumor response to anticancer drugs and influence clinical outcome of patients. Aim. The aim of this study was to evaluate the predictive value of the GSTP1 Ile105Val polymorphism on clinical outcome after the therapy of elderly patients with NHL. *Materials and methods*. The case group was comprised of 60 patients with B-cell NHL (small cell: 35, large cell: 25; median age: 70 years, range: 60-85; males: 33, females: 27, stages I-II: 17, stages III-IV: 43) and 158 blood donors (median age: 38 years, range: 17-59; males: 69, females: 89). The NHL type was diagnosed according to the World Health Organization (WHO) classification. Patients were categorized by the Ann Arbor staging system and the International Prognostic Index. Chemotherapy regimens CHOP or CNOP were administered for all

patients enrolled in the study and radiotherapy by indications. The response to the therapy was scored according to International Working Group criteria (1999, 2007). Genomic DNA from peripheral blood of all individuals was analyzed for identification of GSTP1 genotypes using TaqMan Polymerase Chain Reaction (PCR) allelic discrimination assays. Results. The genotypes of the GSTP1 gene in both control and patient groups did not differ significantly from those predicted by the Hardy-Weinberg distribution. Observed Val allele frequency was 0.32, similar to previous reports on allele frequencies for healthy Caucasians. The frequency of GSTP1 homozygous wild genotype was higher in patients with advanced stages (III-IV) than in patients with localized stages (I-II) (60,5 % versus 29,4 %, P = 0.02). Overall response rate after the first line therapy was better in patients with increasing number of Val alleles 68.25% comparative to 27.1% in patients with homozygous wild genotype (P=0.02). We observed a correlation between GSTP1 homozygous wild genotype and unfavorable prognosis for patients with advanced NHL: among patients with relapse 82 % were of Ile/Ile genotype. Moreover we did not noticed early relapses in patients with Val/Val and Ile/Val genotypes, while patients with Ile1/Ile have shown early relapses in 26,1 % of cases (P = 0.05). We carry on investigations with larger cohorts of patients for further assuring of our preliminary results. *Conclusions*. The results suggest that the Ile105Val polymorphism of the GSTP1 gene may predict clinical outcome of the therapy of elderly NHL patients. Hence the investigations of GSTP1 polymorphism are very promising, since it might provide a possible application of this genetic marker as an independent prognostic factor of NHL.

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THE IMMUNOPHENOTYPE OF MALIGNANT LYMPHOCYTES IN SPLENIC MARGINAL LYMPHOMA ASSOCIATED WITH HEPATITIS VIRAL INFECTION

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Background: Splenic marginal zone lymphoma (SMZL) is a rare, indolent subtype of lymphoma which accounts for less than 2% of all non-Hodgkin's lymphomas. It is the most frequent small B-cell lymphoma associated with splenomegaly and probably the most frequent splenic lymphoma. An important issue is the role of hepatitis C virus (HCV) infection in SMZL. Approximately 10%-15% of SMZL do occur in the setting of chronic hepatitis C. This association underlines the importance of antigenic stimulation in the proliferation of the tumor cells in HCV-associated. SMZL express surface IgM and IgD and are usually CD19+, CD20+, CD22+, CD79a+, FMC7+, CD10-, CD123-, and CD103-; DBA44, CD11c, CD23 and CD5 can be positive in a subset of cases. Cyclin D1/BCL-1 is not expressed, whereas the BCL-2 protein is intensely expressed. *Aims*: to study the immunophenotype of malignant lymphocytes in splenic marginal lymphoma with and without hepatitis viruses and highlight the differences that could guide the process of monitoring and treating these patients. *Methods:* we analyzed 14 patients diagnosed with splenic marginal zone lymphoma in the Hematology Department of Emergency University Hospital Bucharest; 5/14 patients with chronic infection with HCV or HBV - vSMZL and 9/14 patients without chronic infection with HCV or HBV - SMZL. We performed immunophenotypical analysis of peripheral blood and bone marrow aspirate on BD FACS Calibur flowcytometer, using the following panel of monoclonal antibodies (CD19, CD20, CD22, CD79a, CD79b, FMC7, CD5, CD10, CD23, CD103, CD11c, CD24, CD38, CD43, CD81, IgM , IgD, BCL-2) . Histological findings were combined with clinical, immunophenotypic, and laboratory data. Results: Patients' age ranged from 45 to 73 years (median, 65 years) with a male-to-female ratio of 1:1.4. 14/14 patients had splenomegaly, 14/14 patients had bone marrow involvement. Regarding the laboratory features, we observed differences between the 2 groups concerning: anemia, which was present in 4/9 patients with SLVL versus 4/5 patients with vSMZL; thrombocytopenia - present in 2/9 patients (SMZL) versus 2/5 patients (vSMZL), and higher LDH level, respectively 4/9 patients (SMZL) versus 3/5 patients (vSMZL). The immunophenotype was classical in most cases, but 1patient/14 was CD5+. We found important differences in the expression of the following markers: Md (median fluorescence intensity) 10 for SMZL versus 37 for vSLVL, CD38: Md 15 versus 43, CD43: Md 13 versus 35. The free light chain κ/λ ratio was: KAPPA+ (Md 7/1 vs. 15/1), LAMBDA+ (2/1 vs. 2,5/1). *Conclusions:* Splenic marginal zone lymphoma is often associated with chronic hepatitis viral infection. These patients must be considered as a special group with special clinical, laboratory findings and special treatment. The expression of CD 23, CD38, and CD43 (higher in vSMZL group than in SMZLgroup) in splenic marginal lymphoma might be related to viral antigenic stimulation and not to an aberrant phenotype. After antiviral therapy, the intensity of activation B-cell markers and light chain ratio could be used as a surrogate marker of the control of the HCV-related lymphoproliferation.

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IMPACT OF THE REACTIVE MICROENVIRONMENT ON THE BONE MARROW INVOLVEMENT OF FOLLICULAR LYMPHOMA

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Background. Follicular lymphoma (FL) is an indolent B-cell non-Hodgkin's lymphoma (NHL) which comprises about 20% of all NHLs in Europe and North America. Gene expression and immunohistochemical studies suggested that immune microenvironment of FL plays an important role in clinical behavior and progression of the disease: expression of genes which are enriched in T-cells and macrophages was associated with a favorable outcome, whereas genes which derived from macrophages and FDCs were associated with inferior outcome. In 40-70% of FL cases the bone marrow (BM) is also involved at diagnosis which has been considered as a poor prognostic factor. Although lymph node (LN) and BM compartments of FL are related to the same neoplastic clone, several morphologic, phenotypic and genotypic differences have been reported between the tumor cells within these two compartments. The cytological grade of the tumor is usually lower in the BM than in the LN; FL cells of the BM frequently lose the expression of BCL-6 and CD10 and the mutation pattern of IgH variable region genes of FL cells shows many differences between BM and LN. Aims: To investigate the role of the microenvironment in the bone marrow involvement of FL, we performed immunophenotypical analysis of the reactive cell populations in the lymph nodes and corresponding bone marrows of 35 patients with FL. The microenvironment patterns of the BM infiltrates were compared to the corresponding features of the LN in cases with BM manifestation; and the LN microenvironment was compared in FL cases with and without BM involvement. *Methods*. Automated image-segmentation-based localization and quantitation was performed in whole digital slides of immunostained tissue microarrays of formalin-fixed paraffin-embedded tissue biopsies. Results: We found significantly more CD8+ cytotoxic T-lymphocytes, FOXP3+ regulatory T-lymphocytes, and CD68+ macrophages and less PD1+ follicular B-helper T-lymphocytes in the BM than in the matching LN samples. Furthermore, we observed significantly less infiltrating CD8+ T-cells and CD68+ macrophages in cases involving the bone marrow compared to those localized only to the lymph nodes. *Conclusions*. Our study showed that the lower grade and proliferation of the BM could be explained by the different composition of the microenvironment in the BM. On the other hand, different tumor cell growth in the LN and BM may generate different microenvironment. Our study also suggested that cytotoxic T-lymphocytes and macrophages play a relevant role in the prevention of tumor cell propagation and migration and their elevated number in LNs prevents BM infiltration of neoplastic cells in FL.

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THYROID LYMPHOMA CONSISTENTLY DIAGNOSED BY FLOW CYTOMETRY OF FINE NEEDLE ASPIRATION BIOPSY SAMPLE

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Diagnosis of primary thyroid lymphoma (PTL) is most commonly based on histopathological (HP) and immunohistochemical (IH) examination of surgical specimen from thyroidectomy and occasionally on flow cytometry (FCM) of cell suspensions. The routine method (HP/IH)

is able to capture a tissue structure but is based on invasive biopsy, is time consuming and sensitive to the quality of the slides. In addition, a range of antibodies used in IH are limited. Furthermore, post-surgical complications may postpone initiation of the curative systemic treatment, and a permanent hormonal substitution is a rule. FCM of a cellular suspension obtained by the fine needle aspiration biopsy (FNAB) of thyroid gland is a safe, rapid, and cost-effective procedure. A broad range of antibodies may be used to determine a simultaneous expression of numerous antigens as well as clonality. Disadvantage of FCM may be possible loss of diagnostic cells and missing tissue structure. The thyroid polyclonal lymphoid infiltrate in Hashimoto thyroiditis (HT) represents the substrate from which PTL may arise. Assess the usefulness of FNAB/FCM in the diagnosis of PTL. We identified 11 cases of PTL in a database of 3000 lymphoma patients (pts) diagnosed by FNAB/FCM between 2000 and 2010. PTL cases were retrospectively reviewed by comparing conventional cytological smears and FCM. Clinical presentation included estimation of performance status (PS), lymph nodes status, LDH level a previous history of HT. Lymphoma monoclonal cells and polyclonal lymphoid reactive infiltrate cells associated with HT were evaluated by antibodies to the range of antigens: CD45, CD10, CD11c, CD19, CD20, CD22, CD23, CD25, CD38, CD43, CD44, CD52, CD16&CD56, CD56, CD79β CD138, FMC7, HLA-DR, bcl-2, CD62L, CD71, surface light/heavy chains of immunoglobulin and pan-T. Lymphoma subtype was defined according to WHO 2008 classification. Patients characteristics: females - 11 pts with a previous history of HT in 3/11 pts, median age - 74 yr (range 52-89), PS 0 - 10/11 pts, LDH > normal levels - 5/9 pts, cervical lymphadenopathy - 4/11 pts. Diagnosis: percentage of PTL cells in examined by FCM suspension was - 52% (range 7-95%). The final diagnosis comprised: DLBCL-NOS (GCB) - 5/11 pts, DLBCL-NOS (non-GCB) - 3/11 pts and MALT, FL, PTCL-NOS -one case each. Immunophenotype of all B-cell PTL (10 pts): $CD45^+$ (10/10 pts), $CD19^+$ (10/10 pts), CD20+ (10/10 pts), $CD22^+$ (10/10 pts), $CD23^+$ (1/10 pts), $HLADR^+$ (9/10 pts), $CD10^+$ (6/10 pts), $CD43^+$ (4/9 pts), $CD44^+$ (6/7 pts), $CD38^+$ (6/9 pts), $CD11c^+$ (7/ 9 pts), $CD25^+$ (1/9 pts), $FMC7^+$ (8/9 pts), $CD5^+$ (0/10 pts), $CD79\beta^+$ (0/4 pts), κ+/ λ- (5/10 pts), λ+/κ- (2/10 pts), κ-/λ- (3/10 pts), IgG+ (3/9 pts), IgG^+ /IgA^+ (1/9 pts), IgM^+ (1/9 pts), Ig- (4/9 pts), $bcl\text{-}2^+$ (3/8 pts), $CD71^{+++}$ {100% of positive cells} (7/10pts); HT: accompanying PTL was observed in 6/11 pts (based on infiltration estimated by FNAB/FCM): percentage of normal polyclonal B (κ+ and λ+) cells in suspension - 6,5% (range 0-27%) in 6/11 pts, percentage of normal T cells in suspension - 38% (range 5-64%) in 11/11 pts. Combined use of FNAB/FCM is a reliable and minimally invasive method for defining subtype of PTL.

1385

MOLECULAR DIFFERENTIAL DIAGNOSIS DISTINGUISHES BETWEEN PRIMARY MEDIASTINAL B CELL LYMPHOMA AND OTHER TYPES OF DIFFUSE LARGE B CELL LYMPHOMA

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Background. Primary mediastinal B cell lymphoma (PMBL) has been recognized as a subtype of diffuse large B cell lymphoma (DLBCL) based on its distinctive clinical and morphological features but differs from other types of DLBCL due to its peculiar immunophenotype and gene expression profile. It was shown [A. Rosenwald et al., 2003] that PMBL is characterized by increase expression level of JAK2, MAL, PDL1, PDL2 and TRAF genes. Aims. To see if quantitative expression analysis of JAK2, MAL, PDL1, PDL2 and TRAF genes may distinguish PMBL cases from other variants of DLBCL with primary involved chest lymph nodes. Patients and methods. In this study there have been investigated biopsy samples of enlarged lymph nodes from 25 pts with DLBCL with primary involvement of mediastinal lymph nodes, biopsy samples of affected lymph nodes from 12 pts with DLBCL without involvement of mediastinal lymph nodes and lymphocytes from 6 normal donors. To evaluate expression level of JAK2, MAL, PDL1, PDL2 and TRAF genes we have exploited RQ PCR with TaqMan hydrolyzing probes. Results. Normal median value of JAK2, MAL, PDL1, PDL2 and TRAF gene expression have been established according the data obtained in the set of normal donors. The gene was considered to be overexpressed if its value was more than normal median value + 3SD. It turned out that in the 12 pts with DLBCL without involvement of mediastinal lymph nodes there was no overexpression of MAL, PDL1 and PDL2 genes. In this group of pts there was only 1 out of 12 (8%) with the overexpression of JAK2 and 2 out of 18 (16%) with the overexpression of TRAF. In the case of 25 pts DLBCL with primary involvement of mediastinal lymph nodes JAK2 gene was overexpressed in the 14/25 cases (56%), MAL - in the 6/25 (24%), PDL1 - in the 2/25 (8%), PDL2 - in the 5/25 (20%) and TRAF - in the 2/12 (16%). Therefore, in this group of DLBCL pts with primary involvement of mediastinal lymph nodes 14/25 (56%) have shown significant overexpression of at least 3 genes analyzed whereas in the other 11/25 (44%) pts with primary affected mediastinal lymph nodes all these genes levels never exceeded the threshold. Tacking into account these data as well as the data of immunophenotypic analysis the diagnosis of PMBL was confirmed for 14/25 pts. Summary/conclusions. Our data suggest that the quantitative molecular analysis of JAK2, MAL, PDL1, PDL2 and TRAF gene expression enables to distinguish PMBL from other types of DLBCL with primary involvement of mediastinal lymph nodes.

1386

DOES KI-67 PREDICT TIME TO FIRST TREATMENT IN MANTLE CELL LYMPHOMA?

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Background: Treatment strategy for mantle cell lymphoma (MCL) is highly variable between centers and is debated worldwide. Some centres offer early aggressive treatment with chemotherapy +/- stem cell transplant at diagnosis. Others use a "watch and wait"approach whereby treatment is delayed until the patient becomes symptomatic. Our centre affords a unique opportunity to study MCL, as we have an institutional commitment to a "watch and wait"strategy. A biomarker predictive of early time to first treatment would be useful to make clinical decisions in treatment initiation and monitoring. Ki67 is an established predictor of shortened overall survival in MCL; however, its role in predicting treatment time has not been studied. Aims: The primary goal of our study is to determine if Ki67 can predict time to first treatment in MCL. Methods: All patients at our institution >17 years old diagnosed with pathologically confirmed Ann Arbor Stage III or IV MCL from January 1, 1999 to September 28, 2009 were enrolled. A tissue microarray was assembled using archived formalin fixed paraffin embedded tissue from all cases. Immunohistochemical stains, including Ki67, CD 20, and Cyclin D1 were applied to sections of the microarray. Cases were classified as high Ki67 when >20% of tumor cells showed nuclear staining for Ki67. Immunohistochemistry was performed and reviewed to confirm the original diagnosis. Time to treatment was determined by chart review. Results: 51 patients with MCL were identified from pathologic case records; of these, 26 patients with a confirmed diagnosis of MCL had adequate archived tissue for analysis and sufficient clinical records with long term follow up. The median age at diagnosis was 63 years (range 41-87 years). The median time to treatment was 27 days in the patients with high Ki67 index tumors (N=11) and 27 days in patients with low Ki67 index (N=15). Kaplan-Meier analysis showed overlap of the time to treatment curves for the two groups. The log-rank test showed no difference in time to treatment between the two groups (p=0.22). Proportion of patients treated within 90 days of diagnosis was 91% versus 67% in the high versus low Ki67 groups (p=0.197). Summary/Conclusions: While high Ki67 has been shown to correlate with decreased overall survival in MCL, it was not predictive of time to first treatment in our cohort of 26 patients uniformly treated according to a policy of watch and wait until onset of symptoms. The available data is underpowered to detect such a difference. This study reflects other published data, which indicate that the majority of MCL patients are not suitable for a strategy of deferred initial treatment.

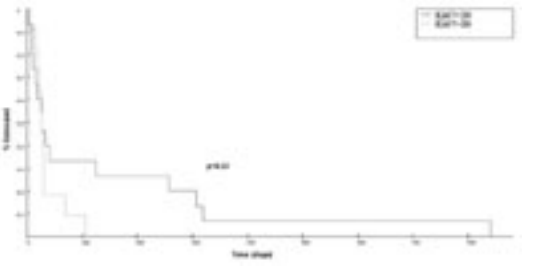

Figure 1. Time to Treatment

1387

PLASMOBLASTIC LYMPHOMAS WITH SKIN INVOLVEMENT

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Background. Plasmablastic lymphoma (PBL) is a rare subtype of non-Hodgkin's lymphomas with a rather poor prognosis. The first cases were characterized as oral leasions of HIV+ patients, and later on more extra-oral cases were also found but up to now very few cases with skin involvement/origin of HIV- patients were reported. PBL usually has a strong association with immunsuppression (81% of cases are HIV+) and EBV-infection. PBL consists of typically CD20-, LCA-, CD138+, VS38c+ and lambda or kappa monoclonal immunoblast like large cells with excentrically located vesicular nuclei with one or sometimes more prominent nucleoli and with a high proliferation rate. There is a strong overlap between plasmablastic myloma multiplex and plasmablastic lymphoma and the differential diagnosis might be very difficult. Markers which can help the differentialdiagnosis are p53, Mum1, Pax5, EBER ISH. *Aims*. Here we report and review seven cases (one female and six males) of plasmablastic tumors of HIV-negative patients with skin involvement. *Methods*. Avarege age was 65 years at the diagnosis of the disease. Three of the cases had a medical history of myeloma multiplex which later progressed to an agressive plasmablastic tumor. Another two patients had immunsupressive therapy because of renal transplantation. We performed a thorough immunohistochemical analysis with 15 markers (CD20, CD79a, Kappa, Lambda, CD38, CD45, CD138, Vs38c, Ki67, CD10, CD30, MUM, MUM1, Pax5, EMA) and additionally EBV-detection with in situ hybridization methods to set up a reliable algorythm for the differentialdiagnosis of this rare lymphoma. Results: The best markers for PBL were CD138, Vs38c, IgG, Ki67, EMA, Pax5 and MUM. The most important differentialdiagnostic problem was to separate PBL from plasmablastic myeloma multiplex, which distinction can be made upon the exact medical history, clinical correlations and bone marrow investigation/staging supported by a coexpression of several immunohistochemical markers, but in doubtful cases only the EBV-detection could help us. *Conclusions*. Althought skin involvement of the very agressive plasmablatic lymphoma is an exceptionally rare occuring phenomen it can occur not only in HIV+ patients but also without HIV-infection and therefore can cause very serious differentialdiagnostic problems. Morphologic and (immuno)phenotypic characterization might be insufficient for distinguishing plasmablastic myeloma multiplex and plasmablastic lymphoma. In these cases exact clinical data/correlations and EBV detection can help the most effectively.

1388

TUMOR INFILTRATING CD 68 POSITIVE MACROPHAGES PREDICT SURVIVAL IN MANTLE CELL LYMPHOMA

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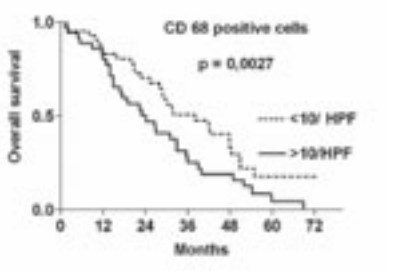
Background. Mantle cell lymphoma (MCL) is a malignant lymphoma associated with a relatively aggressive clinical course and a median overall survival time of 3-4 years. Only limited data about tumor associated macrophages and their influence on survival in MCL exists. *Methods*. We analyzed the amount of CD68 macrophages in relation to the clinical outcome in patients with MCL. Lymph node biopsies of 77 untreated patients (17 women and 60 men) enrolled in two multicenter trials (1975-1985) with a median age of 66 years (range 41-86 years) were included in this study. Biopsy specimens were investigated immunohistochemically with monoclonal antibodies against CD68 (Ki-M1P). 10 High power fields (HPF) were evaluated by random. Results: Patients with low account (less than 10/HPF) of CD 68 positive macrophages had a median overall survival time of 38.2 months, compared to 24.2 months for patients with high (more 10/HPF) CD 68 positive macrophages. The Kaplan-Meier analysis showed a significant difference in the overall survival time (p=0.0027). *Conclusion*s. Patients with mantle cell lymphoma and a low number of CD 68 positive macrophages have a better prognosis and can predict outcome.



1389

ACCOUNT OF CD 8 AND FOXP 3 POSITIVE T CELLS PREDICT SURVIVAL IN MANTLE CELL LYMPHOMA

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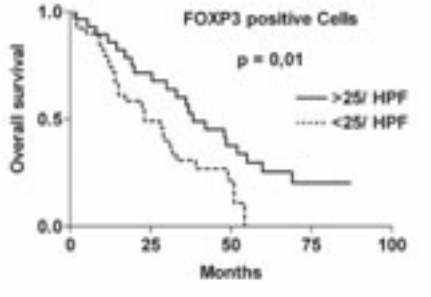
Background. The role of tumor infiltrating T-Cells in malignant B-Cell lymphomas is discussed controversial. There are only limited data on CD 8 and FOXP3 positive cells in mantle cell lymphoma. *Methods*: 81 biopsy specimens of patients (64 men and 17 women) with mantle cell lymphoma and a median age of 64 years (range: 41 to 86 years) were included in this study. The slides were stained immunohistochemically with CD3, CD8 and FOXP3. Positive T-cells of 10 High power fields (HPF) were counted and the average value was calculated.



Results. The CD 8 staining showed a range of 0 to 138 positive cells per HPF with a mean value of 19.4/HPF. A high account of CD 8 positive cells was associated with a significantly longer overall survival time (42 months) compared to MCL with a low account of CD 8 positive cells (28,8 months, p = 0.029). FOXP3 staining had a range of 0 to 104/HPF with a mean value of 28. Patients with MCL and a high number (>25/HPF) of FOXP3 positive cells had a median survival time of 38,2 months compared with the group with low account (<20/HPF) of FOXP3 positive cells (23 months). Kaplan Meier analysis revealed a significant difference (P=0.015) in overall survival time. *Conclusions*. High number of CD 8 and FOXP 3 T-Cells predicts a superior clinical outcome in patients with mantle cell lymphoma.

1390

APOPTOSIS REGULATING PROTEINS AND THE LEVEL OF APOPTOSIS CAN PREDICT SURVIVAL IN MANTLE CELL LYMPHOMA

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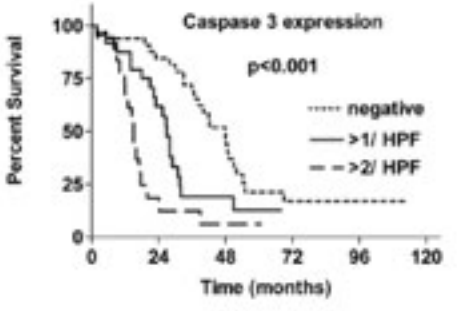
Background. The deregulation of apoptosis is has been implicated in cancer, autoimmunity and degenerative disorders. At the molecular level an external extrinsic death receptor pathway and the internal intrin-

sic (mitochondrial) pathway have been described. Only limited data exist on the expression of proteins involved in apoptotic pathways in mantle cell lymphoma are limited. *Methods*. We investigated the expression of p53, the indicator of DNA damage, and of proteins involved in the regulation of the internal intrinsic mitochondrial pathway (BCL2, Bax) and of effector proteins of apoptosis (caspase 8, caspase 3) in 93 cases of MCL mantle cell lymphoma and correlated the expression with the clinical outcome. *Results*. Similar to previous studies, we found that p53 expression was associated with a shorter overall survival. In contrast to diffuse large B-cell lymphomas, cases expressing the anti-apoptotic protein BCL2 had a favourable outcome. Interestingly, high levels of apoptosis in the tumor before treatment, as indicated by expression of active caspase 3, is are a strong indicator of poor clinical outcome ($p<0.001$). *Conclusions*. These data indicate, that the level of apoptosis itself is a strong prognostic marker in mantle cell lymphomas.



1391

DIET AND NON-HODGKIN'S LYMPHOMA RISK

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Background. The role of dietary factors in the epidemiology of Non-Hodgkin's lymphoma (NHL) remains largely undefined. Dietary habits may play a role in the etiology of NHL by influencing on immune system. Design and methods. We analyzed dietary patterns and the risk of NHL in the case control study, which includes 170 NHL cases (mean age 52 years) and 190 controls (mean ages 46 years). All subjects completed a validated food-frequency questionnaire. Dietary pattern investigated in nine groups and separately. Crosstab tables were used to estimate the odds ratios (OR) and the corresponding 95% confidence intervals (CI) and Ptrend. Results. Consumption of highest versus lowest quartile of proteins (OR, 8.088 Ptrend=0.000), fats (OR, 6.17 Ptrend=0.000) and sweets (OR, 8.806 Ptrend=0.000) were associated with a significantly increased NHL risk. Inverse association was found for fresh fruits (OR, 0.117Ptrend=0.000) and vegetables (OR, 0.461 Ptrend =0.010). Conclusions. An association between dietary intake and risk of NHL is biologically plausible because of immunosuppressive effect of fat and animal proteins and antioxidant properties of vegetable and fruits. It is recommended to encourage the general population to increase dietary fiber and to limit fat, red meat and sweet consumption.

1392

CHEMOTHERAPY WITH ALTERNATING REGIMEN MICMA/IGEV IN ELDERLY PATIENTS WITH REFRACTORY DLBCL: A FIGHT AGAINST WINDMILLS?

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Background. In Europe, more than 50% of the lymphomas arise in patients older than 65 years. Now R-CHOP regimen is the gold standard in treatment of aggressive lymphomas in this patient subset. Salvage stategies are needed for these patients who are not cured with first line therapy, since high dose chemotherapy frequently is not suitable. 2nd line therapy with DHAP produces scarce results. Aims. To evaluate safety, feasibility, efficacy of 2nd line treatment in patients older than 60 yo with combined chemotherapy with MICMA - IGEV. Methods. In two years we treated 20 patients with resistent diffuse large B cell lymphoma (DLBCL) with MICMA-IGEV alternating chemotherapy cycles (MICMA: methylprednisolone 500mg/mq gg1-5, mitoxantrone 10 mg/mq gg1, cytarabine 2 g/mq gg5, carboplatynum 100mg/mq gg1-4; IGEV: hyphosphamide 2 g/mq gg1-4; gemcytabine 800 mg/mq gg1 and 4). Chemotherapy cycles were administered every 21 days only if complete hematopoietic recovery was occurred. M/F was 12/8, median age was 72.5 years (R65-84). Results 15 patients (75%) received 4 chemotherapy cycles, 1 patient (5%) received 6 cycles, 4 patients (20%) received 2 cycles. Median cycles administered were 4 (R2-6). At 29 months all patients were dead. Median survival was 12 months. At 24 months survival was 17.6%. 6 patients (30%) showed comorbidities. All patients showed G3-G4 neutropenia, 6 (30%) G3-G4 thrombocytopenia, 2 (10%) G3 mucositis, 9 (45%) had fever. All patients received G-CSF support. Only 2 patients (10%) had G3 hepatotoxicity. 3 patients (15%) had kidney toxicity. No patients died for therapy-related toxicity. 5 patients required 25-33% chemotherapy dose reduction for organ toxicity. 8 patients (40%) delayed chemotherapy administration for G3-G4 neutropenia or thrombocytopenia. Summary/Conclusion. In literature patients of all age resistent/relapsed after 1st line chemotherapy and treated with 3 ESHAP or 2 DHAP cycles had a 5 years survival of 23%. Elderly patients with more of 60 years with relapsed/resistent DLBCL and treated with 2-4 cycles of DHAP showed a median survival of 9 months. Our patients treated with 4 MICMA/IGEV alternating cycles showed a median survival of 12 months (R1-29 months). 4 cycles of alternating MICMA/IGEV chemotherapy seems to be relatively safe, feasible and effective in patients with more of 65 yo, also with comordidities. These data need of confirmiation on a large coohort of patients.

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HELICOBATER PYLORI ERADICATION WITH SEQUENTIAL THERAPY IN GASTRIC B-CELL, LOW GRADE, MALT-LYMPHOMA PATIENTS: PRELIMINARY RESULTS

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Background. H. pylori eradication is recognized as first-line therapy in early stage, B-cell, low-grade, gastric MALT-lymphoma patients. However, cure rate following standard triple therapies for such an infection is decreasing worldwide. This study assessed the efficacy of a 10-day sequential for *H. pylori* eradication in these patients. *Methods*. All patients diagnosed with gastric lymphoma were considered. Patients underwent upper endoscopy with biopsies, echo-endoscopy of gastric mucosa, total-body CT, and bone marrow biopsy. Only those patients with *H. pylori*-associated, low-grade MALT-lymphoma in stage I-II1 were enrolled. Patients received a sequential therapy including omeprazole 20 mg plus amoxicillin 1 g for the firs 5 days followed by omeprazole 20 mg, clarithromycin 500 mg and tinidazole 500 mg for the remaining 5 days, all given twice daily. Both bacterial eradication and lymphoma remission was considered achieved when 2 consecutive histological examinations were negative. *Results*. Data of the first 10 patients (Male: 6; Mean age: 58? 10 years; Stage I: 10) currently enrolled were analyzed. Median follow-up was 12 months (range 8-22 months). H. pylori eradication was achieved in all 10 (100%) patients, whilst lymphoma remission occurred in 8 (80%) cases. One patient achieved lymphoma remission with radiotherapy following anti-CD20 monoclonal therapy failure, whilst the other patient is still in follow up (10 months following bacterial eradication). *Conclusions*. *H. pylori* eradication rate following standard triple therapy is decreasing. This is the first study showing a high efficacy of the 10-day sequential therapy for curing such an infection in MALT-lymphoma patients.

1394

STUDY OF THE PREVENTION OF HEART DISEASE USING BIOCHEMICAL MARKERS AND LIPOSOMAL ANTHRACYCLINE IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA

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Background. The toxicity of anthracyclines is a known limiting factor in the treatment of patients with lymphoma. The pre-existing heart disease limits its use, while in patients with known risk factors and in

patients with unknown riskscould induce the onset. In the period 2008-2010 were analyzed prospectively 40 patients with newly diagnosed non-Hodgkin lymphoma by a serial samples of pro-BNP and Troponin I, before each cycle of chemotherapy inclusive anthracycline, at day +1 by the execution, at the end of therapy and during the follow up at 3 and 12 months. Before therapy and at the end was assessed left ventricular ejection fraction by echocardiography. On the basis of risk factors have been identified two groups (see Table I). Patient's age or history of heart disease was classified as high risk and received the CHOP scheme with the replacement of doxorubicin with MYOCET (non-pegylated liposomal doxorubicin). While the second group performed the standard therapy with CHOP scheme. Both groups received a total of 6 cycles every 3 weeks. Results. 32 out of 40 patients achieved a complete remission, 7 patients a partial remission and only one has progressed. At 1 year, 31 patients are alive and well. The PRO-BNP increased during the cycles of therapy in 35 to 40 (see Table II) and troponin I is increased in 3 patients (all in the group treated with conventional CHOP). At 3 months and 1 year 2 out of 40 patients have pro-BNP increased while no one with increased troponin I. At 1 year after the treatment in the group treated with CHOP-MYOCETTM 7 patients showed an impairment (not life treathing) of cardiac disease, while in the group treated with conventional anthracyclines three patients developed a new severe heart disease. Conclusions. The PRO-BNP in our study did not show a prediction as it increases in almost all patients. Appeared the most promising Troponin I, although given the small sample size will require more study. The use of the anthracycline liposomal MYOCETTM confirmed its efficacy and tolerance without inducing new heart disease in a group of patients at high risk for heart disease and ancient age.

Table I

	CHOP-MYOCET™	CHOP
N°	25/40 15/40	
SEX M/F	8/17	9/6
MEDIAN AGE	74 (36-84)	62 (33-76)
HEART DISEASE OF ALL GRADES	11/25	4/15
MEDIAN TOTAL DOSE OF ANTHRACYCLINE:	480 mg (320-640)	540 mg (320-600)

Table II.

	CHOP- MYOCET™	CHOP
CR	19/25	13/15
PR	5/25	2/15
PD 0/25 1/15		
INCREASE OF PRO-BNP DURING CYCLES	23/25	12/15
INCREASE OF TROPONIN I IN CYCLES	0/25	3/15
INCREASE PRO-BNP TO 3 MONTH	1/25	1/15
GAP E.F ≥ 10% AT THE END OF THERAPIES	0/25	0/15
LIVE AT 1 YEAR	20/25	11/15
DEVELOPMENT OF HEART AT 1 YEAR IN ALL GRADES	0/25	3/15
INCOMING OR SEVERE HEART DISEASE	7/25	0/15

1395

STUDY REGARDING THE ASSOCIATION BETWEEN MULTIPLE PRIMARY CANCERS THAT INCLUDES A MATURE B-CELL NEOPLASM AND THE METABOLIC SYNDROME

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Background. It is known that cancer patients have a 20% higher risk of new primary cancer compared with the general population. We have published studies showing that patients with non Hodgkin lymphoma have more frequently components of metabolic syndrome compared to subjects without cancer. Aim: We aimed to study the presence of metabolic syndrome components in patients with multiple primary cancers that include a mature B-cell neoplasm comparing with those with only a mature B-cell neoplasm. *Methods*. Of the 668 patients with hematological malignancies who were registered in the Department of Hematology of Hospital Emergency Sibiu, during January 2006 - January 2011, we had selected 424 people with mature B-cell neoplasms, and among them we had chosen all the 21 multiple primary cancers (group A), which we compared with a group of 62 consecutive patients with mature B-cell neoplasms which were in our evidence in January 2011 (group B). We made a comparative analysis of the components of metabolic syndrome and hypercholesterolemia in the two groups. The results were statistically analyzed. *Results*. The average age of patients in group A was 66.57+/-12.32 years, and those in group B - 65.87+/-9.98 years. The average time to onset of the 2nd cancer was 3.52+/-6.24 years. Among patients with mature B-cell neoplasms, 4.95% had multiple primary cancers: 6 - skin carcinoma, 2 - lung cancer, 2 - malignant melanoma and one - gastric cancer, colon, rectum, kidney, breast, ovarian, metastatic with unspecified starting point, acute myeloid leukemia, acute promyelocytic leukemia, myelodysplastic syndrome, chronic myeloid leukemia and polycythemia vera. One patient had triple neoplasia. Patients in group B were more frequently obese (71.43% vs. 57.14%) and had hypertriglyceridemia (40.32% vs. 38.10%), while those in group A had more frequently high blood pressure (57.16% vs. 41.94%) and diabetes mellitus (33.33% vs. 20.97%). The average of metabolic syndrome components was higher in group A patients compared with those of B (1.86+/-1.24 to 1.61+/-1.11). The presence of two cancers predisposes to accelerate catabolic processes, which may explain the reduction in obesity and hypertriglyceridemia. In this group, which is more exposed to stress and various therapies, including corticosteroids, are more frequent high blood pressure and diabetes mellitus, and hypercholesterolemia (41.94% vs. 33.33%). The differences were not statistically significant. Only ischemic cardiopathy is more common in group A patients (p=0.025). *Conclusions*. A significant proportion of patients with mature B-cell neoplasia still has one or two neoplasms. While patients who have only mature B-cell neoplasia frequently suffer from obesity and hypertriglyceridemia, others with multiple primary cancers, including mature B-cell neoplasms, have high blood pressure, diabetes and more components of metabolic syndrome more frequently compared to the first. Studies on large groups of patients are needed so that results can have statistical significance.

1396

SINGLE CENTER EXPERIENCE WITH THE INTENSIVE PROGRAM PTL11-08 IN PRIMARY TESTICULAR DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

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Background. Primary testicular lymphomas (PTL) are rare aggressive, mostly B-cell extra nodal lymphomas with DLBCL comprising more than 90% of cases. Frequent central nervous system involvement predominantly in relapsed patients and continuous pattern of relapses are associated with poorer prognosis. CHOP-rituximab treatment with scrotal irradiation and CNS prophylaxis is the standard of care however resulting in poor response. *Aims*. To evaluate the toxicity and efficacy of intensive multidrug regimen (PTL11-08) in untreated patients with primary testicular DLBCL. Patients and Methods: From Aug 2008 six patients with primary testicular DLBCL aged 52-63 with Ann-Arbor stages IIE (2 pts) and IVE (4 pts) were evaluated and treated with intensive program PTL11-08. All three orchidectomised patients showed disease progression in 2-3 months after surgical treatment. All lymphomas immunophenotype had the activated B-cell-like subtypes (CD10-, bcl6, MUM1+). The treatment plan consisted of 2 alternating cycles: 1 (iv. methotrexate 1000 mg/m2 on day 1, vincristine 2 mg on day 1, ifosfamide 800 mg/m2 on days 1-5, etoposide 50 mg/m2 on days 4-5, cytarabine 150 mg/m2 on days 4-5 b.i.d., idarubicin 20 mg/m2 on day 3, temozolomide 150 mg/m2 on days 1-5, dexamethasone 20 mg on days 1-5) and 2 (procarbazine (orally) 35mg/m2 on days 1-14, iv. fotemustine 100 mg/m2 on day 1, cisplatin 100mg/m2 on day 2, temozolomide 150 mg/m2 on days 1-5) for 4 cycles with CNS prophylaxis (intrathecal dexamethasone 4mg + methotrexate 15mg + cytarabine 30mg) and colony-stimulating factor until recovery. The rational for this drug combination was the ability to penetrate the blood-brain barrier and blood-testicular barrier possibly. *Results*. Severe hematological toxicity (III-IV) was associated/ with the cycles 1 in all patients. Cycles 2 were associated with non-hematologic toxicities grade 1/2. All 6 patients fulfilled the treatment plan with complete response and after a median follow-up of 14 month (4-24 months) are alive and relapse-free. *Conclusions*. The program PTL11-08 for treatment testicular DLBCL is toxic but safe and effective. Future research is needed to increase the number of patients and period of observation.

1397

T CELL/HISTIOCYTE RICH LARGE B-CELL LYMPHOMA (THRLBCL): CLINICAL CHARACTERISTICS, PROGNOSIS AND MANAGEMENT: A REPORT ON 17 PATIENTS

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Background. T cell/histiocyte rich large B- cell lymphoma (THRLBCL) is a rare subtype of diffuse B cell non Hodgkin lymphomas. Because of a considerable similarity to nodular lymphocyte predominating Hodgkin's lymphoma (NLPHL) and classic Hodgkin's lymphoma (cHL), an accurate diagnosis is based on exhaustive immunohistochemical and molecular genetic analyses. *Aims*. To analyze the laboratory and histology in patients (pts) with THRLBCL with selected aspects of the course and survival in relation to different choices of therapy. Patients and *Methods*. Seventeen pts were studied (m/f =11/6, median age 41 years, range 28-72). Comprehensive immunocytochemistry and imaging methods were used to reach the diagnosis and stage of THRLBCL. The B symptoms were present in 94% of pts, peripheral lymphadenopathy in 82%, splenomegaly in 70%, and hepatomegaly in 82%. Mediastinal and abdominal lymphadenopathy were evidenced in 65% and 82% of pts, respectively. Most pts (65%) had a nodular type of disease. Primary extranodal disease was diagnosed in 23.5% of pts. Increased serum levels of LDH and β2-microglobulin were used as single proliferative measures of disease. Anemia, thrombocytopenia, and leukocytopenia were present in 58.8%, 53%, and 18% of pts, respectively. Most pts were in a IVB stage of disease with a high average IPI score. The R-CHOP protocol was applied in eleven pts. Six patients were treated with CHOP, COD, ESHAP, R-EPOCH, ProMACE CytaBOM and DHAP protocols each. Complete remission was achieved in 11.8% of pts and partial remission in 64.7%. In 23.5% of pts disease progressed despite the chemotherapy. In the collective median survival was 36 months. *Conclusions*. The THRLBCL is an aggressive lymphoma and the comparison of pts with this disease and those with diffuse large B cell lymphoma reveals no significant difference in survival.

1398

PROGNOSTIC FACTORS IN THE TREATMENT OF PRIMARY EXTRANODAL DIFFUSE LARGE B-CELL LYMPHOMA WITH RITUXIMAB BASED IMMUNOCHEMOTHERAPY

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Background. Primary extranodal lymphoma (ENL) constitute 25-50% of all non-Hodgkin lymphomas. About half of extranodal lymphoma are diffuse large-B-cell lymphoma subtype. The most common extranodal DLBCL localizations are stomach, central nervous system (CNS) and testis. Aims: The aim of this study was to determine the clinical and laboratory differences between patients with nodal (NL) and ENL DLBCL at the time of diagnosis, and the effect of clinical and laboratory parameters on overall and progression free survival (OS, PFS) in patients with extranodal lymphomas. Material and methods: The study included 95 patients with CD20 positive diffuse large-B-cell lymphoma treated at the Oncology Institute of Vojvodina between 2003. and 2010. Thirty seven (39%) of these patients had primary extranodal, and 58 (61%) had nodal localization. 16 of 37 ENL patients were presented with primary gastric, 3 with breast, testis, skin and lung, 2 with ovarium, thyroid gland, bone, soft tissue and one patient with parotid gland, colon, maxillary sinus and epipharynx localization. Three patients were presented with two extranodal sites and one patient with CNS lymphoma was excluded due to different treatment strategy. All patients received combination of rituximab and CHOP or CHOP-like protocols, without CNS prophylaxis. The study was divided into two phases. In the first phase, we have observed differences in clinical and laboratory parameters between primary nodal and extranodal diffuse large B cell lymphoma. In the second phase, patients with ENL were selected and we compared their OS and PFS depending on different disease stages, presence of B symptoms, prognostic scores (IPI, R-IPI), age, sex, and laboratory parameters: the level of lactate dehydrogenase (LDH), the absolute lymphocyte count, hemoglobin and albumin level. *Results*. The overall response (ORR) to therapy in all patients was 86.3% (CR 72.6%, PR 13.7%), while 13.7% had disease progression. Three-year overall survival was 64,3 %. ENL are more often diagnosed in stages I/II (67% vs 26%, p = 0.0094) and more likely to have low risk IPI score (54% vs 22%, p = 0.044). Despite of the favorable prognostic parameters, we neither found any difference in the number of patients who achieved complete remission with ENL and NL (75.6% vs 70.7%, p = NS) nor the difference in three-year overall survival (62,3% vs 67,7% P=NS). ENL patients with IPI score ≤ 2 had better OS and PFS (p = 0.0167; p=0,026). Patients younger than 60 years had better OS but not PFS (p = 0.0239; p=0,26). Patients who achieved complete remission had much better three year overall survival (84% vs 14%, p <0.0001). We found no statistically significant differences in OS or PFS in patients with ENL depending on the stage of the disease, the occurrence of B symptoms, R-IPI score, gender and laboratory parameters. *Conclusions*. The outcome of primary extranodal lymphoma is affected by IPI score, age and response to therapy. The results of ENL treatment with rituximab based immunochemotherapy are no different from the results of NL treatment with the same protocol.

1399

CLINICAL FEATURES AND OUTCOMES OF NASAL NK/T-CELL LYMPHOMA

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Background.Nasal natural killer (NK)/T-cell lymphoma is a unique type of non-Hodgkin lymphoma (NHL) that is almost always strongly associated with Epstein-Barr virus (EBV). The disease predominantly localizes to the upper aerodigestive tract, most commonly in the nose. It shows a strong association with EBV. Unfortunately diagnosis of NK/T-cell lymphomas often proves difficult, the treatment is unsatisfactory and its prognosis is poor with 5 years overall survival of 30 45%. The aim of this study was to investigate the frequency and clinicopathologic features of nasopharyngeal extra nodal NK/T-cell lymphoma, and evaluate the outcome of this disease. Patients and methods: Between 2003 and 2009, 08 new cases of nasal NK/T-cell lymphoma were diagnosed in our institution. The diagnosis is essentially based on the clinical presentation of extra nodal ulcerative lesions in the upper aero-digestive tract and histopathology analysis of biopsies using immunohistochemistry. Results. The median age was 46 years (ranges 19-77); male/female ratio was 4/4. The clinical features are: pain, obstruction, foul smell, discharge, and bleeding. Primary nasal lesions were ulcerative and are locally invasive and necrotic. This aggressive lymphoma remains localized in the nasal primary site in most cases. Systemic dissemination (stage IV) occurs in one patient (skin, testis) The histological findings showed angiocentric, necrosis, and pleomorphic infiltration. The immunophenotypes were: CD3+, CD56+, and CD45+ in all patients. The Ann Arbor stage was IE, IIE in 7 cases, and IVE in one patient. The patients were classified as having low IPI scores, and only two of them had bulky disease. Treatment modalities were given as follows: chemotherapy alone for 5 pts, chemotherapy with involved radiation therapy for 2 with a median dosage of 40 Gy. The chemotherapy regimens included CHOP (Cyclophosphamide, Doxorubicin, Vincristin, and Prednisone) for 3 pts on first line, but they were relapsed and received an L-Asparaginase based salvage regimen (L-asparaginase, velbe, and dexamethasone). Five patients (62%) were responsive to the treatment: Three patients achieved complete response; two of them obtained partial remission. The overall response rate (CR+PR) was 62, 5%. The 5-year overall survival rate was 37%. Conclusion. The nasal, nasal-type T/NK-cell lymphoma is a rare and distinct clinico-pathological entity. The Results of this clinical study indicated that the L-asparaginase-based regimen significantly improved the response rate. But the overall outcome in nasal NK/T cell lymphoma is poor.

1400

DETECTION OF RELAPSE IN PATIENTS POST TREATMENT WITH DIFFUSE LARGE B CELL LYMPHOMA.- THE ROLE OF BLOOD TESTS

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Introduction. Blood testing in routine outpatient follow up of patients who have had successful treatment for Diffuse Large B Cell Lymphoma (DLBCL) is not helpful to predict or confirm relapse. There has been no conclusive evidence suggesting that blood tests help with predicting or diagnosing relapse of DLBCL. Despite poor evidence it has become routine practise in clinics. Aims. This study was done to provide evidence on whether blood tests are useful in the outpatient follow up clinics. *Methods*. A Retrospective study was undertaken to investigate all

patients with a diagnosis of DLBCL, treated at The Haematology Department in Plymouth University Hospital, Derriford, from 2005 to 2009. We selected patients with DLBCL who completed their chemotherapy and were in good partial remission or complete remission. Information was obtained from medical notes, X-ray PACS system and hospital laboratory results system. We looked at the all the full blood counts and clinical outcomes in the outpatients clinics. *Results*. Total number of patients: - 62 patients. Median age of diagnosis = 67yrs. 38/62(61%) had lymphadenopathy on presentation, 5/62(8%) had thyroid involvement, 8/62(13%) had GI symptoms, 5/62(8%) had bone involvement and 6/62(9%) had other involvement which included skin and parotid node. 5/62(8%) of the patients in the study showed partial response after chemotherapy and 57/62(92%) showed complete remission or very good partial response after standard chemotherapy. Median follow up period was 25 months (range 3months -60months) Median number of OPD clinic per patient was 6(range 1-22 clinics) in the study period. Median number of OPD blood tests done per patient in the study period was 6 (range 1-22 blood tests). 76/400(19%) of the full blood count were abnormal. Of the abnormal blood tests- 74/76(97%) of the FBC showed chronic anaemia, 1/74(1%) showed thrombocytopenia and 1/74(1%) showed CLL. 8/62(13%) of the patients relapsed in the study period. None of the patient who relapsed had any change in their blood counts to indicate or aid to diagnose relapse. All patients who relapsed presented with symptoms: lymphadenopathy 6/8 (62%), 1/8(12%) had stridor and 1/8(12%) had CNS symptoms at relapse. Conclusions. This observational study shows that blood tests do not help in predicting or diagnosing relapse in patients following successful therapy. Majority of the abnormality seen on blood counts were due to other unrelated medical conditions. All relapses were diagnosed in patients presenting with a specific symptoms and/or signs. Following this study, it was noted that, patients found reassurance if their blood counts were normal, however once patients were educated on the blood tests being uninformative with regards to their follow up, their *safety blanket* association with blood tests was significantly reduced. Therefore, in an Out -Patient Department, rationalisation of blood tests can be done economically and without having an adverse outcome on patient care. In the current economical climate this could produce beneficial savings for the department without compromising patient care.

1401

IMPROVEMENT OF PRURITUS IN SEZARY SYNDROME: LITERATURE REVIEW AND CASE REPORT

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Introdution. Pruritus (itch) can be defined as an unpleasant cutaneous sensation associated with the immediate desire to scratch and is an important symptom of cutaneous T-cell lymphomas (CTCL); it's frequent and severe and its pathophysiology remains unclear. Treatment is very difficult, especially in Sézary syndrome. The Sézary syndrome is a leukemic, cutaneous, epidermotropic T-cell lymphoma and is more aggressive form of CTCL, characterized by the association of an exfoliative erythroderma with the presence of atypical mononuclear cells in the skin and the peripheral blood (Sezary cells). Pruritus, insomnia, and depression impair the quality of life of patients with sezary syndrome. Pruritus originates within the cutaneous skin's free nerve endings. Activation of specific areas in the central nervous system results in perception of itch, leading to a scratch response. By a direct axon reflex mechanism, sensory nerve endings release neuropeptides, which may aggravate the itch response by stimulating release of pruritic mediators (kinins, prostanoids, and cytokines) from mast cells, immune cells epithelial cells and endothelial cells. Substance P is a key mediator of pruritus. An increase in the expression of its receptor, neurokinin-1, has been reported on keratinocytes in pruritic skin diseases. Aprepitant is an oral neurokinin-1-receptor antagonist. It is commonly used as an antiemetic agent in chemotherapy-induced nausea and vomiting. However, its action suggests a potential reduction in substance P-induced pruritus, even though pruritus is considered a rare side effect of this drug. Case report: After learning from an article in the N. Engl. J. Med (october 1, 2009) we have treated a patient with Sezary syndrome with aprepitant, obtaining the total resolution of itch. Our patient. 75 years old, accused incoercible itch and erythroderma, lymphadenopathy and splenomegaly. He was treated at beginning with topical therapy, extracorporeal photochemotherapy and for progression, with bexarotene and after with chemotherapy. The patient achieved a complete remission of the disease; maintenance therapy with alemtuzumab was performed. The itch was present during the first 3 cycles of chemotherapy and only after to introduction of aprepitant there was attenuation and then a disappeared of pruritus. The patient reported diminished insomnia and better quality of sleep. Discussion: The options of treatments for pruritus in CTCL are topical agents, extracorporeal photochemotherapy (option that reduce both disease activity and pruritus) and systemic agents (gabapentin, mirtazapine, rosiglitazone and bexarotene). A dramatic improvement was noted by A.Duval and L.Dubertret of Saint Louis Hospital of Paris in three patients with Sézary syndrome suffering from severe and uncontrolled pruritus after treatment with aprepitant, which is an antagonist of natural killer (NK) 1 receptor for substance P. *Conclusions*. In patients with CTCL, pruritus is frequent and often severe. This symptom negatively affects quality of life of patients. First-line treatment recommendation should be topical steroids or phototherapy, sometimes in combination with disease-modifying treatment, such as interferon or retinoids. In case of insufficient response, anticonvulsivants or antidepressants can be used. A possible alternative is provided by NK1 antagonists, aprepitant. Our experience confirms the effectiveness of apepritant against pruritus associated with Sezary syndrome.

1402

FALSE-POSITIVE RESTAGING PET SCANS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA INVOLVING THE MEDIASTINUM AFTER INTENSIFIED IMMUNOCHEMOTHERAPY

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Background. 18F-FDG PET has demonstrated its utility for assessing treatment efficacy in patients with diffuse large B-cell lymphoma (DLBCL). However, there is no consensus on the significance of 18F-FDG-avid mediastinal residual masses after initial chemotherapy. *Aims*. To surgically restage patients with DLBCL of the mediastinum who have PET-positive residual lesions after an intensified immunochemotherapy regimen, in an attempt to avoid complementary radiotherapy or second-line treatment for patients without evidence of viable lymphoma. Methods. We retrospectively studied patients with DLBCL of the mediastinum, treated between 02/2007 and 12/2010, and who underwent surgical restaging because of a persistent positive PET scan. 18F-FDG PET was assessed semi-quantitatively using maximum standardized uptake value (SUVmax). Patients were planned to receive 4 cycles of the R-ACVBP regimen (rituximab, doxorubicin, cyclophosphamide, vindesine, bleomycin and prednisone) given at two-week intervals, followed by a sequential consolidation comprising 2 cycles of high-dose methotrexate, 4 cycles of rituximab, etoposide and ifosfamide, and 2 cycles of cytarabine given subcutaneously. Restaging with CT scan and 18F-FDG PET occurred after the 4th course of R-ACVBP in 3 pts, the 1st course of methotrexate in 2 pts and the 2d course of methotrexate in 2 pts. Results Seven patients were identified. The median age was 27 years (range : 25-39). 5 pts had stage I/II, and 2 pts had stage IV disease (lung 1 pt, pleura and pericardium 1 pt). The age-adjusted IPI was 0 or 1 in 6 pts. Diagnosis was DLBCL in 6 pts and primary mediastinal (thymic) B-cell lymphoma in 1 pt. Four pts had 18F-FDG PET before treatment. The result of CT scan at restaging was partial response in 5 pts, and complete response unconfirmed in 2 pts. The SUVmax in the most intense tumor at restaging ranged from 3,1 to 6,3. For the 4 patients who had PET at baseline, the SUVmax in most intense tumor were comprised between 22 and 24, and the reduction in SUVmax between 62% and 87%. Surgical restaging was performed by median sternotomy (5 pts), mini-thoracotomy (1 pt) and cervicotomy (1 pt). Complete resection of the residual mediastinal masses was achieved in 5 pts. The histological findings were necrosis, fibrosis and/or inflammation in 6 pts. The 7th patient had mediastinal lymph nodes showing traces of silicosis. No patient had viable residual lymphoma cell. All the patients received a sequential con-

solidation chemotherapy as initially planned, without consolidative radiation therapy. At a median follow-up of 14 months (range : 4-40), all patients are alive without disease progression. One patient presented respiratory failure due to pleural effusion after surgery, and had to undergo non-invasive ventilation for 4 days. Summary/Conclusions. In patients with DLBCL of the mediastinum, surgical restaging of 18F-FDG-avid residual mediastinal masses after initial chemotherapy, although not routinely recommended, demonstrated in a small series that tumor necrosis remains metabolically active. Restaging PET should be interpreted with caution to make treatment decisions, and the results of large prospective trials evaluating PET-guided treatment in DLBCL are eagerly waited.

1403

BENDAMUSTINE AND RITUXIMAB IN RELAPSED OR REFRACTORY LOW-GRADE LYMPHOMAS

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Background: Bendamustine is a hybrid of a purine analoge and an alkylating agent, with activity against low-grade lymphomas in combination with rituximab (BR) and a good toxicity profile. Bendamustine has recently become available in clinical practice and experience is limited. Aims: Herein 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: Retrospective and observational study of all consecutive patients with relapsed or refractory low-grade lymphoma or mantle cell lymphoma treated with BR since April 24, 2008 to January 31, 2011. 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/m^2) 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: Descriptive statistics and Kaplan-Meier survival analysis. *Results*. Thirteen patients, 6 woman and 7 men, were included. Mean age was 67,3 years and median age 70 years, range 45 to 82. Diagnosis was follicular lymphoma (FL) in six patients, mantle cell lymphoma (MCL) in three, small cell lymphocytic lymphoma (SCLL) in three and MALT lymphoma of the oral mucosa (relapsed in skin and conjunctiva) in one. Mean time from diagnosis was 5,4 years (2,6-10,5). Mean previous therapies was three (1-5), and two patients (one FL and one MCL) had undergone an autologous bone marrow transplantation. Seven patients received six cycles of BR, four patients 4 cycles and one patient 3 cycles. One patient received only two cycles, and the treatment was early terminated because of hepatitis C reactivation. Complete response (CR) was achieved in ten patients (76%) and partial response in one (7,5%). Two patients (15%) showed no response. Two patients with CR have relapsed (a SCLL and a MCL) at 19 and 21 months respectively, while the remaining eight maintain a CR. The median time to treatment failure was 19 months and the median survival was 21 months, with a median observation of surviving patients of 13 months. Adverse events were grade 3 or grade 4 neutropenia in seven patients (53%) and grade 3 thrombocytopenia in two (15%). An episode of febrile neutropenia and another of coagulase negative staphylococcus bacteriemia associated with indwelling catheter were reported. There was a transient hepatitis C reactivation with remission after BR termination. Five patients reported nausea and vomiting and two asthenia, both in grade 1 or 2. *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 in elderly patients and responses were durable. More experience is needed to confirm the good profile of the combination in the long term.

1404

FEASIBILITY OF HIGH-DOSE METHOTREXATE, TEMOZOLOMIDE AND INTRATHECAL LIPOSOMAL CYTARABINE (HD-MTX-TMZ-IT LC) FOR THE TREATMENT OF CENTRAL NERVOUS SYSTEM (CNS) LYMPHOMA

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Background. CNS lymphoma is an aggressive tumor. Combined systemic chemotherapy-radiotherapy may be associated with acute/delayed neurotoxicity. HD-MTX-TMZ appears to be an effective and relatively safe regimen. Adding IT LC may further improve therapeutic efficacy. *Aims*. We report our preliminary experience with HD-MTX-TMZ plus IT LC used upfront or as salvage in 4 CNS lymphoma patients. Methods: Induction: MTX 3g/ms IV d 1, 10, 20, TMZ 100 mg/ms d 1-5; maintenance (≥SD pts): MTX 3g/ms d 1, TMZ 100 mg/ms d 1-5, every month, for up to 5 cycles as long as response was documented. Fifty mg IT LC was given concomitantly, at least 14 days apart and at least 7 days from HD-MTX, up to 6 doses. Results: Pt 1, 56 y, male, testicular diffuse large B cell lymphoma (DLBCL), stage IVA, cerebellar relapse after CR, Karnofsky performance status (KPS) 50%. Rituximab-TMZ and IT MTX were initiated but soon discontinued due to CMV pneumonia. After recovery, HD-MTX-TMZ was started with precautionary stem cell harvest. Six cycles and 4 concomitant IT LC injections were given with no G3-4 toxicities. He obtained CRu. Pt 2, 76 y, male, primary CNS peripheral T-cell lymphoma not otherwise specified, multicentric, KPS 60%. He was treated with steroids and TMZ, but progressed in three months. He receive the complete HD-MTX-TMZ program with 4 IT LC injections. He experienced G2 renal insufficiency, managed with adequate pre-treatment hydration and reversed after the end of therapy, and reversible G3 iatrogenic diabetes mellitus due to pre-induction dexamethasone. He obtained CRu. Seven months later he relapsed in involved sites, received two further cycles of TMZ and is now in SD. Pt 3, 68 y, female, PCNSL, DLBCL, left cerebellar hemisphere, KPS 50%. HD-MTX-TMZ and concomitant IT LC were initiated as first-line therapy. She experienced G3 atrial fibrillation, resolved with appropriate treatment and never reappeared afterwards. Maintenance was completed. However, after the 5th IT LC injection the patient presented with leg pain and difficulty walking, saddle hypoesthesia, urinary and fecal retention, suggesting conus-cauda equina syndrome. IT therapy was thus withdrawn. Neurologic symptoms subsequently improved with physiotherapy and pregabalin, and stool softeners. She obtained very good PR after induction. She currently complains of residual perineal heaviness and mild difficulty waking, and is able to perform controlled micturition and defecation. Pt 4, 71 y, female, DLBCL stage IIIE (i.e. subcutaneous facial localization), with multicentric bilateral subcortical relapse. Five HD-MTX-TMZ cycles and 6 IT LC injections have been administered so far with no neurotoxicity. CR was attained after induction and sustained thus far. *Conclusions*. HD-MTX-TMZ-IT LC therapy appeared feasible and effective, even in elderly pts. CR/CRu was attained in 3/4 pts, with a response duration of 19+, 7, 11+, and 6+ months in pts 1, 2, 3 and 4, respectively. IT LC is associated with a number of neurologic complications, including Conus-Cauda equina syndrome. However it appears to be in part related to the lumbar puncture procedure itself and not a LC-specific side effect. No delayed neurocognitive impairment has been observed so far.

1405

THERAPEUTIC COMPARISONS OF SURGICAL RESECTION PLUS R-CHOP AND R-CHOP ALONE FOR PRIMARY INTESTINAL DIFFUSE LARGE B CELL LYMPHOMA

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Background. Gastrointestinal tract (GIT) is the most commonly involved extranodal site of all non-Hodgkin's lymphoma (NHL). GIT involvement is represented 10-15% of all NHL cases and 30-40% of all extranodal sites. In the Rituximab era, the addition of rituximab led to an impressive improvement of response rates and survival outcomes in patients diffuse large B-cell lymphoma (DLBCL), Aims: In this retrospective studies, the purpose is finding appropriate treatment strategy according to comparing the efficacy of treatment in patients with primary intestinal diffuse large B cell lymphoma (DLBL) undergoing surgery followed Rituximab-containing chemotherapy or Rituximab- containing chemotherapy alone. *Methods*. Forty four patients were newly diagnosed with DLBL and received chemotherapy between March 2004 and December 2009. Primary intestinal lymphoma which had predominant intestinal lesions was diagnosed by specialized hemato-oncologist. All patients were treated with rituximab combined cyclophosphamide, adriamycin, vincristine, and prednisolone (R-CHOP). Patients were divided into two groups. One included patients who were undertaken surgery plus R-CHOP (surgery/CT group). The other included patients who were undertaken R-CHOP alone (CT group). Results : The characteristics of the patients were as follows: the median age was 58.5 years (range 15-85 years) with a female-to-male ratio of 25:19. Patients characteristics had no significant difference between two groups. The estimated 3 years event free survival rates (EFS) and overall survival rates (OS) of surgery/CT and CT group were 79.3% and 40.8%, (p=0.311) and 84.0% and 43.3%, (p=0.301) respectively. In univariate analysis, EFS and OS

were estimated in Lugano stage I, II1, II2, IIE and IV (p=0.079 and p=0.018), Low, Low-intermediate, high-intermediate and high risk (p=0.068 and p=0.019), LDH < 450 IU/L and ≥ 450 IU/L (p=0.027 and 0.140), and surgery/CT and CT alone, (p=0.311 and p=0.301), respectively. In multivariate analysis, there was no independent predictive factors for survival. Conclusions. atients treated with surgery followed R-CHOP were seemed to have higher survival rate than R-CHOP alone although there was no significant differences for survival rate. There was no significant prognostic factors for survival, but Lugano stage, IPI risk, LDH, and treatment modality could be possible prognostic factors for event free survival.

1406

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS IN LIVER AND KIDNEY RECIPIENTS: A SINGLE INSTITUTION EXPERIENCE

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Background. Post-transplant lymphoproliferative disorders (PTLD) represent a heterogenous group of disease ranging from reactive polyclonal hyperplasia to aggressive non-Hodgkin lymphomas (NHL) and in the majority of cases they are associated with Epstein-Barr virus (EBV). PTLD develop as a consequence of the severe impairment of T cell function and the related reduction of T cell control on EBV latently infected B cell due to immusoppressive therapy after solid organ (SOT) or hematopoietic stem cell transplantation (HSCT). *Aims*. We analyzed the clinical features, treatment and outcome of a series of adult patients who developed NHL after liver and kidney transplantation. *Methods.* We retrospectively studied the clinical data of 16 patients that developed NHL occurred at our institution between 1998 and 2010. *Results.* 16 patients (M/F ratio 2.2) with a median age of 42 years (range 20-59) developed NHL after liver, 3 cases, and kidney, 13 cases, respectively. Previous liver diseases were: hepatitis C virus cirrhosis in two cases and primary sclerosing cholangitis in one case; previous kidney diseases were: glomerulonephritis in 10 cases and congenital syndrome in three cases. Median time from transplantation to PTLD was 111.5 (range 3-360) months. Six patients experienced acute transplant rejection that required intensification of immunosuppressive therapy. Hematologic diagnosis included 12 monomorphic PTLD (10 diffuse large B lymphoma, 1 peripheral T cell lymphoma, 1 anaplastic large cell lymphoma), 2 classical Hodgkin lymphoma-type PTLD, 1 lymphoplasmacytic lymphoma and 1 extranodal marginal zone lymphoma. Histologic diagnosis was made on lymph node and on extranodal tissue in seven and nine cases, respectively. Available histologic analysis of tumor tissue demonstrated that EBV was positive in 62% of cases. CD20 expression was available in all but two patients and was positive in 71% of cases. At diagnosis seven patients were in stage IV, two in stage III, one in stage II and 6 in stage I; median IPI and Mayo prognostic score were 2 and 1, respectively. Ten patients presented with extranodal disease; central nervous system (CNS) involvement was not observed. Eleven patients received immuno-chemotherapy (including anthracyclines) with rituximab and two only chemotherapy; among them only four patients required reduction of doses because of liver/renal toxicity, and one patient experienced infective toxicity (WHO grade 1-2). One patient was treated with only radiotherapy and two patients were observed because of indolent disease. After a median follow up of 51 months (range 1-128) from the end of therapy, 13 patients are alive and in complete remission, two are lost to follow up and one patient died for progressive disease.CONCLUSIONS: Although the limited number of patients involved, and the peculiar subset of renal and liver allograft in which the risk of PTLD is low-intemediate, our study demonstrated that the majority of our patients developed aggressive NHL and an immuno-chemoterapic regimen is feasible, well tolerated and allow to obtain an elevated percentage of remission.

1407

CLINICO-EVOLUTIVE ASPECTS AT 73 PATIENTS DIAGNOSED WITH HAIRY CELL LEUKEMIA ADMITTED IN FUNDENI CLINIC OF HEMATOLOGY BETWEEN 2000-2008

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Background. Hairy cell leukemia(HCL) is an indolent B cell lymphoproliferative disorder that affect middle aged males; the main symptoms at diagnosis are : fatigue, splenomegaly, pancytopenia, hemorrhagic syndrome, infections. The morphological marker of the disease in hairy cell, an activated memory B cell with characteristic immunophenotype (panB$^+$, CD5$^-$, CD23$^-$, CD25$^+$, CD11c$^+$, CD103$^+$, CD123$^+$). The new methods of diagnosis (flowcytometry, immunohistochemistry) allow the diferrential diagnosis between classical an variant form, and/or another lymphomas(eg. Splenic marginal zone lymphoma, with villous cells). The response to the treatment with interferon and/or purine analogues is very good, with long term remissions. *Aims*. To compare data obtained with data from other studies. *Material and method*: clinical and epidemiological retrospective study of 73 patients diagnosed in Fundeni clinic of Hematology between 2000-2008. *Results* : 62 patients were diagnosed with classical form, 11 with variant form of HCL; most of the patients were males, with a medium age of 57,1 years; the main features at diagnosis were: splenomegaly(>12 cm diameter at abdominal ultrasound)-55cases; infections(24 cases), cytopenias(mono, bi or pancytopenia) -all of the73 patients, hemorrhagic syndrome- 15 cases; 11 cases had autoimmune manifestations. The treatment methods were: alfa interferon- 44 cases, Cladribine- 12 cases, combined secvential therapy with alfa interferon and Cladribine- 8 cases, splenectomy - 8 cases, alkilants agents - 5 cases. The response to the treatment was evaluated at 57 patients: 19 partial responses, 34 complete responses, 4 without response. 7 patients died, 3 because of severe toxico-septic shock. The complications in evolution were: infections(21 cases), hemorrhagic syndrome (2 cases), cardiovascular disease(2 patients); 2 patients achieved a second malignancy. Conclusion : HCL is an indolent lymphoproliferative disease, with well established diagnostic features (clinical, morphological, immunological), good response to the treatment, long remissions, but high incidence of infections, that appear at diagnosis or whenever through the evolution.

1408

DICHOTOMOUS MODEL TO EVALUATE TREATMENT OUTCOMES IN NON-HODGKIN'S LYMPHOMA PATIENTS

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Efficacy of treatment of malignant lymphomas is traditionally based on objective clinical data. At present patient-reported outcomes are of increasing importance to evaluate treatment outcomes in this patient population. Recently, the dichotomous model to evaluate treatment outcomes in patients with hematological malignancies was proposed by the experts of the EHA SWG "Quality of Life and Symptoms". We aimed to test this model on new population of Non-Hodgkin's lymphoma (NHL) patients receiving conventional chemotherapy (CT). 54 new NHL patients were enrolled in the study (stage IIB-IV, mean age 30.2 (SD 13.5), males/females - 55/59). The lymphoma histopathology was as follows: diffuse large B cell lymphoma - 24, peripheral T cell lymphoma - 11, anaplastic large cell lymphoma - 11, follicular lymphoma - 4, mantle cell lymphoma - 2, angioimmunoblastic Tcell lymphoma - 2. All the patients underwent conventional CT: CHOP or CHOP-like regimens. QoL was assessed using the generic questionnaire SF-36. All the patients filled in the questionnaire at baseline and at different time-points after the end of CT. QoL treatment response was assessed using Integral QoL Index and was classified as improvement, stabilization or worsening. At 3 months after CT the following distribution of patients according to clinical response rates was observed: complete remission - 62%, partial remission - 6%, stabilization - 13%, progression - 19%. QoL improvement was achieved in 44%, QoL stabilization - in 44%, and QoL worsening - in 12% of patients. In a number of cases discrepancies between clinical response and QoL response rates were registered. Noteworthy, there were patients with complete remission who experienced QoL worsening after the end of CT. Thus, information about clinical and QoL response rates allows to obtain comprehensive information about treatment outcomes in NHL patients receiving conventional CT. The dichotomous model sounds feasible to measure effect of treatment both from physician's and patient's perspective. The evaluation of both clinical and QoL response may provide comprehensive information about the benefits and risks of treatment of malignant lymphoma patients.

1409

SALVAGE CHEMOTHERAPY WITH NON-PEGYLATED LIPOSOMAL DOXORUBICIN (ADRIAMYCIN), FLUDARABINE, OXALIPLATIN AND CYTARABINE (AFOXA) IN POOR-RISK B CELL NON-HODGKIN'S LYMPHOMA

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The aim of the present phase I/II study was to evaluate the feasibility and toxicity of the combination of non-pegylated liposomal doxorubicin, fludarabine, oxaliplatin and cytarabine (AFOXA) in patients (pts.) with prognostically unfavourable recurrent and refractory B cell non-Hodgkin's-lymphoma (NHL). Between 02/2005 and 06/2010 a total of 29 pts. (16 male, 13 female) with diffuse large B cell lymphoma (n=15), mantle cell lymphoma (n=8) and follicular lymphoma (n=6) were treated according to the AFOXA protocol. Pts. (median age 61, range 42-70) with primary refractory disease (n=22), refractory relapse (n=1) and second (n=5) or third (n=1) relapse were enrolled. The intensive pretreatment contained a median of 6 (range 1-18) cycles of chemotherapy. 25/29 pts. were pretreated with the anti-CD20 monoclonal antibody Rituximab. The AFOXA regimen consisted of non-pegylated liposomal doxorubicin (25 mg/m^2, days 1 + 3), fludarabine (25 mg/m^2, days 1-4), oxaliplatin (escalating doses of 100 or 130 mg/m^2, day 5) and cytarabine (escalating doses of 1000 or 1250 or 1500 mg/m^2, day 5). In the phase I part of the study (n=12) the maximal tolerable dose (MTD) was determined for oxaliplatin and cytarabine according to World Health Organization Common Toxicity Criteria (CTC). The primary objective of the subsequent phase II part of the study, which uses the determined MTD, is efficacy. RESULTS: In the phase I part we established the MTD for oxaliplatin with 130 mg/m^2 and for cytarabine with 1000 mg/m^2. Out of 29 pts.; 20 pts. were treated with the established MTD. 7 pts. (35%) achieved complete remission (CR or CRu) and 1 patient (5%) partial remission, with an overall response (OR) rate of 40%. A successful peripheral blood stem cell harvest through mobilization with the AFOXA regimen was possible in 10 pts. (50%). CONCLUSIONS: AFOXA is a feasible salvage protocol for patients with poor-risk recurrent or refractory NHL. The observed toxicity (MTD) seems to be acceptable considering the unfavourable prognosis and intensive pretreatment. The efficacy will be evaluated in the ongoing phase II study.

1410

INTENSIVE THERAPY OF THE PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA WITH PRIMARY INVOLVEMENT OF MEDIASTINAL LYMPH NODES AND PRIMARY MEDIASTINAL B-CELL LYMPHOMA EFFICACY AND TOXICITY

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Background. Primary mediastinal B-cell lymphoma (PMBCL) and diffuse large B-cell lymphoma (DLBCL) with primary involvement of mediastinal lymph nodes have different origins and, therefore, different immunological, molecular and genetic characteristics and response to therapy. *Aims*. To evaluate the efficacy and toxicity of the modified program NHL-BFM-90 (mNHL-BFM-90) for adult patients with PMBCL and DLBCL diagnosis and primary involvement of mediastinal lymph nodes. Methods. There were 37 patients with large B-cell lymphoma with primary involvement of mediastinal lymph nodes. The illness was diagnosed in accordance with WHO classification criteria, the differential diagnostics of PMBCL and DLBCL was performed on basis of the data obtained via immunohistochemical study through and assessment of the gene expression level by PCR. The diagnosis of DLBCL with primary involvement of mediastinal lymph nodes was established for 17 patients (7 men, 10 women, mean age 31 years (from 18 to 60 years)), PMBCL 20 (9 men, 11 women, mean age 31 years (from 21 to 70 years)). All patients were treated according to mNHL-BFM-90 program in the Russian Hematological Research Centre between November 2004 and July 2010. DLBCL and PMBCL staging criteria developed by Ann Arbor were used to stage the patients. All patients were diagnosed with II stage. Bulky mediastinal disease was found in 16 (94%) patients with DLBCL and in 19 (95%) patients with PMBCL. Serum lactate dehydrogenase level was increased in 17 (100%) patients with DLBCL and in 19 (95%) patients with PMBCL. ECOG 1 was established in 1 patient (2,7%), 2 - in 7 (18,9%), 3 - in 23 (62,1%), 4 - in 6 (16,7%) patients. The NHL-BFM-90 protocol was modified by us (a dose of methotrexate was reduced to 1500mg/m2 (12 h) in course A and B, doxorubicine (50 mg/m^2) was included in the course A). 4-6 courses were performed, their quantity being determined depending on achieving the remission time. The patients with residual tumor in mediastinum underwent radiotherapy as consolidating treatment in total dose of 36 Gray. *Results*. 16 (94%) of the patients with DLBCL with primary involvement of mediastinal lymph nodes achieved a complete remission, and 13 (65%) of the patients with PMBCL. One of the patients DLBCL has died from chemotherapy complications. One patient with PMBCL turned out to be primary-resistant. Early relapse was ascertained in 1 patient with DLBCL and 3 with PMBCL. The 3-year disease-free survival (DFS) and overall survival (OS) were similar, 94% in patients with DLBCL and 84 % with PMBCL. Most infectious and hemorrhagic complications occurred during the first course (course A), which can be explained by the initial poor condition of the patients and a large tumor mass at the start of treatment. *Summary/conclusions*. The modified mNHL-BFM-90 is a highly effective protocol. The 3-year DFS and OS were similar, 94% in patients with DLBCL and 84 % with PMBCL.

1411

INTRATHECAL LIPOSOMAL CYTARABINE PROPHYLAXIS IN VERY AGGRESSIVE LYMPHOMA: A SPANISH MULTICENTER STUDY

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Introduction. Lymphomatous meningitis (LM) in very aggressive lymphoma is a common complication with poor prognosis that usually occurs early in the course of the disease . Liposomal Cytarabine (LC) is an extended release formulation which has demonstrated better efficacy compared to standard Cytarabine for the treatment of LM in one randomized clinical trial. Retrospective studies have shown that prophylaxis with IT LC in DLBCL patients is feasible and effective. Aims: To evaluate effectiveness and toxicity profile of IT Liposomal Cytarabine as prophylaxis of LM in very aggressive lymphoma. Methods:.Main endpoints were effectiveness (LM incidence) and toxicity (CTCAE of NCI 3.0) of the IT prophylaxis. A retrospective study was carried out in 10 Spanish hospitals including patients diagnosed with Burkitt and Lymphoblastic Lymphoma that received LC as IT prophylaxis for LM in the period between January 2007 and January 2011. *Results*. Data from 16 patients were analyzed. Baseline characteristics were: 7 Burkitt and 9 Lymphoblastic Lymphoma. Mean age: 45 ± 20 years (range 20-79). Male/Female 15/1. Ann Arbor stage IV 11 (69%). All patients received alkylating based regimens and in 13 patients (81%) treatment included high dose systemic AraC and Methotrexate. LC was administrated at a dose of 50 mg. The total number of administrations was 52 with a mean of 3.25±1.65 doses per patient (range 1-6). In order to minimize side effects, as described before, administration of sistemic AraC and LC were separated in time for at least 7 days. Effectiveness: With a median follow up of 22 months (range 8-35) we have not found any case of LM. Eleven patients completed IT prophylaxis treatment (two patients continues the IT schedule at the time of this report, two patients were exitus before finishing prophylaxis and one patient was lost to follow up). Toxicity: Prevention of chemical arachnoiditis with steroids was given to all patients. There were 4 cases of headache, 2 patients with grade 1 and 2 with grade 2, reversible in all cases. *Conclusions*. This retrospective study with 22 months of median follow up and no cases of LM in very aggressive lymphoma, shows that CNS prophylaxis with LC is effective and well tolerated without serious adverse events reported, with the precautions and prevention meausures mentioned above.

1412

SCREENING FOR PRIMARY IMMUNODEFICIENCY IN PEDIATRIC PATIENTS WITH LYMPHOID MALIGNANCIES

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Background. Primary Immunodeficiency diseases (PID), although rare in the general population are the best characterized and strongest known risk factors for lymphoid malignancies. Clinical immunodeficiency related score (IDR) is a recently introduced tool to identify possible patients with PID. Aim of the work is to evaluate the possibility of primary immunodeficiency in children with lymphoid malignancies by applying the clinical scoring system and studying the humoral and cellular immune status at diagnosis. *Patients and methods*. We studied sixty two pediatric patients diagnosed as non Hodgkin lymphoma (NHL)(n=26) , Hodgkin disease (HD)(n=16) and acute lymphoblastic leukemia(ALL)(n=20).They were 41 males and 21 females aged 0.15-18 years. They were recruited from Ain Shams University, Children `s Hospital and National Cancer Institute in Egypt. Evaluation included a thorough history of previous illness followed by calculation of an immunodeficiency related score (IDR).IDR score ≥6 was suspicious of PID and IDR score≥8 was highly suggestive of PID. Laboratory testing was performed in thirty two patients prior to start of chemotherapy, complete blood picture , immunoglobulin (Ig) A,M,G assay by nephelometry and quantification of peripheral T cell subsets by flowcytometry. Results: IDR score ≥ 6 was found in 2 ALL(10%), 7 NHL(27%) and 6(37.5%) HL patients(p=0.08), while IDR ≥8 was found in 4 NHL(15%),3 HL(18.8%) and none of ALL patients (p=0.58). IgG was higher in ALL 90% compared to HD 37.5% and NHL 33.3% (p=0.03) . IgM level was lower in HD 50% compared to NHL 12.5% and ALL 0%(p=0.04), while no significant difference in IgA Level (p=0.25). ALL had the highest peripheral T cell subset levels. CD 3 and CD8 levels were significantly low in HD (66.7%- 66.7%) compared to NHL (12.5%- 12.5%) and ALL (40%- 20%) (p=0.05) respectively while CD4 levels were comparable (p=0.23). IDR score ≥8 was associated with low IgM level (p=0.05) but not other Igs or T cell subsets levels. Three patients with IDR score more than 8 were diagnosed as ataxia telangiectasia after their malignancy diagnosis (one Burkitt lymphoma, two HL) and one patient had leucocyte adhesion defect diagnosed prior to development of NHL (Burkitt lymphoma). *Conclusions.* Immunedysregulation either raised Igs and peripheral T cell subsets due to chronic immunostimulation or decreased immune parameters as part of PID is associated with lymphoid malignancies. Creating a scoring system for screening of PID at diagnosis of lymphoid malignancy may be a useful tool for case detection and specific therapy.

1413

RISK OF RELAPSE IN 81 PATIENTS WITH PRIMARY NODAL, LOCALISED (I-II STAGE) DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) IN 1ST COMPLETE RESPONSE

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Background. DLBCL is an aggressive and potentially curable lymphoma. It presents itself as a localized disease in 30% of all cases and as primary nodal in 50-60% of these. Is known that in advanced disease, one third of them eventually relapse, but few data exist regarding localised disease. *Aim*s. To analyse the risk and the characteristics of relapse of patients with primary nodal localised DLBCL in complete response (CR). *Methods*. Eighty one patient (43/38 M/F; median age, 59 years) in CR after chemotherapy, mainly consisting anthraciclinic containing regimen, were included in the study. Main clinicobiological characteristics at diagnosis and at relapse were analysed. Uni and multivariate studies were performed. *Results*. Seventeen patients (21%) eventually relapsed. Late relapse, more than 2 years after CR, were 6 patients, and early relapse, less than 2 years after diagnosis were documented in 11 patients. The most important variables predicting relapse at diagnosis were age and being it the only predictive variable in the multivariate analysis. No differences were found according to the treatment given and if they received regimens with or without rituximab. The second CR rate obtained in the late relapsing patients after salvage therapies was higher that in early relapsing (27% vs 50%). Median time from diagnosis to relapse was 1 year for patients for early relapsing and 4.5 years for late relapsing. Five-year overall survival (OS) was 18% for early relapsing patients and 83% for late relapsing patients (p=0.012). For DLBCL relapse, two-year OS was 50% versus 18% with autologous transplantation or not, respectively (p=0.079).

	DLBCL (N=81)	Relapse DLBCL (N=17)
Age (median, range)	59 (18-87) y	64 (38-84) y
Sex (M/F)	43/38	10/7
ECOG 0-1 (%)	81	82
I/II stage (%)	33/67	35/65
B symptoms (%)	14	23
Bulky disease (%)	38	29
LDH > 3.4 ukat/L (%)	59	70
beta2-microglobulin > 2.2 mg/L (%)	44	50
IPI low/low-intermediate risk (%)	91	88

Summary. Late relapse is a common event in localised DLBCL with these patients achieving more frequently a second CR and having better survival than early relapsed patients. The outcome in relapsed patients remains poor and aggressive treatments like autologous transplantation should be performed whenever possible.

1414

GASTROINTENSTINAL (GI) TRACT INVOLVEMENT IN ADVANCED-STAGE DIFFUSE LARGE B-CELL LYMPHOMAS (DLBCL)

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Background and aims. The differentiation between primary extranodal GI tract lymphoma and secondary GI tract involvement in generalized lymphoma is difficult in many clinical situations but has a significant meaning while choosing treatment methods and strategies. The aim of this report is an attempt to characterize patients with GI tract involvement in advanced-stage DLBCL and indicating clues for treatment optimization. *Materials and methods*. We performed a retrospective analysis of 62 patients with CS IIB-IVB DLBCL treated in Chemotherapy Clinic in 2003-2009 regarding involvement of GI tract structures by lymphomatous process. Such changes were observed in 9 patients. The group consisted of 5 women and 4 men aged 31-76 (median-58). CS has been evaluated as II B (but multifocal nodal involvement in abdomen and pelvis)-3 patients, III A -2 patients, IIIB-1 patient, and IVB -3 patients. After standard diagnostic procedures patients were qualified for ICHT according to R-CHOP scheme (6-8 cycles). The following factors were considered in the analysis: 1) localization and characteristics of GI tract involvement, 2) molecular DLBCL subtype: ABC or GBC, 3)clinical parameters: generalized symptoms, IPI, LDH and β-2-microglobuline, 4) efficacy of surgical treatment if previously performed, 5) treatment efficacy understood as response to chemotherapy (CR/PR/SD/PD) and its duration (PFS/OS). Results: 1) the infiltration of stomach was found in 4 subjects, large intestine-3 subjects, small intestine-1 subject, a bifocal infiltration of small and large intestine-1 subject. The infiltration extanded across the entire GI tract wall to local lymph nodes or adjacent lymphatic tissue in 6 patients, however in as many as 3 patients it did not cross GI tract serosa. 2) molecular subtype ABC has been diagnosed in 3 cases, GBC-5 cases, in 1 case the molecular subtype has not been tested. 3) the incidence of systemic symptoms-66% has been comparable to that in the overall DLBCL group-61%, similarly mean IPI (3,2 /3,1), LDH and β-2-microglobuline. The only parameters that differed significantly between the groups were the incidence of anemia-56% in the group with GI tract involvement vs 35%, and need of RBC transfusions- 33% vs 14%. 4) surgical treatment preceeded ICHT in 4 cases,. In 3 patients lymphomatous infiltration has been found at the site of section or close to it during control ensoscopy performed between surgery and ICHT. 5) CR after ICHT (mean 7,1 cycles) has been achieved in 5 cases (55% vs 69% in the overall DLBCL group), in 3 patients with PR a radioimmunotherapy with ibritumomabem has been used as consolidation resulting in 1 CR +1PR+1PD (death during further treatment). Death of 1 patient caused by PD occured during ICHT. A 2- years-long PFS has been achieved by 5/9(56%)while in the overall DLBCL group by 43/62 (69%). A 2-years-long OS for patients with GI tract involvement was 6/9(67%) vs 48/62 (78%) in the overall DLBCL group. *Conclusions*. In advanced-stage DLBCL GI tract involvement is a negative prognostic factor for good ICHT R-CHOP response as well as its duration. Consolidation with ibritumomab in patients with PR only slightly improves treatment outcome.

1415

RITUXIMAB-TREATMENT FOR RECURRENT ACQUIRED ANGIOEDEMA UNDERLYING LYMPHOPROLIPHERATIVE DISORDERS: TWO CASE REPORT

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Background: Acquired Angioedema (AAE) due to the C1-inhibithor deficiency is often the spy of an underlying autoimmune disorder or a lymphoprolipherative disease (LPD). In these cases the acute symptoms can be resolved by the infusion of C1-inhibithor factor. Corticosteroids as immediate and etiological therapy in many cases is incapable of reducing the intensity and frequency of the AAE attacks. Aims: To obtain a faster and deeper control of AAE attacks and to avoid interferences of chemotherapy in their instable Complement's Cascade, two Non Hodgkin Lymphoma (NHL) patients, refractory to corticosteroid therapy, were treated with Rituximab at 375 mg/mq weekly. CaseA: a 50 years old man with a history of recurrent AAE received, two years later, the diagnosis of Diffuse Large B Cell Lymphoma (DLBCL) from the biopsy of laterocervical lymph node with a presentation at stage III of disease. The C1-Inhibitor level was 4,8 mg/dl and functional C1-INH percentage was 5% without demonstrable antibodies against C1-inhibitor (C1Q=91mg/L). At moment of DLBCL diagnosis the patient presented weekly severe attacks of AAE without any benefit from corticosteroid therapy (Methylprednisolone 2mg/Kg BW). Due to progressive symptoms, we administered Rituximab at 375 mg/mq weekly for 4 weeks. After the 3rd dose the AAE attacks disappeared and C1-Inhibitor level and functional C1-INH percentage were 33,8 mg/dl and 45% respectively . Subsequently the patient received chemotherapy according to CHOP like scheme (Cyclophosphamide, Liposomal Pegilated Doxorubicin, Vincristine Prednisone) every 21 days for 6 doses and obtained a Complete Remission (CR). After one year the patient died due to acute myocardial infarction in CR from NHL and AAE. Case B: a 75 years old man with a recurrent AAE and a monoclonal gammopathy (0,98 mg/dl of IgG4 monoclonal component) presented a 40% of bone marrow involvement as unique manifestation by follicular NHL. C1-Inhibitor level was 6,4 mg/dl and functional C1-INH was 18 %. No antibody against C1-inhibitor was present (C1Q 116 mg/L). The AAE attacks (2-3 monthly) were refractory to corticosteroid therapy. Rituximab was administered at standard dosage weekly for 4 weeks and every 3 months for 1 year as maintenance therapy. After the 3rd dose the patient obtained a remission from AAE with C1-Inhibitor level 30mg/dl and functional C1-INH 44% and. after 8th dose NHL marrow involvement disappeared. The patient is still in CR for AAE and NHL, after 1 year from the end of maintenance therapy. Conclusion: our experience demonstrates the efficacy of Rituximab in the treatment of AAE LPD-related in patients refractory to corticosteroid therapy even in absence of antibodies against C1 Inhibitor.

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RITUXIMAB PLUS LIPOSOMAL PEGYLATED DOXORUBICIN IN PRIMARY CUTANEOUS B-CELL LYMPHOMAS

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Background. The most common types of primary cutaneous B-cell lymphomas (PCBCL) are follicle center cell lymphoma (FCL), marginal zone B-cell lymphoma (MZL) and diffuse large B-cell lymphoma of the leg (DLBCL-LT). They represent approximately one third of all cutaneous lymphomas. FCL and MZL generally have an indolent behavior and an excellent prognosis (>90% 5-year survival), different from DLBCL (<60% 5-year survival). According to the EORTC/ISCL recommendations, the first line treatment of FCL and MZL is most often radiotherapy (surgery in a minority of cases with a single skin lesion), while patients with multifocal skin involvement deserve a systemic antiblastic treatment (similar to those with relapsed/refractory disease). The option of first-line chemotherapy, viceversa, is the rule in DLBCL-LT. CHOP-like regimens are by far the most commonly used, although hard to propose in patients over 80, who are frequent in this subset. In this regard, the association of rituximab (R) and liposomal pegylated doxorubicin (PLD) appear very promising. Aims: Based on the favourable results reported with R and PLD in several recent trials, we decided to test efficacy and safety profile of this combination. Methods: From June 2005 to January 2010, 11 patients with PCBCL were treated with R plus PLD at the hematology divisions of Siena and Florence. Five of them were males and six females, with a median age of 56 years (range 39-81). Four patients had FCL, 4 MZL, and 3 LBCL-LT. Ten patients had a stage II-III disease, while a female patient had a stage I disease with facial localization, and refused surgery and radiation therapy due to the high risk of aesthetic damage. Seven of 11 patients had relapsed after previous radiation treatment (3), phototherapy (2) and chemotherapy (2). Treatment plan consisted of 2 monthly cycles of R 375mg/m2 and PLD 20 mg/m2 d 1;15, followed (in responders) by two cycles at same dosage given only at day 1. All patients received prophylactic acetaminophen, clorphenamine, low dose steroids and pyridoxine to prevent rituximab infusional side-effects and Palmar-Plantar Erythrodysesthesia (PPE), respectively. Results: Ten out of eleven patients had a response (8 complete, CR; 2 partial, PR), while a patient did not respond (progressive disease, PD). All patients except two are alive and in stable remission, with a median follow-up of 24 months. One patient died due to progressive disease, and one due to a second neoplasm. Hematological toxicity was negligible (1 case of grade 2 neutropenia), as well as extra-hematological toxicity (2 cases of grade 2 PPE). Mild rituximab infusional reactions were registered in 3 cases at first infusion. *Conclusions*. These preliminary data suggest that R-PLD is very effective and well tolerated in the treatment of all subset of PCBCL. Moreover, this therapeutic option may be offered front-line in case of peculiar localization, in which radiotherapy or surgery may cause permanent aesthetic damage.

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HYPOALBUMINEMIA AS THE MOST SIGNIFICANT PREDICTOR OF POOR OVERALL SURRVIVAL IN PATIENTS WITH MUCOSA-ASSOCIATED LYMPHOID TISSUE NON-HODGKIN LYMPHOMA

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Background. Mucosa-associated lymphoid tissue (MALT) non Hodgkin lymphoma (NHL) belongs to the group of B cell non-Hodgkin's lymphoma (NHL), a subgroup of indolent lymphoma. The latest scientific papers on MALT lymphoma are aimed at finding both the clinical and laboratory as well as the biological parameters which would initially, before starting the treatment, isolate the group of patients at a high risk of rapid disease progression. *Aims*. The aims of this research were to investigate prognostic clinical and laboratory factors significant for outcome of patients with MALT lymphoma, as well as to compared these factors between patients with gastrointestinal (GIT) and nongastrointestinal (non-GIT) sites of primary lymphoma. *Methods*. This study involved 87 patients with diagnosis of MALT lymphoma. The followed pretreatment laboratory parameters were recorded: hemoglobin, serum albumin level, serum lactate dehydrogenase level (LDH), beta2-microglobulin (β2-M), virologic (HBsAg, HCV, HIV) and bacteriological (*Helicobacter pylori*) status. Estimated clinical features were: stage of disease (CS) according Ann Arbor classification, performans status (PS) evaluated by Europian Cooperative Oncology group (ECOG) recomandation, tumor mass voluminosity, number od extranodal localizations, presense of B simptomatology, splenomagaly and/or enlarged lymph nodes. Diagnosis of MALT lymphoma was based on histopathology analysis of tissue samples body, obtained by endoscopy or surgery. The followed-up period was 10 years. Risk factors were identified using the univariate and multivariate analysis. Results: The median patients' age was 58,3 years, range 28-82 years. The most frequent location in 29 (33,3%) patients was the GIT. The most common localizations of non-GIT MALT lymphomas were: the orbit in 13 patients (14,9%), the salivary gland in 11(12,6%), lungs in 9 (10,3%) and tonsils in 8 (9,2%). The median progression-free survival (PFS) was 36 months and at 5 years the OS rate was 64%. Significant longer overall survival (OS) had patients with non-GIT localization than those with GIT localization of MALT lymphoma (p=0.001). The univariate analysis showed that significant

predictors for poor OS were: age >60 years (p<0.001), CS III and IV (p=0.026), presence of B simtomatology (p=0.006), ECOG PS ≥2 (p=0.005), Helicobacter pylory positivity (p=0.015) and decreased serum albumin level (p<0.001). The multivariate analysis indicated the decreased serum albumin level to be the most significant predictor of poor OS: p<0.001, relative risk (RR)=5.060 (95% CI 2.055-12.458). In the group of patients with GIT localization of MALT lymphoma, the most significant predictor of poor OS was serum LDH level: p=0,031 (RR)=3,452 (95%CI 1,121-10,630), while the most significant predictor of poor OS in the group with non-GIT localization of MALT lymphoma was the decreased serum albumin level: p=0,001, (RR)=28,195 (95% CI 3,590"'221,456). Conclusions: It is shown that MALT lymphomas with non GIT localization have longer OS in compare with GIT localization. The most significant predictor of poor OS was decreased serum albumin level. At patients with GIT localization of MALT lymphoma, the most significant predictor of poor OS was serum LDH level, while at patients with non-GIT localization the most significant predictor of poor OS was also decreased serum albumin level.

1418

NON-HODGKIN LYMPHOMA OF OCULAR ADNEXAL SITES SUBTYPED ACCORDING TO THE REAL CLASSIFICATION: A 17-YEAR EXPERIENCE FROM SINGLE INSTITUTION

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Background. The Revised European and American Lymphoma (REAL) classification (1994) provided a useful and practical tool to define lymphomas. *Aims*. To report our experience with this unusual malignancy diagnosed since the REAL classification was implemented.Methods: Retrospective review was performed on patients (pts) with ocular adnexal non-Hodgkin lymphoma sites between 1994 and 2011. Medical records, diagnostic imaging procedures, and pathology reports, including immunophenotypes, were reviewed. Histologic definition applied the REAL Classification. *Results*. 10 pts were identified. All but one were females. Median age at diagnosis was 69 years (54-84). Primary sites included conjunctiva (5), orbital soft tissue (3), eyelid (2). No lacrimal gland location was found. Ann Arbor stage at diagnosis was IE in most cases (7). One patient (pt) had stage IIE (conjunctival and maxillary sinus) and 2 pts had simultaneous bone marrow involvement (stage IV). 6 pts had marginal zone B-cell subtype, and the remaining 4 pts had small lymphocytic B-cell lymphoma. Radiation as single treatment modality was given to all 7 pts with stage IE, and all those achieved a complete remission (CR). CR was also achieved using chemotherapy with cyclophosphamide (C), vincristine (V), doxorubicin, prednisone (P), and rituximab followed by radiation in the pt with stage IIE. Of the 2 pts with stage IV, 1 achieved a CR with rituximab-based chemotherapy, and 1 never achieved a CR despite chemotherapy (chlorambucil, CVP). With a median follow-up of 23 months (9-70), the 9 pts that achieved an initial CR remain alive and disease-free. The only pt that did not achieved a CR lost follow-up at 18 months. *Conclusions*. Radiation alone appeared to be an effective treatment modality for stage IE. Achieving a CR seems to be a crucial factor for long term control of the disease.

1419

ROLE OF THE ENDOSCOPIC ULTRASONOGRAPHY IN THE MANAGEMENT OF GASTRIC LYMPHOMAS: METANALYSIS OF LITERATURE

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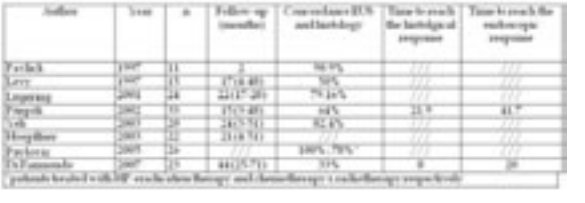
Background. After Sackmann et al, in 1997 introduced the concept that early stage PGL responds to eradication treatment, the last decade has viewed an escalating number of published materials, compared to previous decades, with a great quantity of prospective and retrospective studies. Aims. Due to the fact that the importance of EUS in initial assessment in gastric lymphoma is incontrovertible and that its role in follow-up is not clearly established (*Zucca & Dreyling, 2010*), the present work is focused in reviewing its role in diagnosis and follow-up. Methods A bibliographic research in MeSH database, using the key words [Stomach], [Lymphoma, Non-Hodgkin] and [Ultrasonography] has revealed a total amount of 117 papers published from 1971 to now. Manuscripts have been reviewed taking into account documents concerning staging and follow-up procedures. Results. STAGING: Despite first publications on the reliability of EUS in staging of PGL raised during the early '90's (*Caletti et al., 1993*) reported an accuracy of 80-92% and 77-90% for T and N stage respectively, these data were not confirmed by successive studies (*Fischbach et al., 2002*), reporting an accuracy of 59% and 71% for T an N stage, probably due to the fact that EUS is an operator-dependent technique (*Janssen, 2009*). Nevertheless, EUS has entered in the clinical practice of staging evaluation (*Pavlovic et al. 2005; Di Raimondo et al., 2007*) and has been indicated as the most appropriate technique in defining the loco-regional staging. FOLLOW-UP: Basically, there is a lack of studies concerning follow-up. Early reports with small series indicated a role of EUS both for staging and follow-up (*Pavlick et al., 1997; Lévy et al., 1997*). More recent reports, however, have shown that endosonographic remission is documented with a significant delay respect to histology (*Püspök et al., 2002*), even if at the same time another study confirmed that EUS is a dependable tool, finding a concordance between EUS and endoscopic biopsies of about 80% (*Lügering et al., 2001*), afterwards, two reports stated that EUS is reliable in determining the response evaluation and the detection of disease reappearance (*Yeh et al., 2003; Hoepffner et al., 2003*). Lately, the Serbian group, found a stringent correlation between EUS and histology in both patients treated with HP-eradicating therapy and/or chemotherapy ± radiotherapy. Afterwards, our group carried out a retrospective study in our patients observed in the last 10 years, in order to compare EUS with conventional endoscopy, not confirming the aforementioned studies, cause EUS was concordant with biopsies in a small portion of patients, about one third. In addition, the EUS findings returned to normal with a considerable delayed time in respect to gastroscopy with biopsy, even after a prolonged follow-up. (table 1) *Summary/Conclusion*. It is noteworthy that endoscopic ultrasound (EUS) assay is of great help in determining an appropriate staging of the disease, but its role in follow-up lack of evidences and is not recommended.



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PHASE I COMBINATION OF BORTEZOMIB AND EVEROLIMUS IN NHL: TOXICITY AND EARLY RESPONSE DATA

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The mTOR/AKT and NFkB pro-survival pathways contribute to pathogenesis of various NHLs. Preclinical data suggests the AKT/mTOR pathway rescues cells from bortezomib's antitumor effect, and inhibitors of these pathways show synergy in vitro against myeloma and MCL. We devised a dual proteasome and mTOR inhibition strategy for phase I testing in NHL. This study combines bortezomib (B) with the oral mTOR inhibitor everolimus (E) in relapsed/refractory NHL to determine maximum tolerated dose (MTD), toxicity, and response rates. We present preliminary results. All pts with relapsed/refractory NHL are eligible. A standard dose escalation design was employed. Pts continue study treatment until disease progression, elective withdrawal, or excess toxicity with no maximum number of cycles. B is given D1,4,8,11 of a 21 day cycle and everolimus continuously by mouth. The dose levels are: 1)B 0.7 mg/m2 IV, E 5 mg PO QOD, 2) B 1 mg/m2 IV, E 5 mg PO QOD, 3) B 1 mg/m2 IV, E 5 mg PO QD, 4)B 1.3 mg/m2 IV, E 5 mg PO QD, and 5) B 1.3 mg/m2 IV, E 10 mg PO QD. 11 patients have been treated on study, with MCL (2 pts), Waldenstrom's macroglobulinemia (1 pt), transformed FL (1 pt), DLBCL (2 patients), FL (4 pts), and CLL/SLL (1 pt). The transformed FL pt was later deemed ineligible and received 1 dose of drug. The median number of study cycles given is 3 (range 1-41). Treatment-related grade 1-2 toxicities include thrombocytopenia in 6 pts (54%), anemia in 5 pts (45%), hyperglycemia in 4 pts (36%), and fatigue in 4 pts (36%). Grade 1-2 neuropathy, triglyceride elevation, nausea, diarrhea, AST elevation, or diarrhea occurred in 3 patients each (27%). Treatment-related grade 3-4 toxicities included 1 instance of lymphopenia, neutropenia, thrombocytopenia, hyperglycemia, fatigue, hypophosphatemia, hyperkalemia, and syncope. In addition, grade 3 systolic heart

failure occured in one patient after cycle 14, resolving on discontinuation of study drugs. The patient had no evidence of ischemic coronary disease on stress testing. Two other patients were removed from study at 2 and 4 cycles for adverse events possibly related to treatment, including grade 2 neuropathy and syncope/postural dizziness. Enrollment at the 3rd dose level proceeds; a DLT has been identified at that dose level. Responses are assessed using CT criteria every 3 cycles (9 weeks). 2 PR (both MCL pts), 3 stable disease (2 FL and one Waldenstrom's), 4 progressive disease (2 DLBCL, 1 FL, 1 CLL/SLL), and 2 not evaluable (off study for adverse events prior to first response assessment) were observed. The Waldenstrom's pt was treated for 41 cycles with radiographically stable disease and a decreasing paraprotein"thought ultimately progressed after 29 months, leading to discontinuation. The MTD of B+E has not yet been reached. Enrollment proceeds at the 3rd dose level. B+E appears active in MCL and Waldenstrom's but responses are not evident among other NHLs.

1421

CLINICAL AND MOLECULAR EVALUATION OF THE EFFICACY OF THE HIGH-DOSE POLYCHEMOTHERAPY IN ADULT PATIENTS WITH ANAPLASTIC LARGE-CELL ALK-POSITIVE LYMPHOMA

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Background. Anaplastic large-cell ALK-positive lymphoma (ALCL) - a disease which is characterized by an onset in young age, an aggressive course with high frequency of extranodal involvement (skin, subcutaneous fat, bone, lungs) and poor prognosis. The diagnosis is based on the biopsy findings. Gistological study revealed a diffuse growth of large anaplastic cells, which express T-cell markers, CD30 and ALK. In 80% of cases the disease is characterized by the translocation t (2;5)(p23; q35) and in other cases - by the variant translocations. The overall 5-year survival in adult patients is 40-60% (depending on the stage of the disease) on CHOP and CHOP-like regimes. The using of high-dose chemotherapy protocol NHL BFM-90 in the treatment of ALCL in children leads to 80-100% 5-year disease-free survival (depending on the stage of disease). Relapse are more common in children with advanced stagies, in the presence of factors of poor prognosis and minimal residual disease. For adult patients currently no clear prognostic features. aims: to evaluate the efficacy of high-dose chemotherapy program NHL BFM-90 in patients older than 18 years and to determine the prognostic significance of the minimal residual disease in blood and/or bone marrow samples by RT-PCR. *Methods*. Patients older 18 years with diagnosed ALCL. Blood and bone marrow samples were examined for the quantity of NPM-ALK transcripts by RT-PCR before starting the treatment and after the end of protocol NHL BFM 90. Results: 20 adult patients with diagnosed ALCL were treated by protocol NHL BFM-90. The informed consent was obtained from all patients. All patients revealed generalized lymphadenopathy, 60% of the cases had extranodal sites, such as skin, subcutaneous tissues, bones and lungs. 80% of patients were diagnosed with stage III-IV disease. RT-PCR of NPM/ALK was employed to analyze the minimal residual disease to all patients. Patients with the unfavorable prognostic factors and the positive results of the minimal residual disease evaluation received autologous stem cell blood transplantation. We obtained a complete remission in 19 patients. One patient died of infectious complications during the first course of polychemotherapy. Two patients with stage IV and skin involvement relapsed during the first year after the end of the treatment. One of them died of the disease progression. One relapsed patient achieved the second long-term remission after allogeneic bone marrow transplantation. Thus, the overall 5-year survival rate is 90%. *Conclusions*. The using of child protocol NHL BFM-90 has shown the high efficacy in adult patients with ALCL compared with currently used chemotherapy regimes. The using of autologous stem cell transplantation may by rational in patients with poor clinical and molecular prognostic factors.

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EVALUATION OF PROGNOSTIC VALUES OF CLINICAL AND HISTOPATHOLOGIC CHARACTERISTICS IN DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH RITUXIMAB-CHOP THERAPY

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Background. After the introduction of rituximab, a monoclonal antibody to CD20, improved event-free survival (EFS) and overall survival (OS) among the elderly and younger patients with diffuse large B-cell lymphoma (DLBCL) has been reported. The mechanism of anti-tumor action of rituximab is fundamentally different from other known cytotoxic agents therefore, the prognosis associated with the clinical and histological characteristics of DLBCL need to be re-evaluated. Aims. To evaluate the relation of clinical and histopathologic characteristics to treatment outcomes in patients with DLBCL that were treated with rituximab-cyclophosphamide, adriamycin, vincristine, and prednisolone (R-CHOP) as first-line chemotherapy was investigated. Methods. Patients with newly diagnosed DLBCL treated with R-CHOP combination immunochemotherapy were retrospectively evaluated for their clinical characteristics, treatment efficacy and survivals. Immunohistochemistry (IHC) of CD10, bcl-6, MUM-1 was performed and according to the algorithm proposed by Hans, patients were subclassified into either germinal B-cell like (GCB) or non-GCB type. IHC for bcl-2 was also performed and the relation of patients' clinical and IHC characteristics to overall survivals were analyzed. Results. 87 patients (median age, 57 years) were analyzed for the association of clinical characteristics to OS. Age≥ 60 of the patients, elevated serum lactose dehydrogenase level, presence of 2 or more extranodal sites, and bone marrow involvement of DLBCL were powerful prognostic factors to OS in multivariate analysis. There was no significant difference of OS between the patients with GCB and those with non-GCB type. *Results* of bcl-2 also failed to demonstrate the relation to OS. Whereas no difference enough for OS was shown between high-intermediate risk group and high risk group classified by the standard International Prognostic Index (IPI) (P = 0.64), all 3 groups of revised IPI showed a clear-cut separation for EFS and OS. *Summary/Conclusions*. In this post hoc exploratory analysis, the revised IPI showed more clear-cut separation of patients according to their OS than the standard IPI, especially among patients that had more adverse clinical factors. Hans classification and the result of bcl-2 showed no predictive value for OS in patients with DLBCL treated with R-CHOP.

1423

PROGNOSTIC FACTORS IN FOLLICULAR LYMPHOMA: A SINGLE INSTITUTION STUDY

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Background. For the prediction of overall survival (OS) in the field of follicular lymphomas, many prognostic models have proven useful. In our study we tried to evaluate these prognostic factors based on our experience. *Method.* We conducted a retrospective study on 71 newly diagnosed patients with follicular lymphoma, from a sum of 767 B ell lymphoproliferative disorders on a period of 7 years (2002-2008) in our clinic. We were focussed on clinical characteristics, symptom duration before diagnosis, pathologic findings, including grade, laboratory data, imaging studies at initial presentation and management. The male/female ratio was 1.09/1. Ages ranged from 32 to 84 years (median 57,80). By Ann Arbor classification the preponderence of our patients were diagnosed in stage IV (46.5%, 33 patients). B symptoms were present in 39,4%, 28 patients. Extraganglionary involvement was present in 57 patients from the study lot. Initial therapy was deferred in 39%. The remaining patients received stage-appropriate therapy. Survival was measured from time of diagnosis to death. Prognostic factors at initial diagnosis that were statistically significant in univariate log rank comparisons of Kaplan-Meier survival curves were used to build a multivariate proportional hazard regression model of OS. *Results*. OS differed with high (> 12 g/dl) versus low (< 12 g/dl) hemoglobin (p=.001) and in younger (< 60 years) versus older (> 60 years) patients (p=.05). Both hemoglobin and age were also significant in a multivariate proportional hazards analysis. Low hemoglobin and increased age were independent predictors of lower OS with hazard ratios of 6.6 (95% CI 2.2-20.1) and 3.7 (95% CI 1.2-11.7), respectively. Median survival for older patients who also had anemia was only 3.1 years. A test for interaction between age and hemoglobin was negative (p=.35). The estimated hazard ratio for an older individual with low hemoglobin was 24.7 (95% CI 4.0-153.3). *Conclusions*. The next aspects had prognostic significance: decrease in tumor mass with more than 50% after first treatment, type of response obtained, size and location of the adenopathies, serum of LDH level, platelet number, hemoglobin level at diagnosis, histological type of follicular lymphoma, value of the nuclear proliferation, Ki-67, performance status, IPI value, FLIPI.

1424

CLINICAL CHARACTERISTICS, TREATMENT AND OUTCOME OF PRIMARY RECTAL LYMPHOMA: A SINGLE CENTER EXPERIENCE OF 16 PATIENTS

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Background. Rectum is a relatively uncommon site for lymphoma compared with other gastrointestinal sites and no consensus regarding management of primary rectal lymphoma (PRL) has been made due to its paucity. *Aims*. We aimed to investigate the clinical characteristics and treatment outcomes in PRL patients in a single center patient cohort. Methods. Between January 1993 and December 2010, 16 consecutive patients of PRL were identified and treated at the Asan Medical Center, Seoul, Korea. We retrospectively analyzed the results of these patients. *Results*. These 16 patients with PRL comprised 0.8% of all non-Hodgkin's lymphoma patients (n=1984). The median age at diagnosis was 41 years (range, 30-68 years) and 11 were male. The most common presentations were anal bleeding (n=7) and abdominal pain (n=4) while 3 patients were asymptomatic. Eleven patients had stage I/II disease by Ann-Arbor staging. B-cell lymphoma (n=14) made up of majority of the series and half of them were extranodal marginal zone lymphoma (n=7). The others included 4 diffuse large B-cell lymphomas and 3 mantle cell lymphomas. Ten patients were given systemic chemotherapy with (n=3) or without rituximab (n=7) and 4 of them received additional local therapy including radiotherapy (n=3) or surgical resection (n=1). The others were treated with radiotherapy (n=3) or endoscopic mucosal resection (n=3) as a first-line therapy sparing systemic chemotherapy. Twelve patients (75%) achieved a complete response (CR) after first-line treatment. Especially all of those with extranodal marginal zone lymphoma (n=7) achieved CR during or after initial treatment, while 5 of 9 patients with the other histologic subtypes succeeded to gain CR. During median follow-up of 27.0 months (range, 2.8 - 123.5 months), 3 patients died and 4 patients experienced progression resulting in 2-year progression-free survival rate of 78.1% and a 2-year overall survival rate of 78.6%, respectively. *Summary/Conclusions*. PRL is very rare and seems to be mostly B-cell type. Extranodal marginal zone lymphoma may be one of the most common types of PRL with favorable treatment outcome.

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HEPATITIS B REACTIVATION IN PATIENTS WITH NON HODGKIN LYMPHOMA CD20+ IN MAINTENANCE THERAPY WITH RITUXIMAB

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Background. Anti CD20 antibody (Rituximab) based chemotherapy regimens increase the HBV reactivation risk although sporadic HBV reactivation cases are reported in patients on maintenance with Rituximab single therapy too. We evaluated how many HBV reactivation occurred among patients Hepatitis B core antigen positive (HBcAB +) and Hepatitis B surface antigen negative (HBsAg-) who received Rituximab single therapy during maintenance. *Aims*. The aim of this study is to assess the prevalence of HBV reactivation among patients HBcAb +/ HBsAg - during the maintenance therapy with Rituximab. Methods. In our Unit, 56 patients with non Hodgkin Lymphoma CD20+ received maintenance therapy with Rituximab (schedule: 375 mg/mq every 3 months for 2 years) from January 2007 to December 2010. 42% (25/56) of patients were treated with R-CHOP regimen; 57% of patients were treated with R-FN regimen. None of these patients received prophylactic therapy with lamivudine during induction or maintenance. All the patients were given blood tests for HBV (HbsAg; HbsAb; HbeAg; HbeAb; HbcAb) before starting maintenance therapy and liver function tests before each administration of Rituximab. *Results.* 28% of the patients (15/56) were HbcAb positive. 42% of the patients (25/56) completed the maintenance treatment and 28% of them are HbcAb positive (7/25): in none of these patients occurred the HBV reactivation (median follow up: 14 months). 55% of the patients (31/56) of the patients are still in therapy with Rituximab and 25% of them are HbcAb positive (8/31): even in these patients has occurred to date the HBV reactivation. *Conclusions.* In patients HBcAb +/ HBsAg - treated with Rituximab in single therapy is indicated the prophylaxis with lamivudine. In our observational study the HBV reactivation prevalence among patients HBcAb +/ HBsAg - not in prophylactic therapy with lamivudine during the maintenance therapy with Rituximab is 0%. More ambitious prospective studies are required to establish the clinical utility of prophylactic therapy with lamivudine during the maintenance therapy with Rituximab.

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HEPATITIS B REACTIVATION IN PATIENTS WITH NON HODGKIN LYMPHOMA CD20+ UNDERGOING CHEMOTHERAPY WITH AND WITHOUT RITUXIMAB

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Background. Reactivation of HBV infection is a well-recognized complication in infected patients who undergo cytotoxic chemotherapy for cancer. The highest incidence of reactivation was reported in patients with non-Hodgkin's lymphoma (NHL) and hematopoietic stem cell transplantation. Several case reports demonstrated that severe hepatitis due to HBV reactivation after rituximab administration occurred both in hepatitis B surface antigen(HBsAg)-positive and HBsAg-negative patients. However, systematic evaluation of the relationship between HBV reactivation and rituximab is still limited. We conducted a study to investigate the relationship between rituximab-based therapy and HBV reactivation in 405 CD20-positive NHL patients at our institution. Methods. In our Unit, 405 CD20-positive NHL patients all newly diagnosed underwent measurement of HBsAg, anti-HBs,anti-HBc,HBeAg and anti-HBe. Patients were monitored by liver function tests during and after therapy as follows: on day 1and day 14 of each cycle, every month for a year. *Results*. 154/405 (38%) patients were HBcAb-positive. 107 had an aggressive lymphoma, 42 had an indolent lymphoma. HBV reactivation was observed in 2 patient (1,2%) who had received chemotherapy including steroid and rituximab and in 3 patients(1,9%) who had received chemotherapy including regimen with only fludarabine without rituximab Immediate administration of lamivudine therapy after elevation of HBV DNA level was conducted, and this resulted in reduction of it and improvement of liver function test. *Conclusions*. Rituximab plus steroid-containing regimens may increase the risk of HBV reactivation in HBsAg-negative and HBcAb-positive lymphoma patients but more attention should be paid on treatment with only fludarabine. More ambitious prospective studies are required to establish clinically useful or cost-effective follow-up methods for control of HBV reactivation in lymphoma patients with occult HBV infection.

1427

PERSISTENT B CELL POLYCLONAL LYMPHOCYTOSIS (PPBL) WITH MASSIVE SPLENOMEGALY MIMICKING MARGINAL ZONE LYMPHOMA

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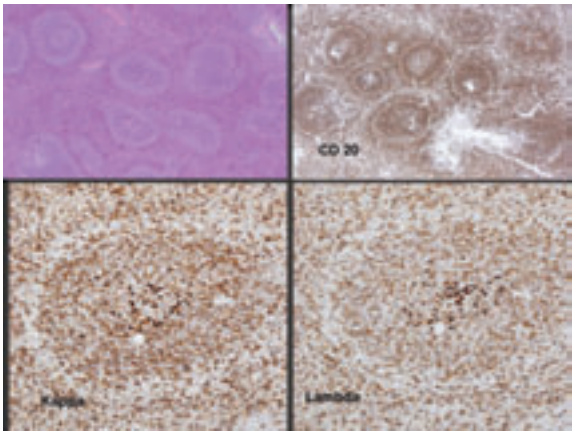
Persistent polyclonal B-lymhocytosyi (PPBL) is a chronic expansion of polyclonal B lymphocytes seen predominantly in smoking women. Although considered as non- malignant it shares some features with malignant lymphomas like cytogenetic abnormalities and organomegaly. We present a case of PPBL with massive splenomegaly infiltrated by polyclonal lymphocytes resembling splenic marginal zone lymphoma. Case report and methods. A 46 years old smoking woman was seen at our institution in June 2006 for lymphocytosis. Blood counts were WBC=13.6X10E9/L (lymphocytes=10160/mm3) with some bilobated lymphocytes in blood film, Hb=104gr/L and platelets=143X10E9/L. Serum biochemistry revealed a Ferritin level of 11mg/dl (N>20) and LDH=240UI/l (<160). IgM levels were increased (1696mg/dl N=<230), the rest of immunoglobulins were in the normal range and no M spike was apparent in serum proteinogram , immunofixation was also negative. Immunophenotyping of lymphocytes by flow citometry revealed a B cell phenotype (CD19,CD22,CD20), positive for IgM, IgD , FMC7, CD27 ,and negative for CD5,CD10,CD23 with no light chain restriction. FISH analysis was negative for Chromosome3 and 8 abnormalities. IgH rearrangement by PCR was polyclonal. The patient was positive for HLADR7. A CT scan revealed an enlarged spleen and small paraaortic adenopathies. The patient developed progressive enlargement of the spleen measuring over 24cms with signs of portal hypertension and worsening of anemia and platelet level. Seplenectomy was performed in April 2009. The spleen weighted 3.9 Kg.. Histologically there was an extense lymphoid infiltration from the marginal zone of the white pulp affecting also the red pulp. By Flow cytometry the cells had the same phenotype as in peripheral blood. By paraffin IHC CD20, IgM, IgD, Bcl2, MNDA were positive and CD3, CD5, Cyclin D1, Bcl6, CD10, XBP1, CD23, CD43 negative without light chain restriction (Picture 1). Molec-

ular analysis showed polyclonal IgHV rearrengement. An isochromosome +i (3q) was detected by FISH. After splenectomy the patient blodd count return to normal range with no abnormal lymphocytes in the blood film. The presence of massive splenomegaly in patients diagnosed of PPBL has been recently recognized. To our knowledge only 6 cases including ours have been reported so far. All of them showed an histological pattern consistent with a marginal zone lymphoma but polyclonal in nature by immunophenotyping and molecular analysis. The biological mechanisms by which some patients with PPBL develop massive splenomegaly is unknown. Due to the rarity of this entity it shoud be kept in mind to avoid misdiagnosis



1428

NON-HODGKIN LYMPHOMAS IN CHILDREN WITH CHROMOSOME INSTABILITY SYNDROMES

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Background. Chromosomal instability syndromes (CIS) - a group of rare autosomal recessive diseases, which have common features of increased chromosome fragility phenomenon, immune deficiency, hypersensitivity to radiation and high predisposition to lymphoid tumour. CIS group include: ataxia-telangiectasia (AT), Nijmegen breakage syndrome (NBS), Bloom syndrome, Fanconi anemia and others. Methods. The diagnosis of NBS and AT is based on specific phenotype, results of clinical, laboratory and genetic investigations. The diagnosis of Non-Hodgkin lymphomas (NHL) is based of clinical, morphological and immunological researches of tumor substrate. Results. 85 children with NHL were treated in the department of hematology, Lviv Children's Hospital, during 1992-2009. 7 of them (8.2%) with CIS: 6 - with NBS (85,7%), 1 - with (14,3%). In 4 cases a diagnosis of CIS was established upon manifestation of oncological diseases in children, while in 3 children lymphoma developed during the follow up period. At the time of diagnosis of NHL median age of this children group was 9.3 years (4.3 years - 12.2 years). All patients with NBS and NHL were homozygoes for the 657del5 NBS1gene mutation. Children with NBS had typical craniofacial abnormalities (microcephaly, 'birdlike' face) and short stature. The skin malformations (spots of hypo- and hyperpigmentation) were diagnosed in 3 children. The other typical detected defects: sindactyly and clynodaktyly (2 patients), renal hypoplasia (1 patient). Clinical picture of AT was comprised of progressive cerebellar ataxia, oculocutaneous telangiectasias, spots of hypo- and hyperpigmentation. Lymphoblastic lymphoma was diagnosed for a 1 child, diffuse large B-cell lymphoma in 5 patients, lymphoma from large and small cells in 1 child. In most cases of the NHL the patients had nodal manifestation: a peripheral group of lymph nodes and spleen - in 7 (100%) patients, abdominal lymph nodes - in 3 (42,9%) children, lesion of mediastinum with the compression syndrome - in 5 (71,4%) patients. Atypical cells (L1/L2-type lymphoblasts according to FAB-classification) in the bone marrow revealed in one (14,3%) boy with lymphoblastic lymphoma. Involved extranodal sites included liver (6 (85,7%) patients), lung tissue and pleura (4 (57,1%) patients), kidney (one (14,3%) child), bones (one (14,3%) girl). Treatment was conducted according to BFM-group protocols (NHL-BFM-95/NHL-DGLLU-2000). Two patients died prior to beginning and two at the first stages of chemotherapy from complications of tumour process and severe concomitant infections, 1 patient - as a result of tumour progression. Specific therapy was effective in 2 (28,6%) children which currently have a long-lasting remission (11 and 7 years). Summary. The severity of clinical course of NHL in patient with CIS is caused by infectious complications related to the background of combined immunodeficiency, which requires a powerful antibacterial, antifungal protection and correction of immune status. Cytostatic treatment of NHL in children with CIS is possible and should be attempted. Intensity of therapy should be adjusted to individual risk factors and tolerance.

1429

DISSECTING FOLLICULAR DENDRITIC CELL SARCOMA INTO SUB-RISK GROUPS ACCORDING TO CLINICAL OUTCOME

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Background. Follicular dendritic cell sarcoma(FDCS) is rare neoplasm and has been regarded as a low or intermediate-grade sarcoma. However, FDCSs show broad clinicopathological spectrum including some highly aggressive cases of short term survival. Currently, no well-established guideline is available for optimal treatment and predicting clinical outcome. *Aims*. To investigate clinicopathological factors predicting their clinical behavior and dissect them into sub-risk groups, and finally provide a base for optimal therapeutic planning, we analyzed 135 FDCSs with relevant clinicopathological parameters and clinical outcome. Methods: Among 34088 articles screened through Pubmed search (search terms; follicular dendritic cell tumor or follicular dendritic cell sarcoma or dendritic cell, 1986 to 2006), 71 articles which reported 135 FDCSs with available clinicopathological information were panned out. Repeatedly cited cases were carefully selected and adjusted to avoid the doubled data. Some missing pathological and clinical follow-up data were added by referring to original authors. 135 FDCSs were analyzed with relevant clinicopathological parameters including age, gender, location, tumor size, tumor margin, mitosis, nuclear atypism, hemorrhage, necrosis, Ebstein-Barr Virus (EBV) association, Castleman's disease association and treatement modality. For assessment of prognostic significance of each parameter, statistical analysis with Kaplan-Meier model and Cox-regression test were performed using SPSS, version 11.5. With combination of statistically validated parameters, we categorized FDCSs into three-tiered risk groups and analyzed their clinical outcome. Results: Mitotic Index (≥5/10 HPF)(P=0.0182) and intra-abdominal location (P=0.0003) were significantly correlated with the clinical courses including recurrence, metastasis and survival, whereas all other tested factors including tumor size, margin and nuclear atypism failed to achieve statistical significance. 66 FDCSs with well documented mitosis, tumor location and follow-up information were categorized into three groups; low risk(n=24, extra-abdominal location and mitosis <5/10HPF), intermediate risk(n=31, extra-abdominal location and mitosis ≥5/10HPF, or intra-abdominal location and mitosis <5/10HPF) and high risk(n=11, intra-abdominal location and mitosis ≥5/10HPF). Three groups showed distinct clinical outcome in event-free survival (P=0.0000) and overall survival (P=0.0001). The median event-free survival were 96 months, 24 months and 11 months (low, intermediate and high risk group, respectively). None of low risk group died of disease (100% survival by 10 years), whereas high risk group showed aggressive clinical outcome (57.1% of 2-year survival). The intermediate risk group showed borderline clinical course (81.2%, 62.5% and 25% of 2-year, 5-year and 10-year survival, respectively). *Conclusions*. Our results suggest that the FDCSs are very heterogenous in clinical outcome and could be subclassified into three-tiered risk group system which would be useful for predicting prognosis and optimal patient management.

1430

CAN WE CURE HTLV-I ASSOCIATED ADULT T CELL LEUKEMIA LYMPHOMA?

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Background. Adult T cell leukemia (ATL) is one of the rare human cancers initiated by a transforming retrovirus, HTLV-I. After many years of

controversy, it is now accepted that the viral transactivator protein Tax plays a critical role in initiating the leukemic process, because Tax transgenics develop a disease with striking ATL features. However, its role in the maintenance of the lymphoproliferation remains still a matter of debate. Long-term prognosis of ATL patients remains extremely poor. In acute ATL, well conducted Japanese trials demonstrated that although combinations of chemotherapy improved response rate, they failed to achieve a significant impact on survival. Patients with chronic and smoldering ATL have a better prognosis but long-term survival is poor when these patients are managed with a watchful-waiting policy or with chemotherapy. *Aims and Methods*. We recently realized a worldwide meta-analysis. *Results*. We showing that the combination of zidovudine and interferon-alpha (IFN) is highly effective in the leukemic subtypes of ATL and should be considered as standard first line therapy in that setting. This combination has changed the natural history of the disease through achievement of significantly improved long-term survival in patients with smoldering and chronic ATL as well as patients with acute ATL and wild type p53. ATL lymphoma patients may benefit from initial induction therapy based on aggressive chemotherapy regimen in addition to or followed by antiretroviral therapy with AZT/IFN. In all patients, in order to prevent the occurrence of resistance and relapse, clinical trials assessing bone marrow transplantation additional targeted therapies such as arsenic/IFN combination or monoclonal antibodies, are mandatory after achieving CR. In that sense, we recently reported that the combination of arsenic trioxide, IFN, known to trigger Tax proteolysis in addition to zidovudine, yielded unprecedented response rates in chronic ATL patients, and may prevent relapses in ATL lymphoma responding patients after chemotherapy. To investigate the molecular mechanism of therapeutic action *in vivo*, we used Tax transgenic mice that develop a disease with striking ATL features. We demonstrate that the combination of arsenic trioxide and IFN cures Tax-driven murine ATLs through selective targeting of leukemia initiating cell (LIC) activity. Importantly, this effect requires proteasome function. *Conclusions*. Overall, our findings strongly suggest that in this model ATL LICs are addicted to the viral Tax oncoprotein and open the prospect of new strategies to cure ATL.

1431

DOSE ADJUSTED EPOCH - RITUXIMAB AS FIRST LINE TREATMENT FOR HIGH RISK, AGGRESSIVE B-NHL: A SINGLE CENTER EXPERIENCE

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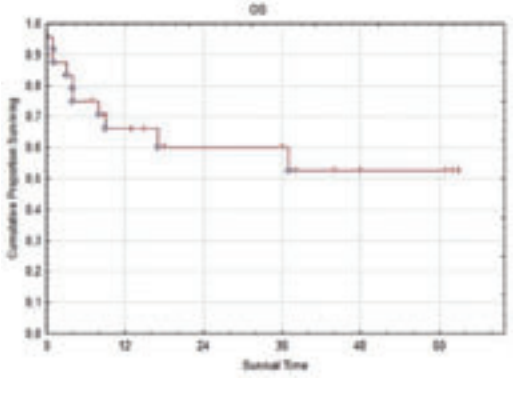
Background. ose-adjusted EPOCH- rituximab (DA-EPOCH-R) is an infusional protocol (etoposide, vincristine, and doxorubicin for 96 hours with bolus doses of cyclophosphamide and oral prednisone) which is based on pharmacodynamical adjustment of drug dosage depending on laboratory values of absolute neutrophil count and platelets (*Wilson WH et al. Blood 2002;99:2685-2693*). Rationale for this regimen is that tumor cells are less resistant on prolonged exposition to low doses of chemotherapy than to short exposition to high doses. Aims. To assess clinical outcome in patients with aggressive B-NHL with poor prognostic factors (very aggressive histollogy, IPI ≥2 and/or high proliferation index; PI>80%, measured as percentage of Ki67+ cells) treated with DA-EPOCH-R in first line. Methods: From May 2005 to March 2011, 24 patients, median age 50.5 years (range 17-75) with poor prognosis, aggressive B-NHL, were included. Male/female ratio was 14/10.



Out of 24 patients 20 were DLBCL, 2 were Burkitt lymphomas and 2 were mantle cell lymphomas. Elevated LDH had 19 out of 24 patients (79%) and 18 out of 24 patients (75%) were in Ann Arbor stages 3 and 4. IPI ≥2 had 19 out of 24 patients (79%). DA-EPOCH-R was administred according to original schedule. Six of these patients proceeded to autologous hematopoietic stem cell transplantation (autoHSCT) as consolidation therapy due to very aggressive histology and biology of disease. In subgroup of 20 patients with DLBCL with median age of 55.5 years (range 17-75), 16 (80%) had elevated LDH, 16 (80%) were Ann Arbor 3 and 4 and 17 (85%) had IPI ≥2. *Results*: Overall response rate (ORR) was 75% (18/24), including 14 (58%) complete responses (CR) and 4 (17%) partial responses (PR). Overall survival (OS) was 52% at 5 years (median not reached) with median follow up of 14 months (range 0-63). Of patients who achieved complete remission, 12 are still in CR with progression free survival (PFS) 75% at 5 years. In subgroup of patients with DLBCL 15 out of 20 patients responded to therapy (ORR 75%), 11 patients achieved CR (55%). Overall survival in this subgroup was 50% with median follow up of 11 months (range 0-63). Summary/Conclusions: In this report we included only patients who had aggressive B-NHL with poor prognostic factors. Our results are in line with previous report (*Garcia-Suarez J et al. British Journal of Haematology 2007;136:276-285*) who also find this regimen effective as first line treatment in high risk DLBCL patients. In certain subgroups of patients it may be option to continue treatment with autoHSCT as consolidation therapy. Only 2 patients relapsed during follow up period. All 6 patients who were transplated are still in CR. When subgroup of patients with high risk DLBCL was analysed separately, the results were similar as for entire group. We conclude that DA-EPOCH-R is highly effective regimen as first line treatment in high risk group of aggressive B-NHL.

1432

OUTCOME OF CHRONIC LYMPHOPROLIFERATIVE DISORDERS ASSOCIATED WITH HEPATITIS VIRUSES INFECTIONS - SINGLE CENTER EXPERIENCE

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Background. Chronic lymphoproliferative disorders (CLD) frequently associate hepatitis viruses infections. The role and timing of antiviral therapy remains to be defined - for HBV early therapy seems to prevent viral reactivation, for HCV the best strategy is not established. *Aims*. Analysis of hematological and virologic response after chemo/immunotherapy alone or combined with antiviral therapy in patients diagnosed with CLD associating hepatitis viruses B, C, D. Methods. Retrospective analysis performed in Hematology Department - Emergency University Hospital Bucharest, on patients diagnosed with CLD (lymph node/bone marrow biopsy) and positive serological tests for hepatitis viruses between December 2007-July 2010; all of them received chemo/immunotherapy +/- antivirals. The hematological response was assessed using CT scan and bone marrow biopsy, the virologic response by determining quantitative viremia using TaqManPCR method. *Results*. We selected a group of 52 patients with CLD and hepatitis virus infection receiving therapy. HCV had 26/52(50%), 23/52(44,23%) HBV and 3 patients double/triple infection. Different hystologic types were found and divided into indolent/aggressive type: see Table 1. Chemotherapy+/-Rituximab was given in 19/52(36,53%) patients, 32/52(61,53%) received also antiviral therapy and one patient Interferon alone. In the group of patients receiving Rituximab representing 17/52 (32,69%), 11/17(64,7%) associated HBV and 6/17(35,3%) HCV. All HBV patients with R-chemotherapy associated antivirals. Four HCV patients received R-chemotherapy and antivirals. Interferon was administered in 13/52(25%) HCV patients and one HBV. Ribavirine+Interferon received 2 patients. Lamivudine was administred in 17/52(32,69%) patients and Entecavir to one. Complete hematologic response was assessed according to the indolent/aggressive type of CLD and HCV vs HVB - see table 2. A possible explanation is that HCV was mostly found in indolent types of CLD, ressponding better to therapy. Twenty six patients achieved complete response, most of them receveing chemotherapy and antiviral, 10 chemotherapy alone and one only Interferon.

Hystologic type	Indolent	Aggressive
Diffuse large B-cell NHL	-	16/53(30,76%)
Small B-cell NHL (marginal, lymphocytic)	16/53(30,76%)	-
Chronic lymphocytic leukemia	2/53(3,66%)	-
T-cell NHL	-	4/53(7,69%)
Follicular NHL	3/53(5,76%)	-
Mantle NHL	-	1/53(1,92%)
Hodgkin's lymphoma	3/53(5,76%)	-
Waldenstrom macroglobulinemia	2/53(3,84%)	-
Total	51(98,08%)	2(1(40,38%)

Table 1. The chronic lymphoproliferative disorders - histologic types.

Hematologic response	Complete	Partial	Progressive disease
Indolent CLD	13/53(28,84%)	13/53(25,15%)	3/53(5,61%)
Aggressive CLD	13/53(21,15%)	3/53(5,76%)	7/53(13,46%)
Total	26/53(50%)	16/53(26,92%)	13/53(25,07%)
HCV infection	16/53(30,76%)	8/53(15,38%)	2/53(3,84%)
HBV infection	10/53(19,23%)	4/53(7,69%)	9/53(17,30%)
Total	26/53(50%)	12/53(23,07%)	11/53(21,15%)

Table 2. Complete hematologic response assessed according to the indolent/aggressive type of CLD and HCV vs HVB infection.

Complete hematologic response associated complete virologic response (negative viral load) in 13/26(50%) patients, partial response only 2 and progressive disease 3 cases of complete virologic response. In total, 18 patients obtained complete virologic response - 13/18 (72,22%) complete hematologic responses, 7/18(38,88%) with HBV and 6/18(33,33%) with HCV; one case of each virus had partial response and progressive disease 3(6,66%) patients with HBV and none with HCV. Most patients with CVR had chemotherapy+antivirals 10/18 (55,55%). Positive viremia was found in 34/52(65,38%) - 22 with combined therapy, 13 with chemoimmunotherapy. Viral reactivation was detected in 2 patients, both with HBV receiving R-chemotherapy+antiviral, one achieving complete hematologic response, and one with progressive disease. Cryoglobulins were found in 8/52(15,38%) patients, all associating HCV, 5 with indolent disease; equal number of complete/partial responses (4) were achieved, but only one complete virologic response. Conclusions. Best hematological responses (complete and partial) were obtained in indolent types of CLD associated to HCV infection treated with combination of chemo/immunotherapy and antiviral therapy. The complete virologic response was revealed in patients with combined therapy, but a significant number associating antivirals still have detectable viremia, revealing that virologic response doesn't parallel the hematological one. Still, adding antiviral therapy seems to be the best option as it prevented viral reactivation in most cases.

1433

INTRAPLEURAL INSTILLATION OF RITUXIMAB FOR THE TREATMENT OF MALIGNANT PLEURAL EFFUSIONS IN CD20+ NON-HODGKIN´S LYMPHOMA

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Background. Malignant pleural effusion is a common clinical problem in patients with neoplastic disease. Approximately 10% of malignant pleural effusions are caused by Non-Hodgkin´s lymphoma (NHL). Aim. Four patients with NHL presented with unilateral pleural effusion, flow cytometry revealed CD19 and CD20 positive malignant cells. Two patients with follicular (grade 2) histologycal type of NHL (FL) had lifetime of effusion above two months. Two patients with diffuse large cell B-cell histologycal type of NHL (DLBCL) had lifetime of effusion less one month. Methods and results. Systemic chemotherapy (FCR or R-CHOP) and repeated percutaneous drainage were unable to control the effusions. Rituximab was instilled in a dose-escalating manner (starting dose 100 mg, maximal dose 400 mg) via the chest tubes into pleural spaces. The effusions resolved in both patients with DLBCL in two-three weeks and the patients are free of symptoms: one - for six months and another - for two years to date. In two patients with FL in 60 days after the application of rituximab, pleural effusion is still present but reduced in size. Flow cytometry of pleural effusion performed after intrapleural instillation of rituximab showed CD19 positive cells with lack of CD20 epitope, which could be explained by either engagement or destruction of the CD20 epitope upon interaction with rituximab making the detection of the CD20 molecule impossible by routine flow cytometry. Complete remission was achieved in both DLBCL and FL patients. High-dose chemotherapy with stem cells support were used as consolidation in one patient with DLBCL. One patient with FL underwent pleurodesis (infusion of 5% suspension of talc in chest tube drainage) with complete disappearance of malignant effusion. Conclusions. Local therapy with intrapleural Rituximab may be a promising novel treatment option for management of malignant effusions in CD20+ NHL. Although our patients had no clinically significant adverse effects, further analysis of rituximab's activity and safety when applied intrapleurally are warranted. Long-lasting malignant effusion may cause negative impact on the local treatment efficacy.

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RITUXIMAB (RTX), METHOTREXATE (MTX) AND TEMOZOLOMIDE IS A SAFE OPTION FOR PATIENTS WITH PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA (PCNSL) AND POST TRANSPLANT LYMPHOPROLIFERATIVE DISEASE (PTLD)

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Background. Although considered a curable form of lymphoma, PCNSL prognosis is still dismal. The backbone of treatment, the use of drugs with high cerebrospinal fluid (CSF) penetration such as MTX and cytarabine, can be toxic in the majority of patients, especially in the elderly. The role of RTX is still not defined in these patients, and new drugs, like temozolomide are still under study. Aims: Describe the initial experience of a single institution in Brazil with a combined treatment with chemotherapy and immunotherapy in older or immunocompromised patients with PCNSL. Methods: From 2007 until now, all patients at our institution were treated following a regimen consisted of: RTX 375mg/m2 and MTX 1g/m2 on D1, D10 and D20, temozolomide 100mg/m2 D1-D5 and prednisone 120mg/m^2 every other day from D1 to D45. Intrathecal chemotherapy with MTX 15mg was delivered on D1, D5, D10 and D15. All patients received urine alkalinization and folinic acid rescue after MTX treatment until MTX levels were <0.01 mmol/L. Patients achieving at least PR received five cycles of maintenance therapy, consisting of RTX and MTX 1g/m2 D1 and temozolomide 100mg/m^2 D1-D5. *Results*. Five HIV-negative patients not suitable for standard protocols were treated in our institution with the described protocol. Two patients, 45 and 60 years old, were diagnosed as monomorphic PTLD, due to past history of liver transplant. The three other patients aged older than 70 years. The median age was 78 (range: 45-82). All patients achieved at least PR after first cycle (4 of them achieved CR) and proceeded to maintenance therapy. With a median follow up of 20 months (1,4 - 48,5) the overall survival was 53% (CI95% 5-100). No relapses were observed, and one patient is still on maintenance therapy. Only one of the patients (PTLD) died, due to infectious complications of chemotherapy and immunossupressive treatment. No neurotoxicity was observed in clinical exams. *Conclusions* Despite the small number of subjects and short follow up, our initial experience shows a regimen with temozolomide, RTX and MTX (1g/m2) without radiotherapy is a safe and feasible treatment for elderly patients with PCNSL and patients with CNS PTLD.

1435

BENDAMUSTINE IN THE TREATMENT OF RELAPSING AND REFRACTORY LYMPHOID MALIGNANCIES: A SINGLE CENTER EXPERIENCE

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Background and Aims. Since 03-2009 we have had the opportunity to treat relapsing and refractory lymphoma patients with bendamustine. 29 patients with either follicular (3), mantle cell (2), marginal zone lymphoma (10), hairy cell leukemia (1), CLL (7), DLBCL (4) and Hodgkin's lymphoma (2) were treated with this bifunctional alkylating agent. Median age of the 27 NHL patients was 73y with a M/F of 11/16. These patients were extensively pretreated (median 3 (range 1-7)). The 2 female Hodgkin patients were younger (25 and 30y) with 5 to 6 pretreatment regimens. *Methods*. Bendamustine was administered every 4weeks for up to 6 cycles at a dose of 90 mg/m^2 days 1 and 2. For the CLL patients a dose of 70 mg/m^2 on days 1 and 2 was chosen. Rituximab was added on day 1 of each cycle in 14 patients. *Results*. 17 patients (59%) received the intended 6 cycles of bendamustine. Early withdrawals were due to adverse events (n=2), disease progression (n=4) or patient decision (n=3). Dose reductions and treatment delays occurred respectively

in 6/138 (4%) and 20/138 (15%) of cycles . Hematological and gastrointestinal toxicity were the most common adverse events. Grade 3-4 leucopenia , thrombocytopenia and anemia were seen respectively in 7/28, 7/28 and 5/28 patients in one or more cycles. Nausea and vomiting was manageable with the association of a 5-HT3 antagonist. 26 patients were evaluable for response. In 18/26 patients (70%) at least a partial response was observed. No response was seen in the patients with DLBCL. The not responding CLL patient appeared to have Richter transformation. Follow up is too short to look at progression free survival. 6/10 patients are still in remission 6 months after the end of treatment. *Conclusions*. Bendamustine demonstrates activity in this extensively pretreated population with lymphoid malignancies without safety concerns. Longer follow up will show how durable these responses are.

1436

INCIDENCE OF GASTRIC INVOLVEMENT IN NONGASTRIC MARGINAL ZONE LYMPHOMA

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Background. There was an observation that a significant proportion of patients presenting with nongastric marginal zone lymphoma (MZL) had gastric involvement as well, thus arguing for esophagogastroduodenoscopy (EGD) as routine diagnostic workup of extranodal MZL. However, the incidence of gastric involvement in nongastric MZL has not been investigated in Asia, where the incidence of MZL is higher than that in Western countries. *Aims*. The present study was undertaken to assess the incidence of gastric involvement in nongastric MZL. Methods. Between April 1993 and December 2010, 153 consecutive patients with nongastric MZL were treated in the Asan Medical Center, Seoul, Korea. We retrospectively analyzed the results of these patients. Results. The median age at diagnosis was 51 years (range, 16 - 79 years), and male patients consisted of 42% (n=64). One hundred patients (86.3%) initially presented as localized disease (Ann Arbor stage I or II), 2 (1.3%), 15 (9.8%), 4 (2.6%) patients were stage IIIa, IVa, IVb, respectively. Most of the patients (n=136, 88.9%) had extranodal involvement of lymphoma at a single site. The most common primary sites were ocular adnexa or orbit (48.4%), intestine (11.1%), lung (9.8%) and nasal sinus (5.9%). Among those 153 patients, 47 (30.7%) had undergone EGD as part of their initial workup. None of these 47 patients was found to have gastric involvement of lymphoma. The most common endoscopic and pathologic finding of EGD was chronic superficial gastritis (n=16, 34%). *Summary*. None of the 47 patients who had undergone EGD had gastric involvement of EGD. Our findings do not support routine EGD in patients with extranodal MZL.

1437

A MONOCENTRIC EXPERIENCE OF CASTLEMAN'S DISEASE

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*Backgroun.*Castleman's Disease (CD), is a rare and poorly understood disease characterized by a pathologic growth of lymphoid tissue. This disease is composed of two different type: unicentric and multicentric disease. Actually the optimal therapy is unknown. We report our experience about patients with diagnosis of CD, seen at our Institution since 2004. Methods and Aims: From January 2004 to December 2010 we observed 6 patients affected by CD (2 males, 4 female, median age 65, range 49-82) Unicentric disease was defined as a solitary mass; multicentric disease compromised patients with multiple masses. We evaluated the involvement of human herpesvirus 8 (HHV-8) in all patients. Clinical, radiologic and laboratory data were analyzed to evaluate treatment response. Results: Among 6 patients, 2 presented unicentric disease, 4 multicentric. All patients underwent lymphonodal biopsy and were so classified: 1 in plasma cell type, 4 hyaline-vascular, 1 mixed; the 2 patients with multicentric disease presented hyaline-vascular histology. At evaluation of HHV-8 infection, 2/6 resulted positive at diagnosis; this patients presented a multicentric disease with systemic symptoms. The patient with plasma cell type (a female of 50 years old) developed CD from 6 years of diagnosis of POEMS syndrome; she was treated with high dose cyclophosfamide and was planned for autologous stem cell transplant. The 2 patients with HHV-8 infection were treated with polichemotherapy, resulting resistant. After chemotherapy they were treated with oral valgancyclovir, both obtaining a complete remission after 2 months of treatment and up to now they are in complete remission after 4 and 2 years respectively. The remaining 3 patients, the 2 monocentric disease underwent to lymphonodal excision and the last one 1 (multicentric CD) was submitted only to diagnostic lymphonodal biopsy. These 3 patients remaining symptoms free without any other treatment from 7,1 and 2 years respectively Conclusions: In our series, the asymptomatic patients (either unicentric or multicentric) did not need any treatment. The 2 symptomatic patients failing chemotherapy and benefited for antiviral treatment. A longer follow up and a larger series are needed to evaluate the better therapy for either symptomatic or asymptomatic disease.

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18FDG-PET/CT IN T-CELL LYMPHOMAS

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Background. T-cell lymphomas has different clinical features, than B-cell lymphomas, thus we took it important to investigate the utility of PET/CT, especially interim PET/CT. Patients and methods: We examined between 01/01/2007-01/05/2010 T cell lymphoma patients at PET/CT Medical, Diagnostical Co in Budapest and Debrecen. Results: 20 T-cell lymphoma patients underwent PET/CT. FDG uptake was observed in every cases. The average SUVmax value was 13.52 (5.02-33.6) at staging. Patients achieving complete metabolic remission (CMR) - evaluated at interim PET/CT - had an average SUVmax value of 16.71 (5.2-33.6) at the time of staging, whereas the patients who did not achieve CMR, had an average SUVmax value of 6.41 (5.02-9) at staging, but this was not significant, p=0.062. A median follow-up was 13.54 (2-27) months. Overall survival was higher in patients, who were in CMR, than who were not (not significant). Overall survival was higher in patients who had higher SUVmax, than who had lower SUVmax (not significant). The median SUVmax was chosen as the cutoff value. Conclusions: Based on all of these findings at T-cell lymphomas, PET/CT has the authority particularly through staging. The role of interim examinations are still challenging, further studies are needed to evaluate its exact role.

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MONITORING OF MINIMAL RESIDUAL DISEASE IN MANTLE CELL LYMPHOMA

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Background. Mantle cell lymphoma (MCL) is characterized by a specific chromosomal translocation t(11;14)(q13;q32) resulting in cyclin D1 over-expression and cell-cycle dysregulation. It is historically considered to be incurable with conventional therapeutic approaches, with a median overall survival of only 4-5 years. The minimal residual disease (MRD) monitoring may help in prediction of clinical relapse and may allow an early treatment of the relapse. *Aims*. The study was made to investigate the clinical reliability and predictive value of MRD monitoring in MCL using polymerase chain reaction (PCR). Methods. A proportion of lymphoma cells in peripheral blood was monitored by quantitative PCR during the course of the disease in 15 patients after chemo- or immunochemotherapy. The t(11;14) breakpoint (n=14) or IGHV clone-specific sequence (n=1) were used as the molecular targets. Molecular remission (PCR negativity) was defined as two consecutive samples with no specific amplification, molecular relapse as two consecutive positive samples after period of PCR negativity or as an increase in number of PCR copies in two consecutive samples (in cases where PCR negativity after induction therapy was not achieved). When molecular relapse in peripheral blood was detected, the parallel bone marrow sample was also tested. Results. The molecular remission was achieved in 14 of 15 observed patients. The average duration of molecular remission in patients who relapsed (n=6) was 30 months (range 11-51). Of the six relapsing patients, 5 of them were previously PCR negative. Two patients have gone through 2 relapses. In total, 7 of 8 observed clinical relapses were preceeded by reappearance of PCR positivity or increase in number of t(11;14) breakpoint copies. The average period between a molecular relapse in peripheral blood and a clinical relapse was 3.3 months (range 1-6). At the time of relapse, the PCR positivity was detected in all cases both in peripheral blood and bone marrow. Summary. MRD level proved to be a strong predictor of clinical relapse in mantle cell lymphoma. This is documented by very good test statistics (positive predictive value 100%, negative predictive value 90%). The detection of

molecular relapse enables an early therapeutic intervention (for example with immunotherapy) resulting maybe in a prolongation of the period without a clinical relapse.

1440

RITUXIMAB PLUS BENDAMUSTINE (RB) REGIMEN IN ELDERLY PREVIOUSLY UNTREATED PATIENTS WITH INDOLENT, NON FOLLICULAR NON HODGKIN LYMPHOMA: PRELIMINARY DATA OF A SINGLE CENTRE STUDY

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Background: Bendamustine (B), a byfunctional chemotherapic agent, has shown considerable activity for solid and lymphoid malignancies. B has recently become available for clinical use, as a first-line treatment for chronic lymphocytic leukaemia (CLL) and as salvage therapy after Rituximab or Rituximab-based regimen, for early relapsed or refractory indolent B-cell lymphoma (NHL). Recent clinical trials have found B to be well tolerated and effective, both as first line treatment in CLL and as a treatment in relapsed or refractory indolent NHL. *Aims*: to assess the efficacy and toxicities of B in combination with Rituximab ® in elderly previously untreated patients (pts) with indolent, non follicular NHL. Methods: from October 2008 to May 2010, 20 pts (M/F=15/5) with previously untreated indolent, non follicular NHL were enrolled in the study. The median age was 74 yo (range: 64-85); seventeen pts (85%) were more than 70 yo and 11 (55%) had B-CLL/SLL, 8 (40%) LPL/WM an 1 (5%) SMZL. Eight pts (40%) present an ECOG PS > 1. ENS involvement was present in one (5%) and the BM involvement in all pts (100%). Fourteen (70%) pts had co-morbidity with more than 2 disease in 30% of cases. R-B regimen consisted of Rituximab 375 mg/mq iv on day 1 and Bendamustine 80 mg/mq iv on days 1, 2; all pts received six-eighth cycles delivered every 21-28 days; the response assessment was planned after 3 cycles and at the end of treatment. Median number of cycles delivered was 5 (range 3-8); 14 pts (70%) completed the planned treatment; dose reduction occurs in 4 pts (20%). Nine pts (45%) received G-CSF at the dose of 300 mcg/die as primary (10%) and secondary (25%) prophylaxis. ESA support was needed in 4 (22%). *Results:* complete response (CR) was achieved in 11 (55%) and partial response (PR) in 9 (45%) of pts with an ORR of 100%; no toxic death or relapses occurs. Ten out of 11 (91%) pts with B-CLL/SLL achieved CR, while all pts with LPL/WM obtained stable RP. The regimen was safe and well tolerated with dose reduction occurring only in 4 pts (20%). The mainly adverse events recorded was neutropenia occurring in (39%); severe neutropenia (WHO grade 3-4) was recorded only in 4 pts (20%). No extra-haematological toxicity was observed. With a median follow-up of 16 months (range: 4-23) OS, PFS and RFS were 94% and 100% respectively. *Conclusions*. RB is an effective regimen for elderly pts with previously untreated indolent non follicular NHL, mainly in CLL/SLL. RB is a safe regimen with major but tolerable toxicities consisting in myelosuppression. A longer follow-up is needed to define response duration and long-term safety.

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FDG PET CT SCAN ROLE ON DIAGNOSIS AND INITIAL STAGING OF LYMPHOPROLIFERATIVE DISEASES

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Background: FDG PET CT scan (PET) was recently introduced as a part of initial staging of many lymphoproliferative diseases. PET is currently used during initial and restaging to detect disease sites which are not recognized with other clinical and laboratory methods. However, the role of PET on diagnosis and staging of lymphomas remains to be seen. Aim: To study the findings of PET during initial diagnosis of lymphoma patients, to compare them with the findings of the conventional staging methods and to examine the possible role of PET on initial staging of lymphoma, in respect to its subtype. Methods: From 2003-2010, 131 patients with recent diagnosis of lymphoma, underwent PET as part of their initial screening. All patients underwent conventional clinical and laboratory initial staging with CT scan and bone marrow biopsy (BMB) and were staged according to standard systems. A comparison between conventional vs PET findings and staging, including special sites of disease, ie spleen and bone marrow (BM), in relation with the discrete subtype of lymphoma, was made. Results: 131 patients performed PET during initial diagnosis of lymphoma in Hematology Clinics of University of Athens and Athens Medical

Table 1. PET vs CT/ BMB findings in lymphoma pts.

	PET(+) findings /CT(-) % per disease(#)					CT(+) findings/PET(-) % per disease(#)			BMB (+)
	Lymph	*Spleen*	*Lung*	*BM*	*Other*	*Lymph*	*Spleen*	*Lung*	
HL (65 pts)	43 (28)	9 (6)	5 (3)	6 (4)	20 (13)	9 (4)	3 (2)	3 (2)	15 (10)
FL (21 pts)	38 (8)	0	0	5 (1)	14 (3)	9 (2)	0	0	29 (6)
DLBCL (18 pts)	39 (7)	0	6 (1)	0	33 (6)	6 (1)	0	0	0
SMZL (10 pts)	20 (2)	0	0	10 (1)	0	0	30 (3)	0	90 (9)
MZL (6 pts)	33 (2)	0	0	0	0	17 (1)	17 (1)	0	17 (1)
SLL (4 pts)	25 (1)	0	0	25 (1)	0	0	0		75 (3)
Other (7 pts)	14 (1)	0	0	14 (1)	14 (1)	14 (1)	43 (3)	0	57 (4)

The lymphoma subtype was Hodgkin (HL) in 65 pts, follicular in 21 pts, DLBCL in 18 pts (3 primary mediastinal), and other lymphoproliferative diseases in 27 [10 splenic marginal zone (SMZL), 6 other marginal zone (MZL), 4 small lymphocytic lymphoma (SLL), 3 multiple myeloma (MM), 2 T-NHL, 1 mantle cell lymphoma and 1 NK leukemia]. Differences between findings of CT and PET were noted in 89 patients (68%) and in 75 of them (57%) PET revealed more sites of disease compared with CT. In 17 patients (13%) CT revealed abnormal findings which did not present increased uptake on PET. In two of those patients, both with HL, lung nodules<1cm were found on CT, not revealed by PET. With PET findings included, patients' stage was increased in 13 (10%) and decreased in 1 (1%). From 33 patients (25%), who presented bone marrow infiltration by biopsy, only in 8 (6%) PET revealed increased bone marrow uptake consistent with disease infiltration. Among those 8 pts, 4 had HL, 1 SMZL, 1 FL grade 2, 1 SLL and 1 T-NHL. Further on, among 30 patients (23%) who presented spenomegaly at CT scan, 21 had increased uptake of PET in the spleen while only 9 (7%), (3 SMZL, 2 HL, 1 MZL, 1 NK leukemia, 1 SLL and 1 T-NHL) did not. Additionally, 6 pts (5%), all with HL, presented increased spleen uptake without splenomegaly on CT (Table 1). *Conclusions*. PET confirms practically all CT findings, as far as lymphadenopathy is concerned and ocassionally reveals additional involved sites. It also helps in defining splenic infiltration. It cannot frequently reveal bone marrow infiltration as compared to BMB. The histological subtype of disease should be taken under consideration for the interpretation of PET results. The clinical significance of PET contribution in staging of lymphomas should be further clarified, adding, in controversial cases, tissue biopsies.

1442

DIFFERENT RISK FACTORS FOR RELAPSED FOLLICULAR LYMPHOMA TREATED WITH CHEMOTHERAPY VERSUS IMMUNOCHEMOTHERAPY

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Background. All widely accepted prognostic indices in follicular lymphoma (FL) were primarily designed for patients with newly diagnosed disease. Nowadays, the precise assessment of risk also in relapsed FL patients seems to be necessary, since lot of treatment strategies are available for these patients. Aims: The aim of this study was to compare the outcome of FL patients in first relapse treated with chemotherapy and immunochemotherapy and to identify high risk patients in both groups. The second aim was to evaluate the impact of adding rituximab to chemotherapy on overcoming the negative prognostic impact of previously identified risk factors in patients treated with chemotherapy. The third aim was to compare the outcome in both groups for identified risk factors in patients treated with immunochemotherapy. Methods: The retrospective analysis was performed on 60 patients with diagnosed

first relapse of FL grade I, II or IIIa, in the period February 2002-April 2009. 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, 33 patients received immunochemotherapy (R-FC, R-FND) and 27 chemotherapy (FC, FND). The characteristics in first relapse examined as possible risk factors were age, higher histological grade in relapse, presence of B symptoms, presence of "bulky"tumor (>10 cm in diameter), ECOG performance status (ECOG PS), spleen enlargement, FLIPI score, hemoglobin level, LDH and ESR. Results: The median follow up after first relapse of FL was 26 months (range 4-97 months). The patients treated with immunochemotherapy had significantly longer time to second relapse (log rank 8.352, p<0.01) and overall survival after first relapse (log rank 8.234, p<0.01). Multivariate Cox regression analysis identified ECOG PS >1 and FLIPI high risk as the independent risk factors for patients treated with chemotherapy. Older age (>60 years) and presence of B symptoms in multivariate Cox regression analysis were identified as the independent risk factors for patients treated with immunochemotherapy. In the comparative analysis, significantly longer overall survival after relapse was revealed in patients with ECOG PS >1 (log rank 12.679, p<0.01), FLIPI score ≥3 (log rank 14.712, p<0.01) and B symptoms (log rank 4.676, p<0.05) if they were treated with immunochemotherapy. The improvement in survival after relapse by addition of rituximab was not recorded in elderly patients (log rank 0.001, p>0.05). Conclusions: The treatment with immunochemotherapy in first relapse of FL brought to clear benefit in survival. Older age and presence of B symptoms are risk factors for poor outcome in patients treated with immunochemotherapy. The addition of rituximab to chemotherapy overcame the negative prognostic impact of high FLIPI risk and poor ECOG PS in first relapse of FL. Studies with large series of patients treated with immunochemotherapy in relapsed FL are needed, in order to identify patients who maybe require more aggressive therapeutic approach.

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SUCCESSFUL TREATMENT WITH PREDNISONE ALONE FOR BING NEEL SYNDROME

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Background.Waldenstrom's macroglobulinemia ((WM) is the result of clonal proliferation of lymphocytes that produce monoclonal immunoglobulin M. Many central nervous system (CNS) complications have been described, the majority associated with blood hyperviscosity syndrome. However, CNS infiltration by plasmacytoid lymphocytes (Bing -Neel syndrome) has only rarely been reported. We report two cases of diffuse Bing-Neel syndrome presentation effectively treated with low-intermediate dose prednisone. Case summary. Case 1. A 81-year old man diagnosed with Waldestrom's macroglobulinemia and progressive cognitive decline was admitted for seizure and coma in November 2003. Electroencephalogram showed mild diffuse cerebral dysfunction and moderate right frontotemporal slowing with epileptiform discharges. Cerebrospinal fluid (CSF) examination was unremarkable. Cryoglobulin testing was negative. Computed tomography of the head showed enhancing heterogenous periventricular hipodensities. Phenytoin was started with sudden neurological improvement. In the following months however the patient showed progressive cognitive deterioration with dizziness, confusion and lethargy associated with persistent monoclonal immunoglobulin In May 2004 low dose prednisone was started with complete and durable mental restoration. Case 2. A 61 year old man diagnosed with WM was admitted for septic fever, haemolytic anemia and mental deterioration in January 2010. He was started on steroid treatment with clinical and haematological improvement. In December 2010 due to the monoclonal immunoglobulin increase the patient received three weekly courses of Rituximab and was admitted with confusion and septic fever in December 2010. Despite of the septic state resolution the patient showed persistent cognitive decline with drowsiness. CSF examination revealed high protein concentration without cellular abnormalities. MRI of the brain showed left parietal white matter T2 hyperintensities. Intermediate dose steroid treatment (50 mg Prednisone) was started achieving remarkable clinical improvement. with persistent IgM concentration. Conclusions. Bing Neel syndrome is a rare and potentially treatable complication of WM. In patients presenting with rapidly progressive cerebral deterioration and monoclonal immunoglobulin M, Bing-Neel syndrome should be considered and effectively treated with corticosteroid therapy.

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PREVALENCE OF HAIRY CELL LEUKEMIA IN HUNGARY WITH SPECIAL EMPHASIS ON THE TREATMENT HABITS OF HUNGARIAN HEMATOLOGISTS

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Background. Hairy cell leukemia is a rare disease, it's incidence in Hungary is 2 patients per million inhabitants. According to the international guidelines, the treatment of choice drugs in HCL are cladribine and pentostatin. Overall survival of treated patients does not differ significantly from that of the general population. *Aims*. To establish the consequences of treatment choices on the quality of hairy cell leukemia care. Methods: We have sent questionnaries on treatment habits of Hungarian haematologists in HCL and on the main characteristics the HCL patients they care for. All Hungarian haematological departments and ambulances responded enabling us to evaluate retrospectively the data of 180 HCL patients. Results: Seventysix percent of the patients were male, 24% were female. Average age at diagnosis was 57 years (range 34-92). First line treatment modalities were the following: interferon-alpha (73% of cases), cladribin (14%), splenectomy (5%), watchful waiting (6%). Cladribine was given as second line treatment in 56% of cases, while further second line treatment options applied were alpha-interferon (32%), watchful waiting (31%), rituximab (7%), pentostatin (3%), splenectomy (1%). First line alpha-interferon was given for less than 3 monts in 19 percent of cases, for 3 to 12 months in 33% of patients, while duration of first line alpha-interferon treatment was more than 12 months in 48% of cases. Looking at second line treatment 43% of patients received alpha-interferon for 3 to 12 months, while the percentage of patients receiving alpha-interferon for more than 12 months was even higher, 57% of cases. At the time of our survey 77% of the patients had non-symptomatic stable disease, 6% had non symptomatic progressive disease, 6% had symptomatic progressive disease and 4% had symptomatic progressive disease, all three latter categories necessitating further treatment. Conclusions: Although cladribine treatment is cost-effective and one treatment cycle results in durable complete remission in 75-80% of cases, in Hungary long lasting interferon-alpha treatment is still wide-spread, because of the peculiar differencies of financing alpha-interferon and cladribine. Cladribine, even the sc. form receives financing only for inpatients, at the primary expense of the hospital. Looking at our findings, earlier and more widespread use of cladribine seems to be justified both from the medical and from the pharmoco-economic point of view.

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PROGNOSTIC SIGNIFICANCE OF THE KI-67 INDEX IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS TREATED WITH RITUXIMAB PLUS CHOP - EXPERIENCE OF SERBIAN LYMPHOMA STUDY GROUP

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Background. Introduction of the anti-CD20 antibody, Rituximab, in therapy of patients with diffuse large B-cell lymphoma (DLBCL) significantly improved survival and altered the predictive values of known prognostic factors in DLBCL. Assessment of tumor cell proliferation based on Ki-67 expression yielded conflicting prognostic predictions of patients with diffuse large B-cell lymphoma (DLBCL). *Aims.* The aim of this study was to evaluate the role and prognostic significance of the Ki-67 proliferation index (PI) in DLBCL patients treated with rituximab plus CHOP (R-CHOP). METHODS: Ki-67 was assayed immunohistochemically in tissue samples of 145 patients with newly-diagnosed DLBCL treated with R-CHOP between January 2007 and January 2011. *Results*. The complete response (CR) rates following R-CHOP administration were not significantly different, based on Ki-67 expression status (P=0.804). The 1-yr event-free survival (EFS) rates were: 82,2% in patients with a low index Ki-67 (Ki-67 < 80%, n = 107) compared with 73,7% in patients with a high index (Ki-67 ≥ 80%, n = 38). In patients with a low IPI (≤ 2), one-year survival was 87,4% and 69,8% in those with a higher index (IPI>2). In patients with diffuse large B-cell lymphoma Ki-67 PI of 80% was found to significantly discriminate patients with good or bad prognosis when combined with low IPI score (AUC = 0.649, P = 0.013). In multivariate analysis, Ki-67 expression combined with low IPI score (≤ 2) was a significant prognostic factor for EFS [hazard ratio (HR) = 1.760; 95% confidence interval (CI) 1.030-3.008; P = 0.039]. *Conclusions*. In diffuse large B-cell lymphoma, a cut-off value of 80% can distinguish patients with a good and bad prognosis when combined with another prognostic factor as low IPI score.

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DIFFUSE LARGE B-CELL LYMPHOMA WITH HEPATITIS C VIRUS- INFECTION TREATED BY CHEMOTHERAPY WITH RITUXIMAB REGIMENS: PROGNOSIS AND TOXICITY

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Many epidemiologic studies have demonstrated an association between hepatitis C virus (HCV) infection and non-Hodgkin lymphoma, suggesting that HCV plays a role in the development of this malignancy. In a number of researches it is shown that level of various markers of a hepatitis C at patients from NHL can be taped at 30 % of patients. In our research we studied function of a liver at patients with NHL at carrying out immunochemistry with markers of a hepatitis C and without them. 41 patients (pts)with NHL have been included in research with markers HCV infection and 108 without it by which it has been spent immunochemistry. The age median patients who were HCV-positive was 47 years. 30 patients HCV-positive have III-IV stages of disease and 11 II stage. Normal level ALT and AST prior to the beginning of treatment was at 6 patients. The median serum level HCV RNA at the beginning of treatment was $2.3x10^5$/ml. The age median patients who were HCV- negative was 56 year, III-IY st was at 59 pts, I-II at 49 pts. Normal level ALT and AST was at 102 pts. All pts were treated by R-CHOP. Among 108 pts without markers HCV infection increased level ALT and AST only at 15 pts. The median was 2.3 norms. HCV RNA it was not defined at all this 15 pts. The treatment has not been stopped at any pts thanks to hepatic toxicity. CR has been reached 56 with DLBCL. Median follow up was 28 months. Serum level HCV RNA has increased at 23 of 41 pts. At all 23 pts before therapy had positive serum level HCV RNA . Level of HCV RNA was from $9x10^4$ to $8.8x10^7$/ml a median has made 6.7x106/ml. HCV-RNA levels significantly increased during immunochemotherapy 21 from 41 pts simultaneously with increased serum level HCV RNA increased level ALT and AST. Level ALT was from 4 to 50 norms, median- 11 norms. Level AST increased at 19 pts from 2 to 9 norms , median of 4.5 norms. The reason of stop treatment at 15 pts was hepatic toxicity. Complete remission (CR) in Pts with HCV infection has been reached 12 DLBCL. Median follow up CR was 12 months In conclusion, our study showed a high incidence of severe hepatic toxicity in patients who were HCV-positive. Hepatic function should be limied in patients who are HCV-positive and receive immunochemotherapy. High-risk patients having chronic active hepatitis receiving cytotoxic drugs, corticosteroids and rituximab should be closely monitored for serum transaminase, bilirubin and HCV RNA levels.

1447

TREATMENT OF SÉZARY SYNDROME WITH LOW DOSE SUBCUTANEOUS ALEMTUZUMAB RESULTS IN PROFOUND DISEASE RESPONSES BUT CAN LEAD TO SELECTIVE OUTGROWTH OF PREEXISTING CD52 NEGATIVE MALIGNANT CELL VARIANTS

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Sézary syndrome is an aggressive cutaneous T cell lymphoma characterized by the presence of neoplastic T cells in peripheral blood, lymph nodes and skin leading to disabling squamous erythroderma with severe itching and systemic complaints. Treatment with intensive therapy is associated with increased infectious mortality, and many patients require long term high dose systemic corticosteroids for disease control. Low dose subcutaneous alemtuzumab (an anti CD52 monoclonal antibody, MoAb) has been used in small cohorts of SS patients and was associated with a high response rate and a good toxicity profile. After single agent treatment with alemtuzumab the occurrence of relapsing or resistant disease due to antigen loss or selection of antigen negative malignant cells has been shown in other malignant diseases like acute lymphoblastic leukemia or prolymphocytic leukemia, but has not been reported in Sézary syndrome so far. We treated 3 patients with Sézary syndrome with high peripheral blood counts of circulating Sézary cells (8*106/mL, 9*106/ mL and 6*106/ mL, respectively) and severe pruritus for which continuous treatment with systemic corticosteroids was needed, with repeated weekly doses of 10 mg alemtuzumab until numbers of circulating malignant cells were < 1*106/mL. This was reached after 5, 3 and 2 subcutaneous injections, respectively, with an impressive improvement of skin lesions and without side effects. In all patients systemic corticosteroids could be tapered. Recurrence of circulating Sézary cells and itching was observed again in all three patients after 3.5, 4 and 3 months, respectively, and all these patients responded to a second course of alemtuzumab (2, 1 and 1 injection, respectively). In one patient a CMV reactivation occurred after this second course, which was successfully treated with valganciclovir. 6 Weeks after the second course alemtuzumab, the first patient relapsed and retreatment with alemtuzumab did not have any effect on the number of circulating Sézary cells nor the skin. We investigated CD52 expression on circulating Sézary cells at the time of Alemtuzumab resistant relapse and before treatment with alemtuzumab. At the time of the alemtuzumab resistant relapse, 95% of circulating CD3+CD4+CD7- cells lacked CD52 expression. A CD52- population was already present before alemtuzumab treatment (0.02% of circulating CD3+CD4+CD7- cells). In patient 2, all circulating CD3+CD4+CD7- cells before alemtuzumab treatment were CD52+ and in patient 3, 0.04% of circulating CD3+CD4+CD7- cells were CD52- before treatment. At present, both patients show an ongoing response without appearance of CD52- Sézary cells (6 and 3.5 months after first alemtuzumab treatment, respectively). In conclusion, single agent treatment with low dose alemtuzumab for Sézary Syndrome effectively cleared circulating malignant cells and lead to an impressive improvement of skin lesions and systemic complaints. However, CD52 negative subclones may be present within the primary malignant cell population leading to resistance to alemtuzumab treatment due to selective outgrowth of the antigen negative variant/subclone.

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IMPACT OF RITUXIMAB MAINTENANCE IN RELAPSED FOLLICULAR LYMPHOMA

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Background:The rituximab maintenance in follicular lymphoma (FL) relapsed after chemotherapy response has been effective in increasing the duration of response in clinical trials. However the results of a clinical trial may not always be reproducible in routine clinical practice. Aims: We present our experience with rituximab maintenance after response to chemotherapy in FL in the first relapse, since its approval in this indication, compared with similar patients treated without maintenance, before the authorization of rituximab in this setting. Material and methods. Retrospective study of our FL patients (1995-2010) in its first relapse with response at least partial to rescue chemotherapy, with rituximab maintenance since its approval in this indication (group A) or without maintenance before adopting the indication (group B). In both group we compared prognostic variables, time to treatment failure (TTF) for relapse, toxicity or dead, and overall survival (SV), as well as the

adverse effects (neutropenia and infectious episodes). Statistical methods: descriptive statistic, Student T, chi2 , survival by Kaplan- Meier method, long rank test and Cox proportional hazard regression method. Results. Forty patient with chemotherapy responsive rescue were included, 20 in the group A and 20 in the group B. 19 male and 21 females. Median age 63 years (30-80). FLIPI abbreviated : 0-1:14 (37%); II-IV: 24 (63%). Rescue treatments: chemotherapy with rituximab in group A and chemotherapy without rituximab (except 2 patients) in group B. Maintenance with rituximab consisted of an infusion of 375mg/m2 every three months for 2 years in 9 patients and 2 courses of 4 -times-weekly doses of rituximab, 3 and 9 months after completion of salvage therapy in 11 patients. 19 patients (47,5%) failed after salvage therapy and 14 (35%) died. 20% of patients had infectious episodes and another 30% grade 3 or 4 neutropenia. There was no significant difference in age or FLIPI between both groups. Five patients received maintenance rituximab in second or later relapse so they are excluded for the efficacy analysis. The probability of salvage therapy failure was lower in the rituximab maintenance group: TTF median has not been reached in group A (60% without failure at 4,75 years) and it was 3,9 years in group B (p: 0,06). In the multivariant analysis only the FLIPI was significant (p:0,016) and almost significant the rituximab maintenance. The median of SV was 6,6 years in group A and 4 years in group B (p: 0,11). In the multivariant analysis FLIPI was the only significant variable (p:0,02). We did not observe significant differences in neutropenia betwen both groups, but infections were more frequent in A group(p:0.03). Summary/Conclusions. Ours observations are consistent with the effectiveness described in clinical trials of rituximab as a consolidation of the chemotherapy response in first relapse of FL. The limited number of patients may justify the results did not reach statistical significance. The neutropenia has been similar in both group but the infectious episodes were more frequent in the rituximab group. A favorable FLIPI was associated with less likelihood of treatment failure and a longer SV.

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CENTRAL NERVOUS SYSTEM INVOLVEMENT IN PREVIOUSLY TREATED DIFFUSE LARGE B-CELL LYMPHOMA - A SINGLE CENTER EXPERIENCE

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Background. The occurrence of central nervous system (CNS) involvement in patients with non-Hodgkin's lymphoma, including diffuse large-B-cell lymphoma (DLBCL), is an uncommon (1.6% to 5%), but almost always fatal event. Available studies report a latency time from initial diagnosis to CNS relapse varying from 5.4 to 8 months. Prognosis is very poor with a median time from CNS involvement to death of 2 to 4 months. IPI, LDH and number of extranodal sites seem to be the more relevant risk factors in predicting the risk of secondary CNS involvement. Aims. To evaluate the patients with DLBCL treated in our center who presented with CNS relapse. Methods. Between October 2007 and January 2011, we identified 9 patients, previously treated with R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone) for DLBCL, presenting with CNS relapse. Data regarding diagnosis, staging, relapse clinical presentation and outcome was collected. Results. Of the nine patients, 55.6% were female with a median age of 66 years old (range: 33-80). At diagnosis, all patients were stage III or IV with IPI equal or superior to 3 and with elevated LDH (median=832; range: 220-1694; reference values: 67-190 U/L). Extranodal disease was present in 55.6% of patients; bone marrow was involved at diagnosis in 44.4%. Patients were treated in first-line with R-CHOP and 2 of them had intra-thecal prophylaxis with metothrexate. Six patients achieved complete remission (CR) at first-line (two of these had systemic relapse with achievement of a second CR) and two patients had resistant disease. Of the six patients in CR, five patients had an isolated CNS relapse. Both patients with resistant disease presented with CNS involvement while undergoing second-line therapy and one patient developed CNS disease before response to first-line therapy was evaluated. Median time from diagnosis to CNS relapse was 7.9 months (range: 4.3-68.4 months). Presenting symptoms of CNS involvement were paresis of lower limbs (n=5), visual impairment or diplopia (n=3), dysarthia (n=2), dysphagia (n=1) and palpebral ptosis (n=1). Work-up revealed parenchymal involvement (n=3), involvement of femoral (n=1) and optic (n=1) nerves; immunophenotyping of cerebrospinal fluid (CSF) at relapse was positive in seven out of eight patients tested: five expressed CD20 and two were CD20 negative. Two thirds of the patients had died at the time of our study. Median survival after CNS involvement was 3.8 months (range: 0.7-39.2 months). Medial overall survival of this group was 12.2 months (range: 5-72.4 months). Of the surviving patients only one is currently in CR after systemic and intra-thecal chemotherapy and autologous stem cell transplant. Conclusions. CNS relapse of DLBCL is an aggressive disease with a fatal outcome in most cases. Although all patients were advanced-stage, only two met criteria for intra-thecal prophylaxis and yet, it failed to prevent relapse. Recent literature questions the role of this form of prophylaxis after the introduction of Rituximab. It is necessary to find more accurate risk factors in predicting the risk for CNS secondary involvement and develop more effective forms to prevent it.

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GENETIC VARIATION IN THE GENE FOR BETA7 INTEGRIN (ITGB7) AND CHRONIC GVHD AFTER ALLOGENEIC HSCT

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Background: MAdCAM-1 / alpha4beta7 integrin signalling pathway has been implicated in the targeting of donor T cells to the mucosal tissues of the recipient after allogeneic haematopoietic stem cell transplantation (aHSCT). We have recently reported an association of MADCAM1 gene variants in recipient with chronic GVHD after HSCT (Ref.). Hereby, we extended our interest to the second partner in this signalling pathway - to the alpha4beta7 integrin. Aims: We wished to determine whether selected SNP variant of the gene encoding for beta7 integrin (ITGB7) is associated with development of acute or chronic GVHD and if it influences survival after aHSCT. Methods: Real-time PCR (TaqMan) was used to genotype ITGB7 SNP GenBank ID rs1554753 A/G in the Czech patients who underwent aHSCT (N=87) and their HLA identical related (N=70) or unrelated (N=17) donors. Results: A trend towards more frequent chronic GVHD was observed in recipients transplanted with a donor possessing at least one ITGB7 rs1554753*G allele than with AA homozygous donor (56.0% vs. 34.8%; p=0.08). Furthermore, the MADCAM1 rs2302217 AA homozygous recipients transplanted with a donor carrying ITGB7 rs1554753*G allele tended to develop chronic GVHD more frequently than patients with other combinations of MADCAM1 and ITGB7 genotypes (80.0% vs. 38.5%; p=0.07). Acute GVHD, overall survival and transplant-related mortality were not associated with the presence of ITGB7 variants in patients or donors. Conclusions: Our data indicate that ITGB7 gene polymorphisms in the donor may be associated with the risk of chronic GVHD, possibly in synergy with a particular recipient MADCAM1 genotype. Replication of our data in a larger cohort and/or assessment of the functional relevance of ITGB7 gene variants in aHSCT are required to confirm/extend these findings.

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Ambruzova et al. Hum Immunol. 2009;70(6):457-60.

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TH1 AND TH2 CYTOKINES REGULATE THE CONTRACTILITY OF THE MESENCHYMAL STEM CELL

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Mesenchymal stem cells (MSC) are nonhematopoietic cells of the bone marrow with capacity to support hematopoiesis, to proliferate and self-renewal. MSC are also considered a promising strategy for tissue repair, as they have been shown to differentiate into almost any cell type derived from all lineages. Besides this potential repair capacity, MSC mediate immunomodulatory effects in vivo and in vitro, and then they may have a therapeutic effect in inflammatory and immune diseases. Several authors have reported the expression of α-SM-actin by MSC. The polymerization of this protein in the cytoplasm is related to the cellular contractility. This capacity appears to be important for different MSC's functions, since changes in the cell shape may influence the cell-cell contacts. In this work, we study the effects of different cytokines on the polymerization of α-SM-actin and on the cellular contractility of MSC. Human bone marrow samples were obtained from bone marrow aspirates. Bone marrow mononuclear cells were isolated by density gradient centrifugation and cultured in Opti-MEM culture medium with

3% of Fetal Bovine Serum at 37 °C and 5% CO2. Bone marrow nonadherent cells were removed after 24 h, and culture medium was refreshed twice a week thereafter. Cells grew adherent and with a fibroblastic morphology. We observed by flow cytometry that almost all cells expressed CD10, CD29, CD73 and α-SM-actin, but lacked CD45. Other antigens such as CD54, CD106, CD21, and STRO-1 were also detected. We used the collagen gel assay to determine MSC contractility. IL-2, IFNgamma, TNF or LT αβ (all them Th1 cytokines) increased MSC contractility, whereas that IL-10 (a Th2 cytokine) or cytochalasin (an inhibitor of actin polymerization) decreased MSC contractility and induced cell relaxation. By confocal microscopy, we observed an increase in the number of α-SM-actin+ stress fibers in the MSC treated with IL-2, IFNγ, LTα1β2 or TNFα, whereas IL-10 decreased that number. Our results show that the MSC contractility is modulated by cytokines that regulate the incorporation of α-SM-actin into the stress fibers.

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ANALYSIS OF CMV SPECIFIC IMMUNITY RECONSTITUTION IN PATIENTS FOLLOWING ALLOGENIC STEM CELL TRANSPLANTATION BY TETRAMER TECHNOLOGY

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CMV specific memory CD8+ T lymphocytes (CMV CD8+ T ly.) play crucial role in the regulation of CMV infection. The tetramer technique is an important tool for detection and quantification of CMV specific T cell response. The aim of the present study was to investigate the role of donor CMV serological status on CMV CD8+ T ly. recovery following allogenic stem cell transplantation and the influence of graft versus host disease (GvHD) treatment and anti-thymocyte globulin (ATG) application in patients more than 100 days after transplantation. In cases with seropositive donors, CMV CD8+ T ly. reconstitution was observed approximately at day 20 following transplantation. In 5 (45%) patients with seropositive donor and without GvHD we found high values of CMV CD8+ T ly.: mean 26 b/µl (min. 16 b/µl, max 44 b/µl). 80% received ATG. In 6 (55%) patients without GvHD and CMV seronegative donors we found low numbers of CMV CD8+ T ly.: mean-1,6 b/µl (min. 0 b/µl, max 6 b/µl). 83% received ATG. 20 (65%) patients with both seropositive and seronegative donors were diagnosed and treated for GvHD. In 14 (70%) cases with low CMV CD8+ T ly. levels (mean 0, 9 b/µl, min. 0 b/µl, max 4 b/µl) 57% received ATG. In 6 (30%) cases with high CMV CD8+ T ly. levels (mean 11 b/µl, min. 7 b/µl, max 24 b/µl) 50% received ATG. CMV infection was not diagnosed in any of the above mentioned cases. Our results are in agreement with the hypothesis that donor serological status play crucial role in CMV specific immunity reconstitution. Our observations demonstrate significant negative influence of GvHD treatment on CMV specific immunity, while no effect of ATG application was proven.

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ASSESSMENT OF CHIMERISM AFTER ALLOGENEIC HEMATOPOIETIC TRANSPLANTATION USING SYBR GREEN-BASED REAL-TIME PCR ANALYSIS OF DNA POLYMORPHISM

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Background. Many haematology-oncology diseases are treated by allogeneic stem cell transplantation (allo-SCT). Assessment of the donor/recipient chimerism is a very important information in the first post-transplantation period.. In the past, cytogenetics and FISH method were available, however, their use was limited only for the sex mixed pairs. Although STR and VNTR detection is more universal, the detection limit is about 1 - 5%. The real-time PCR system with 12 NPs (nucleotide polymorphism) is donor/recipient sex independent and the sensitivity is below 1%. Aims. To measure and monitor chimerism after allo-SCT in sex mixed and sex identical pairs with using SYBR Green-based real-time PCR analysis and Gene Express software. Methods. Samples from patients and donors were provided by the Clinic of Haematology and Transfusiology in Bratislava, Slovakia (n = 69 pairs), before and after allogeneic transplantation by haematopoietic stem cells. DNA was isolated from PB after separation of WBC and stabilization in RNAlater solution. RQ-PCR was performed by the real-time PCR system using SYBR Green and 12 pairs of specific primers for two allelic variants (in the case of Y chromosome marker, only one). Primers for GAPDH were used as endogenous gene control. The amplification profile was identical as in TaqMan quantification protocol but with additional dissociation step .Results. In all cases the first step was aimed to find informative markers in DNA samples from donor and recipient before transplantation. We have screened 69 pairs donor/recipient and only in 1 case we didn't receive any informative DNA marker. After SCT only autologous DNA markers were quantified. An example of the quantification and monitoring of DNA informative markers is presented in the fig.1 Conclusions. We have established a simple method for the relative quantification of 12 autologous DNA polymorphic markers (11+Y chromosome) of recipient chimerism after allogenic haematopoietic transplantation. A relative amount of autologous DNA markers in peripheral blood can be estimated by relative quantification. Quotient RQ (in LOG10 or in %) have been obtained by the real-time PCR analysis using SYBR Green, which was based on measuring DNA informative markers. Denaturing melting point analysis of markers were also provided.

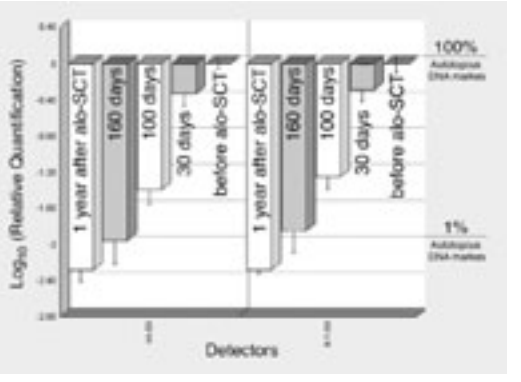

Figure 1. Monitoring of DNA markers after alo_SCT

1454

ACUTE GVHD IS A STRONG PREDICTOR OF FULL DONOR CD3+ T CELLS CHIMERISM (TCC) AFTER REDUCED INTENSITY CONDITIONING (RIC) FOR ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT)

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The monitoring of chimerism is a standard procedure to assess engraftment and achievement of full donor lymphoid chimerism after RIC. Post graft donor lymphocyte infusions are often determined on this study. However there is no consensus on when and how often to perform post-transplant chimerism. We retrospectively analysed our experience about the impact of acute GvHD in the prediction of allograft chimerism. All patients with hematologic malignancies excluding myelofibrosis, transplanted between 2001 and 2010 after Fludarabine-Busulfan-ATG RIC from a HLA identical donor and with T cells chimérisme (TCC) determination between day 30 and 120 were included. 115 patients fulfilled all criteria. Allo-SCT was performed from familial donor in 92 patients (80%) and from MUD in 23 patients (20%). The conditioning regimen consisted of fludarabine 90 to 180 mg / m², Busulfan 8 mg / kg orally or 6.4 mg / kg iv and ATG 2.5 or 5 mg /kg. TCC was serially assessed at 30, 60 and 90 days after allo-SCT. Recipient peripheral blood T lymphocytes were positively sorted by a mix of anti-CD4 and CD8 immunomagnetic beads (Dynal, Compiègne, France). T-cell purity was controlled by flow cytometry and was always > or =95%. Genomic DNA was amplified using fluorescent PCR primers for polymorphic variable number tandem repeats (VNTR) or short tandem repeats (STR). Mixed T-cell chimerism was defined as between 5 and 94% recipient cells, and full chimerism was defined as the presence of more than 95% donor cells. Full TCC was achieved in 94 patients (82%) at a median of 77 (30-120) days post transplant. Fifty eight patients (50.4%) developed acute GvHD. The cumulative incidence of Grade 2-4 GvHD in our population is 32% (95% CI 23-41). Overall the results showed that each of the 37 patients developing grade >= 2 AGVHD had a Full TCC prior day 120. On the other hand, all mixed chimerism were documented in patients not presenting Grade>=2 AGVHD (21 of the 78 patients (27%) without grade >= 2 AGVHD) (p=.002). No other parameter (ATG dose, Donor type...) achieved this level of individual

prognostic. These results, in a very homogenous population, raise the question concerning the utility of routine chimerism surveillance in patients presenting an acute GvHD following RIC Allo-SCT and that can imply a not negligible saving in terms of economic and human resources.

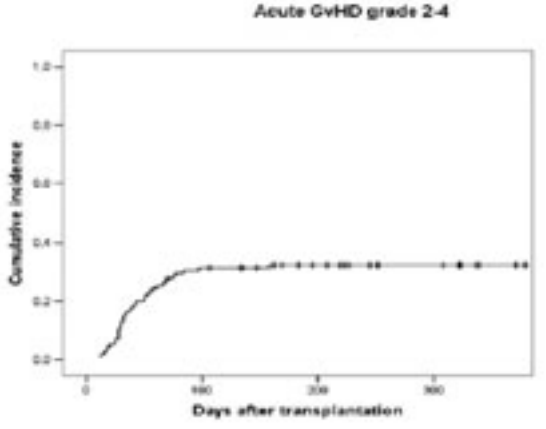

Figure 1: Cumulative incidence of acute GvHD. (Kaplan Meier).

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MONOCYTE SUBPOPULATIONS AND THEIR DIFFERENTIATION PATTERNS DURING POST TRANSPLANT ADVERSE EVENTS

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Backgound. Transplant-related adverse events are a major cause of morbidity and mortality in pediatric patients after high-dose chemotherapy and hematopoietic stem cell transplantation (HSCT). T-cell depletion of the graft, post-transplant immune deficiency, extensive immunosuppression, and neutropenia are known risk factors for bacterial and viral infections. Most of the data about monocytes during infection are known from murine models. Less is known, however, about the role played by monocytes in defense against infections post HSCT. Monocytes consist of several subpopulations, which can be classified according to cell-size, granularity and different expression levels of CD16/CD14. Blood monocytes subpopulations have been defined in two major types CD14++/CD16- (M1), and CD14+/CD16++ (M3) and the intergradation between these subpopulations the CD14++/CD16+ (M2). We conducted a pilot study and analyzed the monocytes subpopulation during post transplant period and transplant related adverse events. Patient and Methods. The patient group consisted of 30 pediatric patients and young adults (7 autologous, 12 allogeneic and 11 allogeneic haploidentical transplanted) with hemato-oncological malignancies and immunodeficiency disorders. The median age was 9.5 years (range 0.5-38 years). The period of analysis was from the day before start of conditioning until 365 days after HSCT. Normal values of monocyte subpopulations were analyzed from a control group of healthy children and young adults (n=20). Surface marker expression (CD14, CD16, CD64, HLA-DR) of monocytes was determined by four-color flowcytometric analysis. *Results*. The median analysis period in the patient group was 228 days (range 43-379 days). Post transplant a significant ($p<0.001$) increase of monocytes was observed at time of "leukocyte -take", defined as the point in time after HSCT when leukocytes first reached levels above 1000/µl. We analyzed a subpopulation of monocytes, (M4), which has been up to this point difficult to distinguish from natural killer cells and shows a surface expression of CD14-/CD16++, looking at both the healthy population as well as pediatric patients and young adults after HSCT. The largest percentage of monocyte subpopulation in healthy subjects was M1 (85.3%) followed by M3 (7.5%), M4 (4.2%) and finally M2 (3.0%). In contrast with healthy populations, between day +30 and day +100 after HSCT the proportional share of M1 (74.5%) presented as too low, while M2 (7.9%), M3 (11.3%) and M4 (6.3%) were rather high. While M4 already showed a significant decrease ($p<0.05$) five days prior to bacterial infection, a significant increase in M4 ($p<0.01$) was observed at the moment of viral infection. During acute graft-versus-host disease (GvHD) grade III and IV a significant decrease was observed in M1 ($p<0.01$), but a significant increase in M4 ($p<0.05$) and M3 ($p<0.05$). *Summary*. Altered patterns of monocyte subpopulations were observed during immune reconstitution and during post transplant adverse events. The role of the individual monocyte subpopulations remains to be elucidated during these conditions.

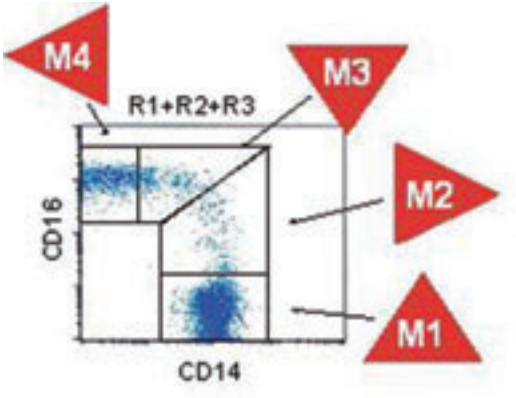

Figure 1. Monocyte subpopulations

1456

LASER TRANSMYOCARDIAL REVASCULARIZATION (LTMR) WITH AUTOLOGOUS BONE MARROW CELLS (ABMC): ONE STEP METHOD FOR HARVESTING AND PROCESSING. EXPERIENCE IN 21 PATIENTS

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Background: Recent studies suggest that Laser Transmyocardial Revascularization (LTMR) with Autologous Bone Marrow Cells (ABMC) could improve the results as cell therapy in diffuse coronary disease (DCD) respect to laser alone. We evaluated a point of care device which utilizes density gradient centrifugation to concentrate bone marrow mononuclear cells for transmyocardial laser injection. Patients and Methods: 21 patients with DCD and medically refractory class III/IV angina (mean age 66 years old), has been included in the study. At the time of surgery, 120 cc of autologous bone marrow were aspirated from the posterior iliac crest and anticoagulated with citrate. Using a density gradient centrifugal system (HARVEST®, Palex, USA), bone marrow harvested was separated into its components (Figure), which included 20 cc of concentrated mononuclear cells inclusive of the buffy coat which was immediately available for direct transmural myocardial laser channels injection. Cell counts and flow cytometry were used to determine the total number of mononuclear cells in addition to specific somatic stem cell populations such as CD34+ and CD133+ cells. Results: Time for bone marrow aspiration and concentration averaged 30 minutes. The complete processing was performed closed to the surgery room, in sterile ambient. There were no complications related to the bone marrow aspiration. Average cell counts pre and post concentration were significatively ($p<0.001$) increased (Table). No correlation between cell counts and patient demographics was detected. All patients received laser therapy with cell support without complications. 19 patients were evaluable for results. Conclusions and comments: These interim data shows that density gradient centrifugation with the HARVEST® device allows fast and efficient point of care concentration of ABMMC for cell-based therapies. Efficacy results regarding cardiovascular applications and clinical endpointswill be presented.

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PROPHYLAXIS OF RELAPSE WITH MODIFIED DONOR LYMPHOCYTE INFUSION CAN SIGNIFICANTLY INCREASE SURVIVAL AFTER HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ADVANCED-STAGE ACUTE LEUKEMIA

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Background Patients with advanced hematologic malignancies after allogeneic hematopoietic stem cell transplantation (HCT) have a poor prognosis because of a high rate of relapse and transplant-related mortality. Donor lymphocyte infusion (DLI) after allogeneic HCT exhibits definite anti-leukemia effects in this group of patients. Our primary data showed the feasibility of modified prophylactic DLI in HLA-mismatched HCT without in vitro T-cell depletion, but there have been no comparative clinical studies to confirm its efficacy. Aims The purpose of this non-randomized, single-center study was to comparatively analyze transplantation outcomes in a consecutive series of advanced-stage acute leukemia patients who either received prophylactic DLI or did not receive prophylactic DLI after HSCT from HLA-mismatched family donors during the same period at our institute. Methods The study was approved by the Institutional Review Board of the Peking University Institute of Hematology. All included patients were informed and signed an informed consent form. Consecutive patients with advanced-stage acute leukemia ((patients in CR3 or beyond, non-remission, n = 75) receiving HSCT from HLA-mismatched family donors during the same period (between January 2003 and September 2010) with (n=48) or without (n=27) prophylactic donor lymphocyte infusion (DLI) were enrolled. The conditioning therapy consisted of a modified BUCY2 plus ATG (thymoglobulin). Modified donor lymphocyte infusion (G-CSF-primed PBSCs instead of harvested non-primed donor lymphocytes and short-term immunosuppressive agents for prevention of GVHD after DLI) was planned from days 30-60 post transplantation before haematologic relapse was diagnosed Results Forty-eight patients received the prophylactic DLI at a median 48 (28-330) days after HSCT. The cumulative incidences of overall grades II-IV acute GVHD were 48% for patients receiving prophylactic DLI and 53% for patients not receiving prophylactic DLI , respectively (P = .55) with a relative risk (RR) of 0.51 (0.22-1.21) (P = .13). The cumulative incidences of overall chronic GVHD (including GVHD occurring before and after DLI) were 51% for patients receiving prophylactic DLI and 39% for patients not receiving prophylactic DLI , respectively (P = .42) with a relative risk (RR) of 1.99 (0.68-5.84) (P = .21). The 2-year cumulative incidence of relapse was significantly lower in patients receiving prophylactic DLI (34%) than in patients not receiving prophylactic DLI (55%) (P=0.018). The 2-year cumulative incidence of non-relapse mortality was comparable in patients receiving prophylactic DLI (38%) and patients not receiving prophylactic DLI (36%) (P = .95).

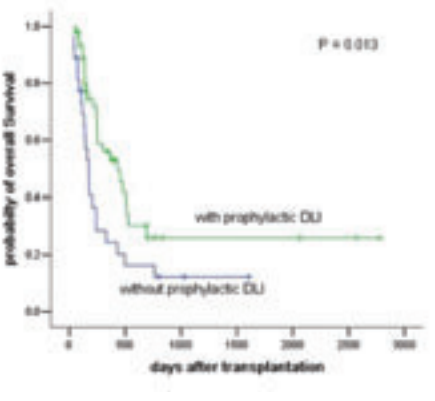

Figure 1. Overall Survival for patients receiving DLI or not.

The three-year probability of overall survival was higher in patients receiving prophylactic DLI (26%) than in patients not receiving prophylactic DLI (12%) (P = .013). *Summary/conclusions* the current study showed that a lower relapse rate, a similar NRM, and a higher survival probability was achieved with patients receiving prophylactic DLI than with patients not receiving prophylactic DLI. The results suggest that prophylaxis of relapse with modified donor lymphocyte infusion can significantly increase survival for advanced-stage acute leukemia patients even after HLA-mismatched T-cell-replete HCT.

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GVHD PROPHYLAXIS WITH HIGH DOSE CYCLOPHOSPHAMIDE AFTER HLA-MATCHED OR HAPLOIDENTICAL ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: recently, it was reported that after T-cell replete bone marrow transplant (BMT) from HLA-matched1 or haploidentical (haplo) related donor2, post-transplant cyclophosphamide (Cy), respectively given alone or with Tacrolimus (FK-506) plus Mycophenolate Mofetil (MMF) for GVHD prophylaxis, was associated with low incidence of graft rejection and acute and chronic GVHD (aGVHD, cGVHD) in patients (pts) with advanced myeloid malignancies (AMM). Methods: in our centre, 8 pts with AMM (1 CML in blast crisis, 1 CML in accelerated phase, 3 AML not in CR, 3 AML in CR2 relapsed after autologous BMT) underwent unmanipulated BMT from HLA-matched (2) and haplo (6) donors. GVHD prophylaxis was performed with Cy 50 mg/kg at days (d.) +3 and +4, alone or with FK-506 and MMF in HLA-matched and haplo BMT, respectively. In the haplo BMT, the conditioning regimen (Cond) was Cy 14.5 mg/kg at d. -6 and -5, fludarabine 30 mg/m2 from d. -6 to -3 and TBI 200 cGy at d. -1 in 4 pts; in a patient Cy was substituted with thiotepa 5 mg/kg at d. -6 and -5. In HLA-matched BMT, Cond was fludarabine 120 mg/m2 and busulphan i.v. 12.8 mg/kg in 4 days. Results: the median time to reach ANC>500/microl was 18 d. (r. 13-26) in 8/8 of pts, and platelets>30000/microl was 29 d. (r.19-40) in 7/8 of pts. After a follow-up of 8 months (m.) (r. 2-23), 5 pts are alive in CR (haplo and HLA-matched BMT in 4 and 1 case, respectively). Two pts (25%) developed steroid-responsive grade 2 cutaneous aGVHD at d. +18 and +39. After FK-506 and MMF withdrawing, 2 pts undergone haplo BMT had delayed grade 2 intestinal GVHD, responding to standard therapy. Interestingly, Foxp3 levels were significantly low in pts developing aGVHD. None of our pts showed cGVHD. Viral infections was documented and cured in 66% of pts. A patient (12.5%) died for transplant-related complications at 2 m. while 3/8 (37%) relapsed and 2/8 (25%) died after 4.3 and 8.5 m. Both these pts had refractory AML at the transplant and received BM from haplo donors. *Conclusions*: post-transplant Cy is a safe and effective prophylaxis of aGVHD and cGVHD in HLA-matched or haplo allogeneic BMT. To reduce relapse rate, especially after haplo BMT in pts with active disease, we are proposing clofarabine and busulfan i.v. as Cond in a multicenter study of GVHD prophylaxis with high-dose Cy after allogeneic BMT.

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1459

CLINICAL SIGNIFICANCE OF T-CELL LARGE GRANULAR LYMPHOCYTE EXPANSION POST ALLOGENEIC STEM CELL TRANSPLANTATION: A SINGLE CENTRE EXPERIENCE

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Background: Polyclonal or oligoclonal T-cell Large Granular Lymphocyte (T-LGL) expansion is a rare disorder generally occurring in patients with autoimmune diseases, viral infections, malignancies, and solid organ or allogeneic hematopoietic stem cell transplantation (allo-HSCT). The clinical significance of finding T-LGL expansion post allo-HSCT is unclear. Aim of the study: to evaluate if there is an association between T-LGL expansion after allo-HSCT and stable chimerism, chronic graft-versus-host disease (GVHD), recurrence of primary disease, and overall survival. Methods: A retrospective study was done on 46 patients whose data were available undergone allo-HSCT with a median follow-up of 61.4 months (r. 17.8-212.5). LGL expansion has been supposed in case of 1) increasing number of peripheral blood lymphocyte counts>2000 cells/mm^3 for at least 3 months, and 2) the predominance of LGLs in the peripheral blood smears. Cases with LGL expansion were confirmed for immunophenotypic profiles (CD2, CD3, CD5, CD7, CD8 and CD57 positive) and T-cell receptor RT-PCR for T-cell monoclonality. Results: Out of 46 patients, 12 cases (26%) showed LGL expansion after allo-HSCT. The median onset of LGL expansion was 35.9 months (r.12.8-

100). In 10/12 patients (83.3%) it was associated with confirmed chronic GVHD, particularly with cutaneous or pulmonary scleroderma-like GVHD or Sjogren-like GVHD. In the remaining 2 cases LGL expansion was documented at a follow-up of 12.8 and 37.8 months when common but not diagnostic signs of chronic GVHD were present. These patients are in actual close follow-up to document an evolution of overt GVHD. Stable mixed hematopoietic chimerism assessed by quantitative real-time polymerase chain reaction was achieved in most patients (73.7% donor component with a range of 45-100%). None of 12 patients experienced disease relapse at a follow-up of 52.4 months (r.14.6-212.5). Conclusion: it seems that LGL expansion is strongly associated with chronic GVHD and with graft versus leukemia effect also in absence of full donor chimerism. We think that pts undergone allo-HSCT presenting LGL expansion need to be monitored carefully for systemic chronic GVHD. Further study in larger series of pts are needed to evaluate this complex mechanism.

1460

SPLENECTOMY AND HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR THALASSEMIA PATIENTS

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Background: beta thalassemia is a genetic disorder resulting in absent or reduced beta globin chain synthesis producing haemolytic anemia. Currently, the only cure for thalassemia is allogeneic bone marrow transplantation, which corrects the genetic defect in the hematopoietic system by the use of allogeneic stem cells, the origin of which must be immunologically acceptable. Patients with thalassemia can be categorized into 3 classes of risk for bone marrow transplantation (Pesaro classification), and the category of greatest risk is class 3. Aims: massive splenomegaly in patients with thalassemia is often a reflection of inadequate medical care and/or advanced disease and frequently is found in class 3 patients. It is also associated with increased blood transfusion requirement. Splenectomy is indicated if there is significant abdominal discomfort, splenic infarction, or symptomatic hypersplenism. Presence of splenomegaly prior to an SCT raises the theoretical concern of the removal of infused stem cells, which could potentially have an adverse impact on engraftment. Given this, splenectomy performed prior a SCT could modify engraftment kinetic, which in turn could have an impact on graft tolerance and development of GVHD. Methods: our experience runs on fifteen patients affected by thalassemia that underwent splenectomy in the Department of Surgery of the Tor Vergata University General Hospital of Rome from May 2005 to April 2010. All patients were prepared for surgery by preoperative blood transfusion to achieve more than 9.5 gr/dL of haemoglobin level and all received prior pharmacological immunization with vaccines for meningococcus, pneumococcus and haemophilus influenzae. Results: mean and median age were 11 yrs. (min 4 - max 26 yrs). Mean operative time for surgical procedure was 75 min (range 60 - 100 min). The average postoperative hospitalization time was 5.7 days (range 5-7). Minimum spleen weight was 495 gr and maximum 2397 gr. Only one patient at day +2 after surgery showed increased level of amylases and lipases, but he was given promptly i.v. sandostatine treatment and those above mentioned enzymes normalized in five days. Conclusions: in this retrospective analysis, we reported that splenectomy prior to an allogeneic SCT in patients with thalassemia is associated with faster engraftment, a reduced peri-transplant transfusion requirement, and with not a significantly increased risk of death from peri-transplant infections.

1461

HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA WITHOUT GROWTH FACTORS: EXPERIENCE FROM A DEVELOPING COUNTRY (ORAN, ALGERIA)

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Background: The need for growth factors after autologous stem cell transplant (ASCT) has been investigated recently. Data from developing countries are scanty. Aims: We analyzed and updated our experience"on 32 consecutive patients with multiple myeloma (MM) treated with ASCT without receiving growth factors after transplant. Patients and methods: Among the 32 patients 11 was females and 21 was males. The median age was 55 years (range, 37-64 years). Before transplant, patients received chemotherapy using VAD (vincristine, adriamycin and dexamethasone, n=10) or bortezomib-dexamethasone (n=22). The median number of CD34+ cells was 3,86x10 6/kg (range, 1,05 - 8,62). High-dose melphalan (200 mg/m2) was used for conditioning and followed after 24 hours by reinfusion of autologous non-frozen hematopoietic stem cells, which had been stored for 24 hours at 4°C. All patients received prophylactic ciprofloxacin,fluconazole and acyclovir. Results: All evaluable patients had a full hematopoietic reconstitution. Median time to achieve neutrophils ≥ 500/µl was 12 days [range 10-17] and median time to achieve an unsupported platelet count≥20 000/µl was 13 days [range, 11 - 28]. After ASCT , 87% of patients responded. Grade II-III mucositis was the major regimen related toxicity. The median follow-up was 07 months (range,1-20 months). Estimated overall survival and EFS at 20 months were 96.5%±0.05% (s.e.) and 93%±0.05% (s.e.), respectively. Conclusion: We conclude that it is feasible and reasonable to perform ASCT for MM without administering growth factors and the procedure is easy to perform without requiring costly growth factors.

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ENGRAFTMENT SYNDROME AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION: INCIDENCE AND ANALYSIS OF RISK FACTORS. A SINGLE CENTER EXPERIENCE

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Background: Engraftment syndrome (ES) is an early complication following autologous stem cell transplantation (SCT). Nevertheless, there are no clearly established diagnostic guidelines. Aim: The aim of this study has been to analyze our experience as a single institution trying to identify risk factors predisposing to ES and a better characterization of the diagnostic criteria. Methods: We included patients that developed non-infectious fever within 5 days of engraftment (first day when neutrophils count>0.5x10^9/l). Moreover, we analyzed and compared diagnostic criteria of ES reported by Maiolino and Spitzer. *Clinical criteria for diagnosis of ES Spitzer criteria (3 major or 2 major+1 minor) within 96h of engraftment Major: non-infectious fever, skin rash, pulmonary edema, hypoxemia Minor: weight gain, hepatic or renal dysfunction and transient encephalopathy. Maiolino criteria Non-infectious fever plus: skin rash or pulmonary infiltrates or diarrhea commencing 24h before or at any time after the first appearance of neutrophils. Results: From January 2008 to December 2010, 10 patients (3 males, 7 females, median age of 50 years (range, 30-62) out of a total number of 50 patients who underwent an auto-SCT had clinical and biological data suggesting ES. Clinical diagnosis were: 7 multiple myeloma, 1 POEMS syndrome, 1 solid tumor and 1 Hodgkin´s lymphoma. At the end of neutropenic phase, five patients had only fever and skin rash, two developed fever and hypoxemia, 2 presented fever, rash and weight gain and 1 patient had fever, rash and diarrhea. All patients had negative cultures and no clinical documentation of infection was observed. The incidence varies according to Maiolino (20%) or Spitzer diagnostic criteria (10%). Peripheral blood was the source of stem cells in all cases. Four patients received G-CSF since day +7 after transplantation and median number of CD34+ cells/kg infused were 4.38x106/kg (range, 3.7-5.16). Median time to neutrophil recovery was 14 days (range, 9-18). All patients were treated with methylprednisolone (1 mg/kgr/12h) when ES was suspected and all had a rapid resolution of all symptoms and signs. They were discharged of hospital 7 days after ES diagnosis (range, 3-10). Summary: The differential diagnosis between ES and other complications at the end of neutropenic phase may be difficult. There is an increasing incidence of ES in the last years but it may vary according to the diagnostic criteria applied. Peripheral blood as stem cell source, higher numbers of CD34+ cells, use of G-CSF and the underlying disease (higher incidence in solid tumors, POEMS, amyloidosis and autoimmune disease) may favour its development. Corticosteroids is the treatment of choice and should be administrated early after the suggesting clinical and biological data appear.

1463

TOLL-LIKE RECEPTOR 4 GENE POLYMORPHISMS IN THE CHINESE WHO PARTICIPATED IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Graft-versus-host disease (GVHD) is a major complication after hematopoietic stem cell transplantation (HSCT) and remains to be a major cause of morbidity and mortality. The toll-like receptors4(TLR4) has been identified as a sole signal-transducing component in the LPS receptor complex with a conical shape(e.g. from Escherichia coli) and has been showed to be a key player in the innate immunity and immune tolerance. Activation of toll-like receptor4 (TLR4) by lipopolysaccharide (LPS) induces the NF-κB signaling pathway to release the critical proinflammatory cytokines and increases the recepient GVHD response. In order to clarify the role of TLR4 in the occurrence of acute GVHD after HSCT, we collected 208 samples from HSCT recipients and their HLA-identical donors to test the hypothesis that TLR4 polymorphism in the recipients or donors influence the risk of acute GVHD in allogeneic HSCT recipients. All patients received methotrexate and cyclosporine for GVHD prophylaxis and had either grade 0 or grades II to IV acute GVHD. The TLR4 Asp299Gly and TLR4 Thr399Ile polymorphisms of each sample were examined by using DNA sequencing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods, we used two restriction enzymes to randomly test samples, NcoI for the PCR-RFLP assay of the Asp299Gly allele and HinfI for the Thr339Ile allele. No homozygous or heterozygous variant alleles of the Asp299Gly or Thr339Ile polymorphisms were detected in any samples of our study. Our results demonstrated that the TLR4 Asp299Gly and Thr399Ile polymorphisms might be very rare in Chinese population. The results of this study cannot confirm the role of TLR4 mutation in the pathogenesis of GVHD in humans.We expect to reach a definite conclusion by a TLR4 knockout murine GVHD model in our ongoing project.

1464

IMPACT OF PRIOR AUTOLOGOUS MOBILIZATION STATUS ON ENGRAFTMENT AND OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR LYMPHOMA AND MYELOMA

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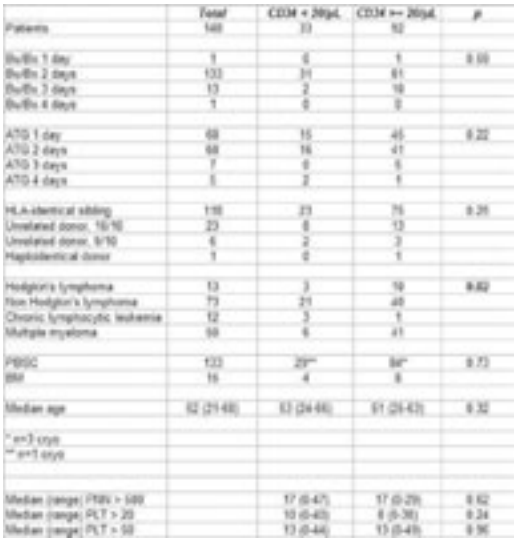
Background: approximately 15% of patients who are candidates for autologous HSCT fail to collect an adequate amount of CD34+ cells (poor mobilizers). Reasons for poor mobilization include patient and treatment-related factors. Infra-clinical damages to the bone marrow (BM) micro-environment may play a role and affect the interactions of stem cells with stromal cells and the bone-blood barrier that contribute to efficient mobilization. If such a hypothesis stands true, then damages to the BM micro-environment should also affect the ability of donor stem cells to home and seed in the BM of recipients after allogeneic HSCT. *Aims*: we thus examined whether patients who underwent allogeneic HSCT at a single institution, using fludarabine-busulfan-ATG RIC conditioning after a failed attempt at autologous HSCT, were different in terms of hematopoietic recovery than patients who received a tandem autologous and allogeneic HSCT. Methods: inclusion criteria were age > 18 years, diagnosis of lymphoproliferative disease or multiple myeloma, prior mobilization (succesfully or not), HSCT from related or unrelated donor and RIC conditioning. Poor mobilization was defined as circulating CD34+ <20/µl on the first day of planned collection. PMN and PLT engraftment were determined for good and poor mobilizers, taking into account confounding factors such as age, type of donor and disease. Results: a total of 148 patients were included in this analysis, of which 125 were evaluable for CD34+ counts and engraftment (n=33 for poor-mobilizers and 92 for good mobilizers, see table 1). All but two patients engrafted. Overall, the median (range) day was 17 (0-47) for PMN >0.5 G/L, 8 (0-49) for PLT >20G/L and 12 (0-49) for PLT >50G/L. There was no significant difference in PMN or PLT engraftment between the two subsets. Nevertheless, NRM and overall mortality were higher in poor mobilizers (p=0.009 and p=0.01 respectively); relapse rate, acute and chronic GvHD were similar in both groups (p=0.81). Data were confirmed after adjustment for patient's age, type of donor and disease. *Conclusions*: our data suggest that lymphoma and myeloma patients with a history of poor mobilization defined as CD34+ <20/µl do not have different outcomes in terms of PMN and PLT engraftment. Despite the absence of difference in terms of early hematopoietic recovery after allogeneic HSCT, NRM and mortality were significantly higher in poor mobilizers. Identifying the exact reasons for such different outcomes will require further investigation.



	Total	CD34 < 20/µL	CD34 >= 20/µL	p
Patients	148	33	92	
Bu/Bn 1 day	1	0	1	0.69
Bu/Bn 2 days	133	31	81	
Bu/Bn 3 days	13	2	10	
Bu/Bn 4 days	1	0	0	
ATG 1 day	68	15	45	0.22
ATG 2 days	68	16	41	
ATG 3 days	7	0	5	
ATG 4 days	1	2	1	
HLA-identical sibling	118	23	75	0.31
Unrelated donor, 10/10	23	8	13	
Unrelated donor, 9/10	6	2	3	
Haploidentical donor	1	0	1	
Hodgkin's lymphoma	13	3	10	0.62
Non Hodgkin's lymphoma	73	21	49	
Chronic lymphocytic leukemia	12	3	1	
Multiple myeloma	50	6	41	
PBSC	133	29**	84*	0.71
BM	15	4	8	
Median age	52 (21-68)	53 (24-66)	51 (24-63)	0.32
* n=3 crys				
** n=1 crys				
Median (range) PMN > 500		17 (0-47)	17 (0-29)	0.62
Median (range) PLT > 20		10 (0-40)	8 (0-36)	0.24
Median (range) PLT > 50		13 (0-44)	13 (0-49)	0.96

Figure 1. Patients' characteristics.

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THE USE OF 18F-FDG PET-TC IN THE ASSESSMENT OF RESPONSE AFTER ALLOGENEIC STEM CELL TRANSPLANT IN HEAVILY PRE-TREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: A SINGLE CENTRE EXPERIENCE

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Background. Chronic lymphocytic leukemia (CLL) is a heterogeneous lymphoproliferative disorder; the only potential curative option especially for high-risk patients is the allogeneic transplant. To our knowledge, no studies have been published on the role of 18F-FDG PET or PET-CT in evaluating response to allogeneic transplant in CLL. Aim: The aim of our study was to evaluate the role of 18F-FDG PET-CT in monitoring response to reduced-intensity conditioning (RIC) transplant and to compare the results with standard criteria according to International Workshop on Chronic Lymphocytic Leukemia (IWCLL). *Methods*. We retrospectively analyzed 9 consecutive high-risk CLL patients, affected by fludarabine-refractory (4 patients) or relapsed CLL (5 patients) who underwent RIC transplant from March 2004 to May 2009. PET-CT scanning was planned at about 8 months after transplant to assess response and at a mean 9 month period during follow-up. Overall, 42 PET-CT studies were performed. The mean long-term follow-up period was 39.7 ± 20.5 months. All studies were analysed qualitatively and scored as either negative or positive: any focal area of activity higher than vascular background, in any site incompatible with normal anatomy, was considered abnormal. *Results*. The first PET-CT performed after transplant showed abnormal FDG uptake in 5 patients: 4 patients classified as stable/refractory and 1 patient in partial remission(PR) at pre-transplant evaluation. No abnormal FDG uptake was observed in 4 patients who showed PR before transplant. Response assessment by IWCLL criteria performed at the same time of the first PET-CT (at about 8 months after transplant), showed persistent disease in 8 patients (5 PET positive and 3 PET negative) and complete response in 1 patient (PET negative). At the end of follow-up, all 4 patients with previously negative scans were still PET negative and are in complete remission by standard criteria. Similarly, all 5 patients with previously positive scans were still PET positive; of these, 1 died 27 months after RIC transplant for disease progression and

4 are alive with persistent disease. *Conclusions*. From our preliminary data in a small series of CLL patients, the first 18F-FDG PET-CT after transplant shows different metabolic findings that reflect the different pre-transplant status and seem to predict the patient outcome earlier than clinical evaluation by standard criteria. PET-CT performed during follow-up is useful to assess disease status and to early detect disease progression.

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ETANERCEPT FOR STEROID-REFRACTORY ACUTE GRAFT-VERSUS-HOST DISEASE FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Treatment strategy for steroid-refractory acute GVHD after Allo-SCT needs to be standardized. We report our clinical experience of the effect of etanercept on steroid-refractory acute GVHD. A total of 18 patients who received allo-SCT and presented with steroid-refractory acute GVHD at Ajou University Hospital were retrospectively studied. Twenty-five milligrams of etanercept were given s.c. twice weekly for four weeks. Clinical responses were evaluated with regard to the severity of acute GVHD. The median age of patients was 43.5 years. By using paired t-test, etanercept showed down grading effect of acute GVHD ($p = .005$) but no patient experienced complete remission. Eighty percent of grade II, 14% of grade III, and 57% of grade IV patients showed a partial response. Skin and gut GVHD were well controlled with etanercept, whereas hepatic GVHD was not. Four patients died of fatal infection. There were no factors affecting the clinical outcome of etanercept. All the non-responders died and 56.6% of the responders survived ($p = 0.0008$). Etanercept can be effective in treating steroid-refractory acute GVHD after Allo-SCT, with tolerable side effects.

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VON WILLEBRAND FACTOR (VWF) LEVEL IN THROMBOTIC MICROANGIOPATHY (TMA) IN CHILDREN UNDERGOING ALLOGENIC BONE MARROW TRANSPLANTATION: A SINGLE - CENTER EXPERIENCE

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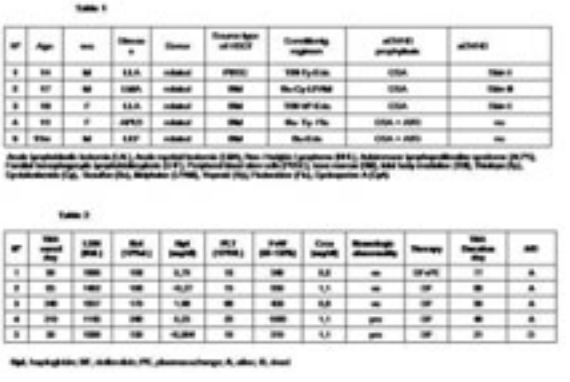
Introduction: Thrombotic microangiopathy is a well-documented complication that may occurr after hematopoietic stem cell transplantation (HSCT). The reported incidence of TMA after HSCT varies from 0,5% to 75%, is characterized by thrombocytopenia, microangiopathic hemolytic anemia, fever, neurologic and renal abnormalities resulting from formation of disseminated hyaline thrombi in the small vessels of the main organs due to generalized endothelial damage. VWF is a multimeric protein contained in endothelial cells, platelets and plasma that mediates platelet adhesion and aggregation at sites of vascular injury. Once released from the abnormally stimulated endothelial [by multiple factors: high-dose chemotherapy, immunosuppressants, graft-versus-host disease (GVHD), infection and irradiation] ultralarge multimers of VWF, present in the endothelium but not found in the plasma in physiological conditions, directly promote intravascular aggregation of the platelets and the consequent microvascolar thrombosis. Materials and methods: We report a retrospective analysis of patients (15 months to 17 years old) with hematologic disease who underwent allogenic HSCT between February 2007 and December 2009 at our insitution. We diagnosed transplantation-associated TMA in 5 of 36 transplanted patients. Table 1 shows the characteristics of patients who developed TMA after HSCT. The diagnosis was evaluated on the basis of clinical and laboratory criteria: microangiopathic hemolysis with negative Coombs test with unexplained drop in hemoglobin level and platelet count (Plt), increased number of reticulocytes (Ret), evidence of red cell fragmentation on the blood smear, increased value of indirect bilirubin (double of the normal value), increased serum lactic acid dehydrogenase (LDH) activity (more than 500UI/l), renal dysfunction (serum creatinine = Cre > 1,5 mg/dl), neurologic dysfunction. In addition, all patients were monitored for vWF levels (vWF Antigen kit-ELISA). Results The most relevant clinical and laboratory data concerning diagnosis, therapy and patient outcome with TMA after HSCT are listed in table 2. Five of 36 transplanted patients (13,8%) developed TMA for a median duration of 58 days (range 21-90). In 3 of 5 patients TMA occurred within 100 days after HSCT. All patients had increase in fragmented red cells, thrombocytopenia, significant elevation in LDH. Moreover we found elevated FvW levels until the early signs of thrombotic microangiopathy, even in a case was the first parameter to be altered with a modest decrease in platelets. Of these 5 patients, 3 responded to administration of DF, 1 responded to a combination of Df + PE and only one developed, during DF treatment, severe TTP and died from multiorgan failure. Conclusion TMA is a severe and relatively common complication after HSCT. The diagnosis in transplanted patients is difficult because its presentation overlaps with other post- HSCT complications. However is important to recognize it beforeTMA can have harmful effects. This retrospective analysis of transplanted patients in BMT Unit of Pediatric Center evaluate the impact of TMA and the relevance, even in our small series, of vWF levels as important parameter for the diagnosis and monitoring the evolution of the disease.



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DOUBLE UNRELATED CORD BLOOD TRANSPLANTATION FOR ADULTS WITH HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA IN KOREA

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Background. Unrelated cord blood (CB) has been shown to be a valuable alternative source of hematopoietic stem cells for transplantation in patients who lack a suitable related or unrelated donor. Graft cell dose is an important determinant of hematopoietic recovery and overall outcome following CB transplantation (CBT), and the limited cell dose of single CB unit has been a major barrier to its more widespread use. Strategies to overcome this barrier include the use of two partially HLA-matched CB units (double CBT). Aims: We report the results of unrelated double CBT for 14 adults with high-risk acute lymphoblastic leukemia (ALL) between 2005 and 2010. Methods: The median patient age was 31 years (range, 16-52 years). All patients had one or more high-risk features, including 7 Philadelphia chromosome-positive ALL. Eleven patients (78.6%) were transplanted in CR1; 1 (7.1%) in CR2; and 2 (14.3%) in refractory/relapsed status. All patients received myeloablative preparation consisting of total body irradiation (12 Gy), fludarabine (150 mg/m2), and cytarabine (9 g/m2). Graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus and mycophenolate mofetil. CB unit was selected according to the number of total nucleated cells (TNC) per recipient's weight and HLA compatibility (HLA-A and -B by serotying and HLA-DRB1 high-resolution genotyping). No patients had a suitably matched related or unrelated donor available at the time of transplantation. All patients and donors provided written informed consent, and the treatment protocol was approved by the institutional review board of The Catholic University of Korea. Results: The median cell doses infused were 3.60x107 TNC/kg (range, 2.62-7.88), 1.80x105 CD34/kg (range, 0.65-5.85), and 8.04x107 CD3/kg (range, 6.24-13.49). All patients achieved a successful engraftment with full donor chimerism. Neutrophil recovery occurred at a median of 25 days (range, 17-109 days), and platelet recovery occurred at a median of 39 days (range, 20-185 days) after CBT. Acute GVHD was observed in 9 patients (64.3%; 7 grade II, 2 grade III). Four (36.4%) of the 11 evaluable patients had chronic GVHD (2 mild, 2 moderate). With a median follow-up duration of 13 months (range, 3-59 months) for surviving patients after CBT, 10 patients are alive with a leukemia-free status, while the other 4 patients have died

of relapse (n=3) or infection (n=1). The cumulative incidence of relapse and the probability of disease-free survival at 2 years were 23.8% and 76.2%, respectively. *Summary/Conclusions*. Our data suggest that adult high-risk ALL patients without suitable related or unrelated donors should be considered as candidates for double CBT and provide the rationale for a larger clinical study in Korea.

1469

SINUSITIS IN PATIENTS UNDERGOING ALLOGENEIC BONE MARROW TRANSPLANTATION

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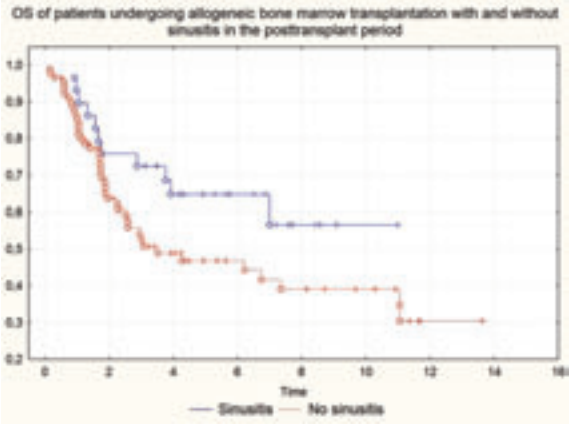
Background. Sinusitis is one of the most common infections in general population, but data on its incidence and impact on the overall survival in hematologic patients undergoing allogeneic bone marrow transplantation is scarce. *Aims.* Analysis of the frequency of sinusitis, its fatality and potential predisposing risk factors in allogeneic bone marrow transplant recipients. *Methods.* The records of 113 patients with different hematological diagnoses with the exception of chronic myelogenous leukemia who underwent altogether 128 allogeneic bone marrow transplantations, were retrospectively reviewed for the occurrence of sinusitis, its complications, survival and the transplant data. *Results*. Twenty nine patients (26,5%) developed sinusitis between day -1 and +2044 (269±376) post transplantation, with 43% of them within the initial 100 days. They were treated with antibiotics and only 2 patients required surgical intervention and one extensive surgery. The only factor which predisposed to sinusitis in the analyzed group was slower neutrophils recovery (19 vs 16.6 days; p=0.03).



The type of donor (related vs unrelated, matched vs mismatched), source of stem cells (peripheral blood vs bone marrow), number of transfused CD34+ cells/kg and CR status had no impact on the occurrence of sinusitis. Sinusitis did not deleteriously affect overall survival of transplanted patients (p=0,08). *Conclusions* Sinusitis is a frequent infection in recipients of allogeneic hematopoietic stem cells, occurring most frequently during the first 100 days post transplantation. It can successfully be managed with antibiotics in the majority of them and has no impact on the overall survival.

1470

THE OUTCOME OF HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN PRE AND POST MODERN THERAPEUTIC INTERVENTIONS

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Background. We report the outcomes of high dose chemotherapy (HDT) and autologous stem cell transplantation (ASCT) in the first 100 cases treated in a single institute over 13 years. The two main groups of patients were those with Myeloma and Non-Hodgkin lymphoma. We assessed the outcomes of HDT and ASCT pre- and post-introduction of modern therapy in Myeloma and anti-CD20 in CD20 positive lymphomas. We also analysed the prognostic indicators at presentation in each disease group and compared our results to other centres. Aims This single-centre study is aimed at assessing the outcome of HDT and ASCT in our institute and analysing the results in relation to: - Prognostic indicators, previous treatments received and outcomes for Multiple Myeloma (MM), Diffuse Large B-cell Lymphoma (DLBCL) and Hodgkin Lymphoma (HD). - Outcome in patients with DLBCL following ASCT in relation to previous exposure to anti-CD20. - Outcome of ASCT in MM pre- and post-introduction of Thalidomide. - Compare the outcome of different disease groups in our institute to those already reported by others and identify which group of patients benefit the most from ASCT. Methods We analysed data on all patients treated with HDT and ASCT since the start of the service in 1997. The details of the patients, their disease and outcome at 100 days for the period including December 2009 have already been reported to the British Society of Blood and Marrow Transplantations (BSBMT). Results The median age of all patients that underwent ASCT was 53 (range 23-66). Overall survival (OS) at 1 year was 89.5% and progression-free survival (PFS) at 1 and 5 years were 83.7% and 57.4% respectively. Mortality rates at 3 months, 1 year and 5 years were 2.2%, 10.5% and 34.4% respectively. Two patients died within 100 days of transplant, both with NHL. Log-rank analysis demonstrated that those with HD had the best survival following ASCT compared to other groups (p-value 0.039). The main disease groups and the number of patients in each group analysed were: Myeloma (n=39), Hodgkin lymphoma (n=14) and Non-Hodgkin lymphoma (n=40). There was no significant difference in PFS or OS following ASCT for DLBCL between the 14 patients that received anti-CD20 as part of their initial therapy and the 11 patients that were anti-CD20 naïve. Treatment of Myeloma with Thalidomide did not have a significant effect on PFS or OS post ASCT. ISS and IPI scores at diagnosis were not significant in predicting outcome after ASCT in Myeloma and DLBCL (p-values 0.101, 0.081 respectively). Summary/Conclusions. The outcome of HDT and ASCT in HD was better than any other disease group, as was expected. Overall outcome for patients was better than the average outcome reported by BSBMT and EBMT. There was no difference in outcome following HDT and ASCT in patients treated after the introduction of anti-CD20 for CD20 positive lymphomas or the use of Thalidomide as first line treatment in Myeloma. However, the small numbers of patients in each disease group makes it difficult to draw firm conclusions.

1471

DOES CENTRAL VERSUS PERIPHERAL VENOUS ACCESS HAVE AN EFFECT ON THE CD34+ COLLECTION EFFICIENCY IN LARGE-VOLUME LEUKAPHERESIS?

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Background. The yield of CD34+ cells obtained from an apheresis procedure can theoretically be predicted from the peripheral blood CD34+ cell concentration, the processed blood volume (PBV) and the efficiency of the collection (CE) (CE = total CD34+ cells collected/(peripheral blood CD34+ cells/µl x PBV in µl) (*Ford et al. Transfusion 1998*). Central venous lines (CVL) are frequently used to provide the high flow rates necessary for large-volume leukapheresis, when peripheral venous access (PVA) is determined to be inadequate after clinical investigation. *Aims*. The aim of this retrospective study was to evaluate the difference in CE between donors/patients undergoing apheresis by CVL or PVA. Methods: During 39 months, 71 donors/patients underwent 115 different apheresis procedures with a Cobe Spectra cell separator (Caridian-BCT). Haematological diagnoses included: healthy donor (n=29), multiple myeloma (n=18), lymphoma (n=24). The choice between CVL or PVA was made by both a medical and a nursing staff member, based on the adequacy of peripheral veins. 67 (58,3%) apheresis sessions were performed by CVL in contrast to 48 (41,7%) by PVA. All donors/patients were evaluated for age, sex, weight, BMI, diagnosis, collection time, PBV and pre-apheresis haematocrit (HCT), thrombocyte count (TRC) and white blood cell count (WBC). Determining factors for CE were assessed after correlation and linear regression analysis. *Results:* Donors/patients receiving a CVL were older (57,7 ± 1,4y versus 51,3 ± 2,1 y; p=0,01) and had a higher BMI (26,6 ± 0,7 versus 24,2 ± 0,5; p=0,01). Healthy donors received significantly less CVL (39,5%) compared to donors with myeloma (60%) or lymphoma (78,6%) (p=0,001). Collection times were shorter using CVL (225,5 ± 6,1min versus 252,8 ± 8,1min; p=0,008) and PBV were greater (20,1 ± 0,81L versus 15,2 ± 0,59L; p<0,001). Pre-apheresis laboratory results showed a lower HCT (33,8 ± 0,7% versus 37,9 ± 0,6%; p<0,001), lower TRC (145 ± 11 x 10^9/l versus 209 ± 12 x 10^9/l; p<0,001) and lower WBC (34,7 ± 2,8 x 10^9/l versus 46,1 ± 2,9 x 10^9/l; p=0,007) in donors/patients with CVL. CE was not significantly higher in donors with CVL (49,6 ± 2,5% versus 47,1±2,5%; p=0,5). HCT (r =-0,245; p=0,008), TRC (r=-0,267; p=0,004) and WBC (r=-0,376; p < 0,001) were all significantly inversely correlated with CE.

Stepwise multiple regression analysis showed WBC count to be the best predictor of CE (R^2=0,096). *Conclusions:* Donors with haematological disease, older age and higher BMI were more likely to need a CVL for CD34+ cell collection compared to healthy donors. Procedures using CVL were shorter in duration time and obtained a higher PBV. However, the use of CVL has no effect on CE. Low WBC seemed to be the best predictor of CE. This fact suggests, to take into account, the PBV in regard to the pre-apheresis WBC in order to avoid the necessity of multiple apheresis sessions.

1472

THE CLEARANCE OF INFUSED HEMATOPOIETIC STEM CELL FROM THE BLOOD CIRCULATION

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Background. Homing is the essential step and is a rapid process in hematopoietic stem cell (HSC) transplantation. Aim and method: In the present study, using flow cytometry, we tried to determine the clearance time of the infused HSCs from the circulation following autologous HSC transplantation in 20 patients. *Results*. The median CD34+ cells were at the highest level at the first hour and reached below pre-infusion values at the first day after HSC infusion. By nonparametric analysis, positive correlation was found between CD34+ cell levels at the first hour and the post-thaw CD34+ cell dose (r= 0.57, p= 0.01). Inverse correlation was determined between CD34+ cell levels at the first hour and neutrophil engraftment (r= -0.54, p= 0.01). Compared with the patients having CD34+ cell count of ≥2/μL at the first hour after HSC infusion, the patients having CD34+ cell count of <2/μL had delayed neutrophil (20 vs. 12, p=0.008) and platelet (47 vs. 11, p=0.01) engraftment. *Conclusions*. Our results showed that infused HSCs were removed from the blood circulation within a day. In addition, CD34+ cell levels at the first hour might be an important indicator to predict the delay of neutrophil and platelet engraphment.

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1473

RISK FACTORS FOR MICROBIAL CONTAMINATION OF PERIPHERAL BLOOD STEM CELL PRODUCTS

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Background. In spite of the well known contamination rates and the microbial agents in stem cell products, the risk factors affecting microbial contamination have not been well described. Study Design and Methods: In a 12-year period, we retrospectively evaluated the culture results and risk factors after processing and thawing of a total of 510 peripheral blood stem cell (PBSC) products of 184 patients/donors. Results: Of 28 (5.7%) PBSC products in which microbiological contamination was detected after processing, growth was not detected in 13 (46.4%) products in the post-thawing period. Large volume leukapheresis (LVL) (odds ratio [OR]= 5.85, 95% confidence interval [95% CI] 1.52 - 22.49, p= 0.01) and the large number of the culture specimens that were sent (OR= 1.4, %95 CI 1.03 - 1.91, p= 0.03) were found to be risk factors for post-processing growth. The presence of post-processing microbial growth was found to be risk factor for post-thawing (OR= 20.22, %95 CI 6.67 - 125.15, p< 0.001) and post-transplant (OR= 3.25, %95 CI 1.24 - 8.50, p=0.01) microbial growth. Conclusion: Culture results should be monitored carefully in patients in whom post-processing growth risk increased when LVL was applied and when a large number of culture samples were sent. In transplantations performed with products having post-processing growth positivity, one must keep in mind the fact that growth of different pathogens may be seen at a high rate (30%) along with a markedly increased risk of culture positivity (OR=3.25).

1474

RISK FACTORS FOR ADVERSE EVENTS DURING COLLECTION OF PERIPHERAL BLOOD STEM CELL

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Background. Although collection of peripheral blood stem cells (PBSCs) by apheresis devices is a safe procedure, patients/donors-associated adverse events (AEs) and apheresis instrument related technical problems might be observed. Aim and Methods: We retrospectively reviewed of PBSCs collections following 528 mobilization cycles (413 patients and 75 related donors) over a 10-year period with the intent of identifying risk factors for AEs occurring in association with the procedures. *Results*. A total of 206 (13.1%) AEs occurred in association with the 1572 procedures. 191 (12.15%) of the AEs were classified as clinical AEs and 15 (0.95%) were classified as apheresis instrument-related AEs. The most common clinical AE was numbness of lips, tongue, or extremities (161 procedures, 10.2%) related to the infusion of acid citrate dextrose-A (ACD). Multivariate analysis revealed high amounts of ACD/weight (odds ratio [OR]= 1.11, 95% confidence interval [95% CI] 1.02 - 1.21, p= 0.009), high numbers of procedures (OR= 1.33, 95% CI 1.13 - 1.56, p< 0.001) and female gender (OR= 2.83, 95% CI 1.70 - 4.71, p< 0.001) as being significantly associated with clinical AEs. *Discussion*. Female gender was shown to be the most important risk factor for clinical AEs. Females who significantly increased risk of AEs would benefit from prophylactic calcium before and/or during PBSC collection.

1475

DOES SECOND ALLOGENEIC STEM CELL TRANSPLANTATION COULD BE TREATMENT OPTION FOR RELAPSES, GRAFT REJECTION OR ABSENCE OF ENGRAFTMENT AFTER FIRST ALLOGRAFT: SINGLE CENTER EXPERIENCE

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Introduction. Allogeneic stem cell transplantation (SCT) is only potentially curative treatment option for different hematological malignancies. Despite efficacy, relapses are still possible and till now there is no standard approach neither for relapses, nor for other SCT failures. Current options for such cases are chemotherapy, donor lymphocyte infusion or second SCT. Aim: We are reporting outcome of second SCT in our center. *Patients and method*. From 1995 till 2010., 18 patients (pts) undergone second SCT for treatment of relapse (13 pts), graft rejection (2 pts) or absence of engraftment (3 pts) after first SCT. Median age in this cohort of pts was 22,5 (16-32) years, M/F ratio 11/7. Pts were suffering from various diseases: 2 AML, 9 ALL, 4 CML, 1 MDS, 2 AA. Median follow up is 30,5 (range 2-180) months. *Results*. Disease relapse had occured at a median of 18,7 months after first allo SCT (range 6-72). Graft rejection was observed after one year from first SCT in both cases with aplastic anaemia. Pts with acute and chronic leukemias had received *salvage* chemotherapy (Flag-IDA) and afterwards despite of marrow findings, underwent second allogeneic SCT with reduced intensity conditioning. Pts with aplastic anaemia were conditioned with Cyclophosphamide and ATG. All pts had received stem cells from same identical sibling donor. Peripheral blood was source of stem cells in 16 pts and bone marrow in 2 pts. Engraftment was observed in all pts with median neutrophil recovery after 16 (11-23) days. Prevention of graft versus host disease (GvHD) was modified according to specific situation (complete absence of prophylaxis in the cases of leukemia relapses or combination of Cyclosporin A with Metothrexate or MMF in the graft rejection or graft failure). Overall survival (OS) of all our pts is 37,5% with median follow up 60 (2-180) months. Conclusion: Our modest results have showed benefit of second SCT as treatment option for selected cohort of pts who have failed after first allografting. Further investigation on larger, homogenuos groups of pts is need.

1476

HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOIDE LEUKEMIA IN 77 PATIENTS UNDER 18 YEARS OLD

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Background. The prognostic of acute myeloid leukemia (AML) in children has significantly improved over this last two decades, essentially with the help of the intensive chemotherapy. Several randomised trials have shown the superiority of HSCT in children with high risk AML in first complete remission (CR). This is a single centre retrospective study involving in 77 children receiving HSCT for HLA identical sibling donors for AML. *Material and methods*. From September 1998 to April 2010, 77 patients (pts) under 18 years old received an HSCT for AML, (62 pts were in 1st CR, 8 pts in 2nd CR and 4 pts were refractory). The cytologic types of AML (FAB: M1:16 pts, M2:27 pts, M3:3 pts, M4:15 pts, M5:4

pts, M6:1 pt, M7:1 pt, M0:2 pts, undetermined type: 8 pts). The conditioning regimens were: Santos (BU-EDX 50) for 63 pts, (BU-VP-EDX 60) for 13 pts and Tutshka (BU-EDX 60) for only 1 pt. GVHD prophylaxis associated Ciclosporin and Methotrexate. Median age is 11 years (4-17). Sex-ratio is 0,83. Median time from diagnosis to transplant is 7 months (3-45). Sixty eight pts received peripheral stem cells with a median rate of CD34 6,21x106/ kg (1,7-27), 8 pts received a bone marrow with a rate of mononuclear cells: 5,8x108/kg (2, 25-8,15), and one pt received a cord blood with a rate of nuclear cells 2,3x108/kg. Results: All the pts have a neutropenia; median duration of the aplasia is 15 days (8-25), overall median time to achieve neutrophils count > 500/mm3 is at day 14 (8-27). Forty two pts (54, 5%) needed blood transfusion, 72 pts (93,5%) required platelets transfusion. Three pts (3,8%) presented a complete resolutive hepatic veno-occlusive disease . Acute GVHD occurred in 26 pts (35,1%), in 19 pts (25, 6%) it was between grade II and IV. Chronic GVHD was observed in 27 pts (39,1%), extensive form in 22 pts (31, 8%). Only 5 pts (6,4%) have presented CMV infection at median time of the day 114 (53-237). Eighteen pts (23,3%) relapsed at a median time of 16 month s (2-36). TRM incidence is 16, 8%. At the 30 th of November 2010, median follow up was 70 months (7-145), 46 pts (59,7%) are still alive, 45 pts (58,4%) are in remission. Overall Survival and Event Free Survival at 12 years are respectively 56,3% and 55%. *Conclusions.* Most of the pts of this study are pts in a first complete remission, and all of them are with unknown cytogenetic profil, the results of the trial are interesting, the TRM and the relapse rate seems not higher than what we observed in other series. An algorithm for treatment depending on the disease risk and the transplant risk might appear in the future in order to decide how to planned HSCT: in first CR or a delayed HSCT.

1477

TREATMENT EFFICACY AND PROGNOSIS IN PATIENTS WITH EXTRAMEDULLARY RELAPSE OF ACUTE LEUKEMIA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Leukemia relapse still remains a problem in patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT). A lot of relapses occurred in extramedullary sites with or without bone marrow involvement. Extramedullary relapse (ER) seems to be less sensitive to chemotherapy and immunotherapy, and there is no standard treatment of pts with ER after allo-HSCT. *Aims.* To study frequency and clinical features of leukemia ER after allo-HSCT and to evaluate efficacy of different treatment options. *Methods*. We performed 10-year retrospective cohort single-center study of children and young adults who had leukemia ER after allo-HSCT. Results: Among 79 patients (pts) with acute leukemia who relapsed after allo-HSCT 8 pts (10%) had ER including 4 pts with ALL and 4 pts with AML. The mean age was 14.5 years (range, 1-21). At the moment of allo-HSCT 5 pts had complete remission (CR), 2 pts had relapse, and 1 pt had refractory disease. Allo-HSCT from matched unrelated and matched related donor was performed in 6 and 2 pts respectively. The conditioning regimen was myeloablative and reduced-intensity in 5 and 3 patients respectively. The median time between allo-HSCT and ER was 15 months (range, 3-26). Two pts had isolated ER with full donor chimerism in BM. In other cases ER with simultaneous bone marrow (BM) involvement (n=2), consecutive occurrence of ER and BM relapse (n=2) and BM relapse 3 and 10 months prior to ER (n=2) were diagnosed. The sites of ER were head and neck, salivary glands, breast, paravertebral tissue, bones, skin, kidneys, testes, ovaries, small pelvis. All pts received chemotherapy and immunoadoptive therapy with donor lymphocyte infusion (DLI, n=7) and NK-cells (n=2). Four pts underwent local therapy: 2 - surgery, 2 - radiotherapy. Complete remission (CR) after first ER was achieved in 6 pts, partial remission (PR) - in 2 pts. Duration of CR ranged from 1 to 10 months (median 4). Then 4 pts developed second ER in another sites, 1 pts had BM relapse, and 1 pts had ER in combination with BM involvement. At last follow-up 4 pts were alive including 2 pts in CR lasting 12 and 24 months. The longest CR is maintained with daily chemotherapy. Conclusions: This small study demonstrates that ERs compose about 10 % of post-transplant leukemia relapses. Current available treatment options with chemotherapy, immunotherapy and local control provide rather high rate of initial response but durable remissions are infrequent. Long-term maintain chemotherapy may be considered in this setting. Further prospective studies in a larger cohort would be required to develop optimal management of ER after allo-HSCT.

1478

THE USE OF ADJUSTED IDEAL BODY WEIGHT FOR OVERWEIGHT PATIENTS UNDERGOING HPC MOBILISATION FOR AUTOLOGOUS TRANSPLANTATION

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Background. Generally, transplant protocols dictate the use of patients' actual body weight (ABW) to calculate the required minimum number of CD34+ cells to be harvested for later hematopoietic progenitor cell (HPC) transplantation. In recent years there has been a significant increase in obesity rates in developed nations. In Australia, the 55-64 age group had the highest combined rates of overweight and obesity. This age group is overall the most common age patients proceed to autologous transplant for haematological malignancies. More overweight patients requiring larger HPC collections to meet transplant target doses per kg body weight, equates to a higher demand on apheresis, transplant and HPC storage facilities. *Aims*. We have sought an improved measure of body weight (termed transplant weight) to reach HPC targets for overweight and obese patients undergoing mobilisation and subsequent autologous transplantation.Methods: In our centre for the last 10 years, for patients weighing 25% more than their ideal body weight (IBW) (defined as 'overweight'), their adjusted ideal body weight (AIBW) has been used as their transplant weight and is calculated as follows:IBW= for males; 50 + 0.91 x (height (cm) -152) and for females; 45 + 0.91 x (height (cm)-152).AIBW = IBW + 0.25 x (actual weight - ideal weight).AIBW is then used for determination of minimum blood volume to be processed for HPC harvest of 2 x 106 CD34/kg as follows:Litres processed = 6.5 x transplant weight/CD34 per microL peripheral blood1. AIBW is also used for determination of CD34 cell dosage given at transplant for overweight patients.Results: AIBW has been used at our institution for 65/153 (42%) of patients with haematological malignancies who have had autologous HPC harvests. For patients > 25% above IBW, median ABW was 90kg (range 62-175 kg) whereas median AIBW was 69kg (range 50-110 kg). Median volume of peripheral blood processed to achieve minimum 2 x 106 CD34/kg using AIBW was 13.2 L (range 5-33 L).For patients who then proceeded to transplant, 35/82 (43%) had AIBW used to determine CD34 dosage/kg. All patients engrafted with no significant difference between the groups. Median time to neutrophil and platelet engraftment for overweight patients collected and transplanted using AIBW was 13 (range 10-24) and 15 (10-40) days respectively. For normal weight patients collected and transplanted according to their ABW, median time to neutrophil engraftment and platelet engraftment was 12 (9-25) and 14 days (9-29) respectively.Summary and Conclusions: By using an AIBW for patients more than 25% above their ideal weight, we have reduced the volume of blood to be processed and hence apheresis time required to achieve the minimum number of CD34 cells per kg body weight. Further, this has reduced cryopreservation storage space needed and dose of DMSO given at transplant. There has been no adverse effect on engraftment times for these patients and we consider this a safe and more efficient strategy for body weight estimation for overweight patients undergoing autologous transplantation.1. Trickett AE, Smith S, Kwan YL (2001) Cytotherapy 3:5-10.

1479

INTRAVENOUS BUSULFAN-CYCLOPHOSPHAMIDE AS PREPARATIVE REGIMEN BEFORE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE LYMPHOCYTIC LEUKEMIA

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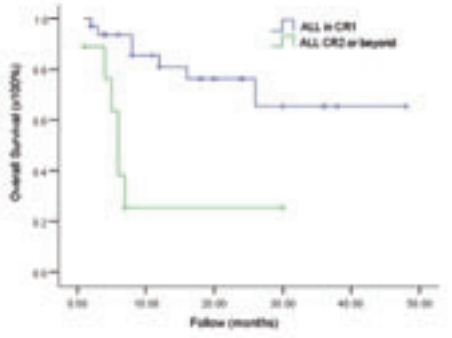
Background. Intravenous bulsulfan-cyclophosphamide (IV BU/CY) may improve the outcome of allogeneic hematopooietic stem cell transplantation (allo-HSCT) by reducing the toxicity and TRM compare to the oral BU/CY regimen. There are still limited reports of IV BU/CY regimen in acute lymphocytic leukemia (ALL). *Aims*. This study was to evaluate the efficacy and toxicity of IV BU/CY in adult ALL patients undergo allo-HSCT from HLA-matched donors. *Methods*. We retrospectively analyzed 42 consecutive patients transplanted between Jan 2007 to Oct 2010. Thirty-three patients were in first complete remission (CR1), 2 in second remission (CR2) and 7 patients in more advanced stage with median age of 28 years (range, 17~55). The median follow-up was 15 months (range, 1~48).



Results. Overall, 13 patients died with 30-month OS at 56.0±10.6% (65.3±12.5% for CR1 vs. 25.4±15.5% for CR2 or beyond, p<0.001). Eleven patients relapsed 2 to 26 months after allo-HSCT with 30-month RR at 40.0±10.9% (32.0±12.7% for CR1 vs. 72.4±17.1% for CR2 or beyond, p=0.001). Overall, only 2 cases of clinical diagnosed VOD were documented (4.7%) and one died of severe VOD. Other conditioning associated toxicities were diffuse alveolar hemorrhage (DAH) in 1 and hemorrhagic cystitis in 8. A total of 4 patients died due to transplantation related mortality (TRM) with 30-month TRM at 9.7±4.6%. *Summary*. This study demonstrated that the IV BU/CY can be considered as a feasible regimen for adult ALL with low incidence of VOD and TRM.

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PEGFILGRASTIM: SUCCESSFUL USE FOR PBSC MOBILIZATION IN ONE CENTER

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Background. The use of G-CSF is most widespread method of peripheral blood stem cells (PBSC) mobilization during past decade. This cytokine may be applied alone or in combination with chemotherapeutic agents. When pegfilgrastim became available, great interest appeared to replace multiple injections of conventional form of G-CSF by single injection of pegilated form. We took an attempt to estimate mobilizing activity of pegfilgrastim given as single dose of 18 mg following chemotherapy (CT). The aims of our research were: 1) to reveal the ability of this regimen to produce a suitable peak of CD-34+ cells in adults with cancer diseases; 2) to prove the possibility to collect after this mobilization the sufficient number of PBSC for subsequent autologous transplantation; 3) to assess side events of such mobilization approach. Methods. Seventeen consecutive cancer patients (pts) were treated from April 2010 to February 2011. They received pegfilgrastim as a single dose of 18 mg in combination with CT to mobilize autologous PBSC. There were 5 female and 12 male, age was 24 - 55 y.o (median 40). Diagnoses were lymphoma (7), multiple myeloma (3), poor-risk Hogkin lymphoma (4), ALL (2), thymoma (1). Nine pts received Cyclophosphomide (CY) 6 g per b.s.m. followed by 18 mg pegfilgrastim. Eight pts received other CT cycles followed by the same 18 mg pegfilgrastim. There were 6 heavy pretreated pts (2 in CY group, 4 in the other group). The target of harvest was to collect CD-34+ cells number sufficient for auto transplantation (not less, then 2 million per kilo b.w.). Duration of neutropenia, adverse events, time to neutrophil recovery, peak and harvest CD-34+ cells were recorded. *Results*. Fifteen of 17 pts (88%), including 5 heavy pretreated pts, had successful collection. It was collected leukocytes 32 - 100 milliard (median 70), CD-34+ cells consisted of 2,3 - 57 (median 10,6) million per kilo b.w. In all 15 pts we observed obvious peak of CD-34+ cells in PB. In pts, mobilized by CY the peak was registered on days 10 - 16 (median12), in those mobilized by other CT - on days 14 - 30 (median 19). Four of 15 pts were transplanted up today with fast and sustained engraftment. There were no considerable side events associated with mobilizing regimens. *Conclusions*. our experience demonstrates that combination CT with pegfilgrastim for PBSC mobilization is safe and effective. CY 6 g per b.s.m. followed by pegfilgrastim (single dose of 18 mg) shows high CD-34+ cell PB peaks, laid in restrict, well predictable time. Mobilized PBSC have high potency of hemopoietic reconstitution.

1481

MULTICENTER RETROSPECTIVE ANALYSIS OF HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS PBSCT FOR OVARIAN CANCER

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Background. Ovarian cancer is one of the most chemotherapy sensitive solid tumors. However, the role of dose intensity in the chemotherapy of advanced epithelial ovarian cancer has remained controversial. Accordingly, the current study attempted to analyze the outcome of high dose chemotherapy and autologous peripheral blood stem cell transplantation for ovarian cancer retrospectively. Methods. Clinical data were collected retrospectively from 6 transplant centers in Korea between January 1996 and July 2008. Results. Twenty three patients of total 6 centers were analyzed. The median age of the patients was 52 years (40-62 years). Nineteen patients (82.6%) were platinum sensitive and 4 patients were not. The conditioning regimens were reported to be ICE (ifosfamide, carboplatin, and etoposide) in 43.5 %, CTM (cyclophospamide, thiotepa, and melphalan) in 26.1 %, melphalan in 13%, other in 17.4 %. The median time to attain a neutrophil count greater than 500/mm3, following the transplantation, was 13 days (4~38), and to attain a platelet count greater than 20,000/mm3, was 13 days (4~38 days). There was primary graft failure of platelet in 3 patients. The median duration of progression free survival and overall survival were 6.3 months (95% CI, 0-19.3) and 19.7 months (95% CI, 10-29.4). The median overall survival was 25.9 months in the platinum sensitive patients and 17.4 months in the platinum resistant patients, however, there was no statistically significant difference in PFS between two groups. Poor prognostic factor for survival which retained statistical significance at multivariate level were platinum resistance (p=0.06, RR 3.99; 95% CI, 0.94-16.97) and clear cell type (p=0.015, RR 9.63; 95% CI, 1.56-59.45). *Conclusions*. Some subgroups (non clear cell types, platinum sensitive groups) of patients with ovarian cancer seem to have good outcomes after high dose chemotherapy with autologous stem cell transplantation, although several biases may have affected these observations.

1482

THE TREATMENT OF STEROID-RESISTENT GRAFT-VERSUS-HOST DISEASE WITH MULTIPOTENT MESENCHYMAL STROMAL CELLS

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Background. Acute and chronic graft-versus-host disease (GvHD) develops in more than 50% patients after hemopoietic stem cell transplantation (HSCT) and remains one of the main mortality causes. The first line of therapy is glucocorticoids, in combination with cyclosporine/tacrolimus. Different drugs are used as the second line (mycophenolate mofetil, ATG et al). But in many cases the therapy is not effective. Actually the search of new methods of GVHD treatment is very important. Now multipotent mesenchymal stromal cells (MMSC) are studied in the therapy of steroid-resistant GVHD. Aims. To research the efficacy of MMSC in the treatment of steroid-resistant GvHD. Methods. Between July 2007 and December 2010 8 patients after HSCT from HLA-identical sibling donors (7 patients), and from HLA-identical unrelated donor (1 patient) with steroid-resistant GvHD were treated with MMSC. Bone marrow derived MMSC from HLA-identical sibling donors (6 patients) and from haplo-identical family donors (2 patients) were cultured in alpha-MEM with 4% human platelet lysate. The intravenous infusion of MMSC was performed to 4 patients with acute steroid-resistant GvHD grades II-IV and to 4 patients with chronic extensive GvHD. The dose of MMSC was 0,8-1,6x106 per kg (median dose 1,1x106/kg cells). Five patients received MMSC once, two patients - twice, and 1 patient received MMSC 4 times. Most of the patients didn't have side effects during and after MMSC infusions. The effect was

estimated 30 days after MMSC infusion. The trial was approved by local ethic committee.Results. Among 4 patients with extensive chronic GvHD 2 patients didn't respond, and both of them died from infections. One patient had a clinical improvement (alive for 15 months after HCST), and one patient had a partial response on the therapy (alive 24 months after HCST). Four patients with acute steroid-resistant GvHD grade II-IV were treated with MMSC. One patient didn't respond (injection of MMSC +97 day after HCST) and died six months after HCST because of infections. One patient had clinical improvement (injection of MMSC +76 and +124 days) and one had complete response (injection of MMSC +82 day after HCST). They are alive 26 and 24 months without immunosuppressive therapy and GvHD respectively. The fourth patient received 1 infusion of MMSC for acute steroid-resistant GvHD grades II after HSCT (injection of MMSC +58 day) and complete response was achieved after this. Three months after HSCT the patient relapsed and was treated with donor lymphocyte infusion, remission was achieved with GvHD grade IV. The patient received 3 infusions of MMSC and had partial response. The patient alive 11 month after HSCT. The immunosuppressive therapy is continued. Conclusions. Good effect was observed in two cases among 4 patients with chronic extensive GvHD after MMSC infusion. In four among five cases with acute steroid-resistant GvHD injection of MMSC was effective. All of the patients who had response are alive, and patients who didn't have response died. The best results are fired in cases when MMSC were infused directly after the diagnosis of acute steroid-resistant GvHD.

1483

CARDIAC COMPLICATIONS AFTER BONE MARROW TRANSPLANTATION. A REPORT ON A SERIES OF 35 CASES IN THE DEPARTMENT OF BONE MARROW TRANSPLANTATION- TIMISOARA, ROMANIA

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Background. Cardiotoxicity is one of the non malignant complications after bone marrow transplantation and the principal clinical manifestations are: restrictive or dilated cardiomyopathy, arrhythmia, heart failure, pericarditis, hypertension, acute myocardial infarction. Aim : The purpose of this study was to estimate the frequency of the cardiac complications after BMT and of their impact on survival. Method: Cardiac complications related to bone marrow grafting were investigated in a group of 35 consecutive adults patients undergoing BMT (33 autologous, 2 allogeneic) in the transplant unit of Children's Hospital- 3rd Clinic of Pediatrics Timisoara, Romania between 2008 and 2010. We followed the next parameters: heart rate, blood pressure, ECG, echocardiography, Holter ECG, Holter for blood pressure, chest x-ray. From 35 cases - 5 patients have essential hypertension and 3 ischemic heart disease before transplant, without aggravation after transplant. *Results*. From 35 cases, 18 didn't have cardiac complications and 17 with cardiac complications; from 17 patients , 9 cases were Multiple Myeloma, 1AML, 4 Hodgkin's Disease, 1 MDS (AREB II), 2 Non-Hodgkin's Lymphoma. During the cells administration were no major cardiac complications. There were 2 cases of minimal pericarditis, 1 case of heart failure NYHA type II (EF<50%) , 5 arrythmias including 4 tahycardias and 1 ESV, 2 secondary hypertension, 7 diastolic dysfunction type I , 6 mitral regurgitation and 1 aortic regurgitation. After stem cell transplantation 9 cases developed dyslipidemia , considered one of the cardiovascular risk factor. The pregraft regimen was Melphalan, BEAM for autologous, Bu/Cy for allogeneic. Treatment before transplantation :VAD, Bortezomib+De - xamethasone for MM, R-ICE, R-CHOP, R-DHAP for NHL, ABVD, BEACOPP for HD, Cytosine-arabinoside + Anthracyclines for AML and MSD, mediastinal radiotherapy , were the factors basically responsible for the cardiac toxicity. Routine echocardiography confirmed the high incidence of subclinical cardiac abnormalities and their reversibility.The monitoring of the cardiac function is very important and the early treatment is essential; at these patients the cardiac treatment were with: beta-blocker=9 cases, ACE inhibitors=3 ,angiotensin-receptor blockers =2, statins=9, diuretics=1, nitrates=1, calcium chanel blockers=1, antiplatelet agents=1. *Conclusions*. Currently we consider that the cardiac toxicity is one of the most important limiting factors for bone marrow transplantation. We suggest, therefore, that the transplantation should be done as early as possible. In our cohort didn't appear major cardiovascular complications which required emergency cardiological intervention, only periodical follow-up. The major cardiotoxic events attributable to BMT are uncommon, comparable with another studies (in the literature occurring with a frequency of < 1-2 % after BMT) .

1484

ULTRASOUND DETECTION OF DELAYED FOCAL AND DIFFUSE LIVER DISEASES AFTER HEMATOPOIETIC STEM CELL TRANSPANTATION IN CHILDREN

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Background. During Hematopoietic Stem Cell Transplantation (HSCT) the liver is the target of many type of injures widely described in literature: toxicity related to the preparative regimen sinusoidal obstruction syndrome, hepatic acute graft-versus-host disease and acute hepatitis. In the setting of pediatric HSCT little is known about the role that HSCT may play in developing of late hepatic complications. At the state of art no guidelines exist regarding follow-up evaluation of liver status in pediatric patients after HSTC. AIMS. The aim of this study was to investigate the late effects of pediatric HSCT on the liver and to propose an adequate approach for monitoring liver status in post-transplanted children using abdominal ultrasound (US). *Methods.* Evaluation of liver status using abdominal US was performed before and after HSCT in 51 children (22 girls, 29 boys) with a mean age of 9.4 years (range 0.5 - 21). All the patients underwent HSCT (15 autologous, 36 allogeneic) at our Institution. The follow-up evaluation with abdominal US have been performed every six months. RESULTS. At a median follow-up of 28.3 months (range 2 - 91) out of 51 patients evaluated, 5 (9.8%) presented focal liver disease at abdominal US: 3 patients developed focal nodular hyperplasia (FNH) and 2 hepatic hemangioma. In 13 children instead we found the presence of diffuse liver disease (25.4%) with 10 cases of mild steatosis and 3 mild hepatomegaly. In 3 patients both steatosis and hepatomegaly have been observed. None of the patients had US hepatic disorders before HSCT. Characteristics of the patients who have developed hepatic disorders and of the HSCT procedure performed have been resumed in Table 1.

No patient experience symptoms and no peculiar laboratory findings have been observed. SonoVue-enhanced US has been performed in patients with suspected FNH and hemangioma in order to differentiate them from metastatic or malignant lesions. In 2 of the patients with FNH, magnetic resonance imaging together with hepatic biopsy have been required to confirm the diagnosis. None of these subsequent lesions presented malignant transformation. *Conclusions*. The findings of this study suggest that both focal and diffuse liver disease such as FNH, angioma, steatosis and hepatomegaly can occur in children as late hepatic complications of HSCT. Abdominal US seems to be a mandatory approach for children undergone HSCT and should be performed every 6 months. Future studies are needed to better investigate the late effects of HSCT on liver and understand how these complications are related both to the patients' characteristics and HSCT procedures.

1485

NON ABSORBABLE STEROIDS IN AUTOLOGOUS GRAFT-VERSUS-HOST DISEASE WITH GASTROINTESTINAL AND CUTANEOUS INVOLVEMENT AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION FOR NON HODGKIN`S LYMPHOMA. A CASE REPORT

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Background: Graft versus host disease (GVHD) is a potential life threatening complication tipically associated to allogeneic stem cell transplantation (AloSCT). Nonetheless, few reports in literature describe a GVHD-like syndrome occurring in patients undergoing to an autolo-

gous stem cell transplantation (AutoSCT). We discuss an autologous GVHD in a patient diagnosed with non hodgkin`s lymphoma who proceed to an AutoSCT with skin and gastrointestinal involvement. Case report: A 56 years old gentleman was diagnosed in may 2007 of diffuse large B cell lymphoma. At diagnosis, the stage was IV-B with a high IPI score. As induction chemotherapy he received 6 cycles of R-CHOP, achieving a complete remission (CR). Unfortunately, two years later he relapsed in a early stage (IIA) and was treated with 4 cycles of ESHAP achieving his second CR. Then he received an AutoSCT with BCNU 300 mgr/m^2, etoposide 800 mgr/m^2, cytarabine 1600 mgr/m2 and melphalan 140 mgr/m^2 (BEAM, total doses) as conditioning regimen. Graft composition was: total nucleated cells of 3.33x10^8/kgr compressing 6.38x10^6 Kg CD34+ cells. G-CSF was administered from d+5 to d+10. Neutrophil and platetelets engraftment occurred on d+9 and he was discharged on d+12. He received transfusions of 5 irradiated and filtered blood products (3 packed red cells and 2 platetelets units) without any adverse event during their infusion. Throughout the three months after the AutoSCT he was admitted twice (on d+20 for 16 days, and on d+92 for 10 days) because of recurring fever, diahorrea, hyporexia and weight loss of 11 kilograms, with persistent mycrobiological negativity of blood and stool samples for bacterial, viral, fungal and parasites examinations. We weren't able to identify any link between the drugs given to the patient as infection prophylaxis in the post-trasnplant period and his symptoms. However we stopped all of them with no clinical improvement. The symptoms showed a trend to spontaneusly ameliorate within the two admissions to finally outbreak again. Interestingly, a PET-TC done for residual disease monitoring on d+90 showed extensive bowel inflammation without sings of relapse. During his last admission a slight skin rash was found affecting mainly the back and the lower limbs. We performed skin and colonic byopsies and histological findings were consistent with GVHD in both of them. The patient was started on oral non absorbable steroid therapy with beclometasone 8 mgr four times in a day for two weaks, and then tapered off during two more weeks with rapid symptoms disappereance, including the skin rash. *Conclusions:* GVHD is a matter of concern in AloSCT. Few reports in the literature has described a GVHD-like syndrome in the AutoSCT setting. Some reports hypothesize a key role of certain immunosuppressant drugs given during the conditioning regimen as cyclosporine or alentuzumab. Our patient didn`t received any drug apart from the conditioning regimen itself and the standard infections prophylaxis. Interestingly the skin rash disappeared with the oral non absorbable steroids, suggesting a *trigger* role of the gastrointestinal tract in the whole picture of the GVHD as it has been highlighted in some animal models.

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THYROID DYSFUNCTION AFTER HSCT - A SINGLE CENTRE EXPERIENCE

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Background. Thyroid dysfunction after hematopoietic stem cell transplantation (HCST) is recognized as a late complication. It has been established that both imaging and serological investigation are required in order to monitor the thyroid function of patients after HSCT. Aims: to present the experience of a single center SCT Unit in long term endocrinological follow up focusing on thyroid complications. Material and method: this is a single centre retrospective study of 146 consecutive patients admitted in the HSCT Unit of Timisoara during 2005-2010. Forty-nine (33.56%) were children and 97 (66.44%) were adults. Gender distribution was 64 (44.83%) females and 82 (56.17%) males. The transplant was autologous in 118 (80.83%) patients and allogeneic in 28 (19.17%). The spectrum of diseases for which they received HSCT was: aplastic anemia 3 (2%), Hodgkin lymphoma 43 (29.4%), non-Hodgkin lymphoma 21 (14.4%), acute lymphoblastic leukemia 17 (11.7%), acute myeloblastic leukemia 12 (8.3%), chronic myeloblastic leukemia 1 (0.7%), multiple myeloma 28 (19.2%), neuroblastoma 3 (2%), primary neuro-ectodermal tumor 3 (2%), rabdomyosarcoma 5 (3.4%), Ewing's sarcoma 7 (4.8%) and nephroblastoma 3 (2%). The conditioning regimens were BEAM in 62 (42.5%), Melphalan in 29 (19.8%), Bu-Cy in 25 (17.2%), Bu-MEL in 12 (8.2%), regimens with carboplatin in 6 (4.1%), Bu-Cy-Eto 4 (2.7%) and other regimens in 8 (5.5%) patients. None of the patients received TBI. Cervical or upper-body irradiation was administered to 46 (31.5%) patients. All patients were tested before conditioning for TSH, FT3 and FT4. Patients with thyroid dysfunction prior to HSCT were excluded from this study. All patients were tested immediately after transplantation, then at 6 months intervals for TSH, FT3 and FT4 using the CMIA Abbott method. Thyroid ultrasonography was performed at 3 months intervals. Results: Thyroid dysfunction was observed in 31 patients (21.2%). The most frequent problem was represented by hypothyroidism in 19 (13%) patients. Overt hypothyroidism was discovered in 7 (4.8%) patients. All 7 had received BEAM conditioning and 6 of them were submitted to prior radiotherapy. Subclinical hypothyroidism was observed in 12 (8.2%) patients all of which had received melphalan conditioning. Only 4 had received radiotherapy. Euthyroid sick syndrome was seen in 5 (3.4%) patients and none of them had received prior radiotherapy. Seven patients (4.8%) developed hyperthyroidism in the first 0-6 months after transplantation. All 7 patients were conditioned with BEAM and 4 received prior radiotherapy. None of the patients who received conditioning with busulphan presented thyroid dysfunction. Radiation therapy has been recognized to have a key role in inducing thyroid damage. Out of the 46 patients who were submitted to radiotherapy, 14 (30.43%) developed thyroid dysfunction. *Conclusions*. The incidence of thyroid dysfunction in our center is lower than that reported in the literature, probably because most patients were monitored for less than 5 years and thyroid dysfunction is a long-term complication in HSCT patients. Long-term survivors of HSCT are at increased risk for thyroid abnormalities. This knowledge should promote efforts for regular screening to detect and treat thyroid illness early, especially in young adults transplanted during childhood or adolescence.

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BIOSIMILAR G-CSF IN POST AUTOLOGOUS STEM CELL TRANSPLANTATION SETTING: PRELIMINARY RESULTS

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Background. G-CSF is used in neutropenic phase post-high-dose chemotherapy to reduce infections and support engrafment. *Aims.* Nowadays biosimilar filgrastim products are available as Tevagrastim and data in the setting of autologous trasplantation for haematological malignancies are lacking. Methods. In our Institution from November 2010 and February 2011 15 patients underwent autologous peripheral stem cell transplantation for haematological malignancies (8 Non Hodgkin Lymphoma, 1 Hodgkin Lymphoma, 6 Multiple Myeloma). 13 patients received BEAM +/- Rituximab conditioning regimen and 2 pts high dose melphalan. The median age was 61 (range 17-76). A median dose of 4.2 (range 2.2-8.85) x 10^6 CD34(+)cells/kg was infused. On the fifth day after progenitor cells infusion, tevagrastim treatment was initiated in all patients at daily doses of 300 μg by subcutaneous injection. To assess tevagrastim efficacy, we evaluated duration of neutropenia as cut-off values neutrophil counts of 500 neutrophils/mm^3, time to platelets recovery > 20000/mm^3 and number of fever episodes during neutropenia. In addition, we select the last 15 patients with similar characteristics treated with originator G-CSF before starting with biosimilar use. *Results.* No adverse events were registered. Administration of tevagrastim resulted in neutropenia recovery in a median of 11 days (range 7-13) while platelets recovery was observed in a median of 12 days (range 7-22). Nine patients (60%) experienced febrile neutropenia resolved with endovenous broad spectrum antibiotics. No differences were reported in term of white blood cells and platelets recovery among patients receiving biosimilar G-CSF versus originator one. *Conclusions.* In our preliminary experience Tevagrastim resulted safe and effective in autologous stem cell transplantation with similar efficacy versus other registered growth factors. Considering that every patient received at least 10 days of growth factor support, the use of biosimilar G-CSF may lead to a cost reduction of about 200 €(approximately 50%).

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VIRAL INFECTIONS - STILL A CONSTANT RISK IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Viral infections remain an important cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT). *Aims.* To evaluate the frequency and type of viral infections after HSCT.

Methods: We performed a retrospective descriptive unicentric survey in Timisoara BMT Unit during 2001- 2010, on 166 consecutive transplants: autologous - 127 and allogeneic - 39 with patients mean age 28,04+/-16,81 years. Their pathological background consisted of leukemia-24,09%, malignant lymphoma-42,77%, aplastic anemia-1,8%, solid tumors-13,25% and others-18,07%. All of them had an identical supportive. A pretransplant serological screening for HIV, hepatitis, CMV, VZV, EBV and HHV was undertaken. Complex investigations were performed in febrile neutropenia, graft failure or in symptomatic patients. Results. We registered 49 episodes of documented viral infections, either of exogenous (34,69%) or endogenous (65,3%) origin. Their timing was early-7,5%, intermediate-40% and late phase 52,5%. Seven patients experienced more than one viral infection: one-CMV, polioma-BK and VZV, one- CMV and polioma-BK, one-HBV and polioma-BK and in 4 cases with HBV infection occurred also VZV reactivation. The reactivation or infection with herpesviruses families happened in 34 cases: CMV-5, VZV-28 and HHV6-1. Five patients presented CMV reactivation from which 80% before day+100. CMV appeared more often among the recipients of allogeneic grafts. None of the patients presented clinical signs but all cases were associated with elevated serum levels of hepatic enzymes and with moderate or severe neutropenia. All cases were treated with iv ganciclovir for a median of 3 months and experienced a negativation of CMV-PCR after a median of 2 weeks of therapy. All VZV reactivations/infections appeared after cessation of prophylaxis with acyclovir. 92,85% of patients were VZV-IgG seropositive before HSCT. The cutaneous presentation was: disseminated in 2 patients, extended in two dermatomes in 2 cases and limited to one dermatome in 24 patients. All cases responded to oral acyclovir treatment none of them requiring hospitalization. Post-herpetic neuralgia occurred in 25% of cases. HHV-6 was detected by PCR at day +16 in one patient who presented with severe altered functional liver tests. The evolution was fulminante to hepatic failure despite intensive antiviral treatment. We also identified adenovirus and polyoma-BK infections in 2 and respectively 4 cases. Viruses were detected by PCR in urine samples and the clinical expression was hematuria. A concerning aspect was constituted by hepatic viruses infections found in 9 cases: HBV-8 and HCV-1. In one case the HBV was a precore mutant type and in the rest of patients the HBV was wild type. None of the patients developed hepatic failure or died from HBV complications. *Conclusions*. In the structurally and functionally immunodepressed HSCT patients, viral infections continue to be a life threatening risk. There are evident limits of proven diagnosis in viral infections and a consequently high proportion of prophylactic and preemptive therapies. Early identification of infections, a specifically tailored therapy and an increased accessibility to diagnostic tools for viral diseases are only some conditions for improvement of life expectancy and increasing of cure rates.

1489

ANALYSIS OF CD31 AND CD62 DISPARITIES IN HLA-IDENTICAL SIBLING CORD BLOOD TRANSPLANTATION FOR HEMOGLOBINOPATHIES. PRELIMINARY DATA

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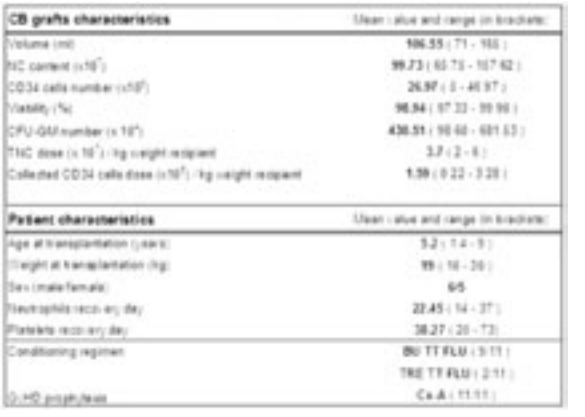
Nowadays, cord blood transplantation (CBT) represents an effective treatment for several malignant and non malignant haematological diseases. Substantial evidences have been accumulated indicating that HLA donor/recipient compatibility primarily influences the transplantation outcome. However, little is known about the extent to which minor histocompatibility antigens (mHAgs) mismatches are involved in engraftment. Compared to other stem cell sources, a CBT recipient may have a decreased probability of engraftment and a delay in neutrophil and platelet recovery. Platelet endothelial cell adhesion molecule-1 (PECAM-1, or CD31) and leukocyte endothelial adhesion molecule-1 (LECAM-1 or CD62L) are polymorphic proteins that can generate potentially immunogenic small peptides by intracellular cleavage. These molecules act as mHAgs and may activate alloreactive T lymphocytes in the transplantation setting. CD31 is expressed on several cell subsets including platelets and most leukocytes and is required for the transendothelial migration of leukocytes. CD62L acts as a 'homing receptor' for leukocytes to enter secondary lymphoid tissues, regulating leukocyte trafficking through the blood. Hence, the CD31 and CD62L mismatches should reasonably have a role in influencing neutrophil and platelet recovery times (RT). To test this hypothesis, 11 patients and their CB sibling donors were typed for the polymorphisms of CD31 (codons 125, 563 and 670) and CD62L (codons 206 and 213) genes by PCR-SSP technique using mHA Minitray kit (University Clinic Heidelberg Hospital).



CB grafts characteristics	Mean ± stv and range (in brackets)
Volume (ml)	186.55 (71 - 155)
NC content (x10⁷)	99.73 (65.75 - 167.62)
CD34 cells number (x10⁵)	26.97 (5 - 46.97)
Viability (%)	96.84 (97.33 - 99.99)
CFU-GM number (x 10⁴)	436.51 (98.60 - 681.53)
TNC dose (x 10⁷ / kg weight recipient	3.7 (2 - 6)
Collected CD34 cells dose (x10⁵) / kg weight recipient	1.39 (0.22 - 3.28)
Patient characteristics	**Mean ± stv and range (in brackets)**
Age at transplantation (years)	5.2 (1.4 - 9)
Weight at transplantation (kg)	19 (10 - 26)
Sex (male/female)	6/5
Neutrophils recovery day	22.45 (14 - 27)
Platelets recovery day	38.27 (20 - 73)
Conditioning regimen	BU TT FLU (5:11)
	TBI TT FLU (2:11)
GVHD prophylaxis	Cs A (11:11)

The CBs characteristics are shown in the table. The patients were homogeneous for age, weight, diagnosis (10 thalassemia and one sickle cell anemia), conditioning regimen and GvHD prophylaxis. Moreover, each donor/recipient pair was HLA-identical (HLA-A, B and DRB1). Regarding the GvHD direction, all couples were matched for CD31 and 10/11 couples for CD62L polymorphisms. Despite it is reported the association between CD31 and CD62L incompatibility and acute GvHD in HLA-identical transplantation, no patient included in our study developed GvHD, including the CD62L mismatched one. This may rely primarily on the naïveté of the CB immune system.In the rejection direction, we found that the mean values of platelet RT for the CD31 matched and mismatched couples were 41 and 31 days, respectively; whereas the mean values of neutrophil RT for the CD31 matched and mismatched couples were 24 and 19 days, respectively. On the contrary the mean values of neutrophil RT for the CD62L matched and mismatched couples were 21 and 37 days for codon 206, and 21 and 26 days for codon 213, respectively. Basing on our data, one can imagine that CD62L interactions with endothelial vascular cells may occur also in the recipient's bone marrow, thus providing a possible explanation for the delay in neutrophil recovery observed in the mismatched group. Anyway this hypothesis is not supported for CD31. Our data are preliminary and further studies are desirable, enrolling a larger number of patients, to understand the effective role of CD31 and CD62L polymorphisms in CBT setting. Especially since none of our patients experienced graft failure and overall the RT remained under the limits observed in the literature.

1490

EXTENSIVE FASCIITIS AS LATE MANIFESTATION OF GRAFT -VERSUS - HOST DISEASE, A PURPOSE OF A CASE

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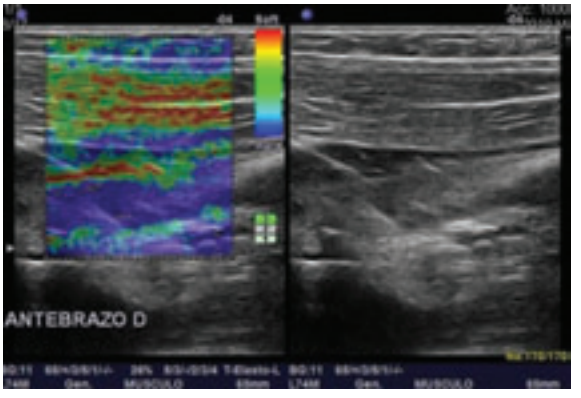
INTRODUCTION: The cGVHD is a multisystem disease that occurs from the day +100 post transplant on and generates inflammatory phenomena that evolve in tissue fibrosis. Fasciitis as a manifestation of chronic graft versus host disease (cGVHD) is rare (incidence of approximately 0.55%).

PATIENT: Female 18 years old, diagnosed with T Acute Lymphoblastic Leucemia in June 2007. Cytoreduction is induced with hydroxiurea and leukocytapheresis (2 processes), and a high-risk LAL-PETHEMA-2003 scheme treatment is established, achieving complete remission (CR) after induction and remained in CR after consolidation. Maintenance: Methotrexate and mercaptopurine. An allogeneic familiar related transplantation with identical HLA and ABO peripheral blood hematopoietic progenitors was performed as much as a GVHD prophylaxis with cyclosporine (CSA) and short course methotrexate. In the

days: +43 post-transplant dyschromia skin rash arms (virus PCR negative) appeared; +66: loss of nails; +75: increases in liver enzymes and acne; +103: From day +100 on, the patient received CSA alternating with prednisone 30 mg. On day +240 CSA treatment was deleted and maintained with steroids, starting his reduction in day +330 and its total suppression at day +480 post-transplant. In the month +19: stiffness of small joints of the fingers, elbows and knees and orange peel in the lower extremities, moreover, waxy skin and underlying tissues attached to his forearms. It is suspected as possible scleroderma plates cGVHD compatible. Skin biopsy is performed with a normal and non inflammatory infiltrate result. Due to the fact that myositis or fasciitis was suspected it was performed: MRI demonstrating an inflammatory involvement of the fascia and septa interfascial, with extension to adjacent muscle fibers, discrete subcutaneous tissue involvement. Elastography: technique based on real time ultrasounds, with colour-coded images, evaluating elasticity of soft tissue stiffness, which discarded muscle involvement (Figure 1).



The results of nerve conduction, electromyography and muscle enzymes were normal. In this way extensive fasciitis as manifestations of cGVHD is diagnosed to the patient and she is on therapy with steroids and mycophenolate mofetil combined with PUVA. Nine months after a new MRI and elastographic exam showed an improvement of lesions mainly in upper arms. *Conclusions.* : The fasciitis is a rare complication in transplanted patients. The diagnosis can be obtained by biopsy, including skin, subcutaneous tissue, fascia and muscle, or alternatively with MRI data supported by other laboratory and / or symptoms. Elastography is a novel technique, useful to assess muscle damage in these cases. It is very important early treatment but we do not know any specific one. Support measures and infectious prophylaxis to prevent deterioration of the quality of life are also needed.

1491

CAN WE USE AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION AS AN INTENSIVE CONSOLIDATION THERAPY FOR ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA PATIENTS IN REMISSSION

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Background: Despite advances in our understanding of its pathogenesis, acute myeloblastic leukemia remains difficult to treat. During the past several decades, improvements in chemotherapeutic regimens and supportive care have resulted in significant but modest progress in treating AML. Conventional chemotherapy is highly effective in the treatment of acute myeloblastic leukemia (AML). About 50-80% of adult patients with de novo acute myeloblastic leukemia achieve complete remission (CR) with currently available chemotherapy regimens consisting of antracyclines and cytarabine. Although initial complete remission can be achieved in a high percentage of patients, relapse occurs in 70-80% of the patients. Two main approaches have been the attempt to eradicate the leukemic clonal cells population via chemotherapy with or without autologous stem cell rescue or to pursue a combined approach using an antileukemic therapy combined with an antileukemic immune response via allogeneic bone marrow transplantation. Autologous transplantation compares favorably against allogeneic bone marrow transplant in several ways. Autologous transplantation can be used as a consolidation therapy in the older population, and lack of a matched donor does not preclude the patients from this treatment. Aim: To evaluate autologous hematopoetic stem cell transplantation as an intensive consolidation therapy in adults with acute myeloblastic leukemia in remission. Methods: We report a retrospective analysis on 48 patients diagnosed with de novo AML, who did not have an available histocompatible donor, and who underwent autologous transplantation between years 2000-2009 at the University hematology Clinic, Skopje, Macedonia. All patients had ECOG score 1 or less. The patient's age ranged from 17 to 65 years with the median age 41 years. There were 26 males and 22 females. For stem cell mobilization patients received chemotherapy or chemotherapy plus G-CSF. The preparative chemotherapy regimen prior to autologous transplantation consisted of BuCy in 24 patient, BEAM in 22 and BuCyMel was used in the remaining 2 patients. We used bone marrow as primary source of stem cells in 18 patients, and peripheral blood stem cells in remaining 30 patients. Results:The five years overall survival was 52% and the 5 years disease progression free survival were 42%. We analyzed sever factors that can influence the overall survival and the disease free survival such as: age, disease status, stem cell source, chemotherapy regiments prior to transplantation, conditioning regiments, number of mobilized stem cells. Advanced age and bone marrow stem cell source seems to be more influent factors. We report that the clinical results of autologous hematopoietic stem cell transplantation are sufficiently encouraging to warrant future trials that include autologous transplantation as an option for appropriately selected patients with AML in CR1. We conclude that autologous hematopoietic stem cell transplantation is a reasonable and save intensive consolidation for patients with acute myeloblastic leukemia who do not have a suitable HLA -matched donor.

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OUTCOME OF ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR ACUTE MYELOGENOUS LEUKEMIA: A SINGLE CENTER RETROSPECTIVE ANALYSIS

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Background: The selection of patients (pts) upon the best stratification and the timing of transplantation are the key issues of clinical management in AML. Aim: We evaluated the outcome of all pts allografted consecutively for AML in our BMT-unit during 1991-2009. Methods: The data of 149 pts were analyzed. Donors were siblings (107), relatives (11), unrelated (25), double cord blood (CB) (1) , haploidentical (5). Sixty-three pts aged 35 (8-63) were transplanted in CR1 after myeloablative (MA) (56) and non- myeloablative (NMA) regimen (7). Peripheral blood (PB) was the main graft source (51). Karyotype was available in 40 pts (intermediate 32, poor risk 8). Eighty-six pts were allografted beyond CR1: 42 Prim.Ref, 15 CR2, 23 Rel1 and 6 advanced (CR3; Rel2+). The majority of pts received PB (72) and MA regimen (82). Karyotype was available in 71 (favourable 4, intermediate 53, poor 14). Results: For CR1 pts OS was 63%, NRM 23%, DFS 60% and RR 21% at 13 years, whereas for 46 pts transplanted after 1999 NRM was lower (17% at 9 years). DFS for CR1 pts with unrelated donor was 47% and 62% for siblings. The outcome post NMA was poor (DFS 21% vs 65% post MA). According to cytogenetics OS and DFS were 62% and 64% for the intermediate, 44% and 45% for poor risk respectively. For CR2 pts OS was 51% and DFS 46%, RR 43% and NRM 16%. For Prim.Ref. pts OS was 20%, DFS 17% (plateau at 2 years) and NRM 34% at 12 years. Poor risk karyotype pts (7) had dismal outcome (DFS, OS 0% vs 25% and 31% respectively for the intermediate risk group) (28). For pts in REL1 OS was 15%, NRM 56%, DFS 4% and RR 86%. For the 6 pts transplanted for advanced disease OS/DFS was 17% and RR, NRM rates high. The 5 pts undergone haploidentical hematopoietic cell transplantation (HCT) had OS and DFS 40% at 8 years. In multivariate analysis significant factors associated with better outcome were early phase disease (p=0.001), lower risk karyotype (p=0.001), presence of acute graft versus host disease (GVHD) and chronic GvHD (p=0.007, p=0.006). Factors associated with lower NRM were the time of transplantation (>2000, p<0.001), bone marrow graft (p=0.035) and absence of chronic GvHD (p=0.004). Conclusions: According to our experience, allogeneic-HCT for AML in early phase seems to have the potential to cure a significant proportion of pts with low NRM, even in the alternative setting, with improving results during the late time frame. In contrast, a small proportion of refractory pts may be rescued by HCT, specifically in the intermediate cytogenetic risk group.

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IMPROVED OUTCOME IN PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA POST HIGH DOSE CHEMOTHERAPY WITH THE COMBINATION OF BCNU, CYTARABINE, MELPHALAN AND AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION

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Background: Autologous hematopoietic cell transplantation for selected patients (pts) with acute myelogenous leukemia is a viable and reliable option of therapy, even in the era of alternative or reduced intensity transplants. The main advantage of the procedure is very low toxicity and treatment related mortality rate (TRM). Aim: We evaluated the outcome of pts autografted for AML in CR1 in our BMT-unit. Methods: In this retrospective study we analyzed the data of 51 pts, aged 37 (12-54) years with de novo AML (49) and secondary AML (2) auto-transplanted in CR1. The conditioning regimens used were: a)BEAM modified (marrow harvest on day -3, infusion of BCNU day -3, Etoposide and Cytarabine day -2, Melphalan day -1) and fresh marrow graft (20), b)BUCY-2 (10), c)BUCY-4 (6) and d)BCNU-AraC-Mel (BCNU 300 mg/m^2 day -3, Cytarabine 3 gr/m^2/12 hours day -2, Melphalan 140 mg/m^2 day -1) (15) with cryopreserved graft either marrow (14) or peripheral blood (PB) (17). The PB graft was mobilized post high dose Etoposide (1,6gr/m²) and has been used in the recent period 2000-2009. Results: For the whole cohort of 51 pts auto-transplanted form 1987-2009 the probability of DFS and OS was 28% and 33% respectively with a Δm follow-up 15 (9-20) years and TRM 6%. In terms of the time of transplant for the early period (1987-1999) the DFS rate was 27%, relapse rate 57% and TRM 10% at 21 years. On the contrary, the OS and DFS of pts who received the BAM regimen and mobilized PB as graft during the late period 2000-2009 was 53% and 83% respectively, while the TRM was 0% at 8 years. Interestingly, pts with intermediate risk group cytogenetic (17), mostly normal karyotype, succeeded rates of OS 82% and DFS 57% at 8 years. Summary/conclusions: The challenge of refinements of clinical management of patients with AML is to identify individual patients likely to benefit from specific therapeutic modalities, such as autologous hematopoietic cell transplantation, as an intensified consolidation treatment with zero morality rate. It can be employed to maintain the remission and cure the disease. Better supportive care has enhanced the ability of nearly each AML patient to deliver high dose chemotherapy plus autologous rescue with mobilized PB graft. Moreover, comparison of various used conditioning regimens demonstrated a DFS survival advantage of BAM (49%) over the others, ie modified BEAM (32%) or BUCY-2, BUCY-4 (31%) at 7, 21 and 18 years respectively.

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SUCCESFUL TRANSFUSION-FREE ALLOGENEIC STEM CELL TRANSPLANTATION IN A JEHOVA`S WITNESS PATIENT. A CASE REPORT

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Background: Allogeneic stem cell transplantation (AloSCT) is a curative approach for patients with relapsed acute lymphoblastic leukemia (ALL). Less intensive modalities of the conditioning regimens have arisen to minimize the risks associated with the procedure. We discuss a patient with a relapsed ALL who proceded to a nonmyeloablative AloSCT from her HLA matched sibling without any transfusion support as a consequence of her denomination as Jehovah's Witnesses. Case report: A 35 years old lady was diagnosed with pre-B ALL in july 2007 without adverse cytogenetics. She refused any transfusion support during the chemotherapy treatment, which led to a lesser intensive induction chemotherapy mainly to minimize the risk of bleeding by thrombocytopenia albeit a higher risk of relapse. After induction chemotherapy with vincristine, daunorubicine and prednisone she achieved a complete remission (CR) and was started in maintenance therapy (MTT) for two years. On march 2010 she relapsed and was retreated with the same induction chemotherapy with a minimal residual disease (MRD) of 2% by flow cytometry in the bone marrow samples. Thereafter she received new MTT until august 2010 when she overt relapsed again. Rescue chemotherapy with vinblastine, cytarabine and VP-16 was administered achieving CR again with negative MRD. On October 2010 she underwent a nonmyeloablative AloSCT from a matched sibling. We adopted three strategies to prevent severe cytopenias during the AloSCT: firstly we administered a reduced intensity conditioning with total doses of 6.4 mgr/kg iv busulfan, 1000 mgr/m2 iv Cyclofosfamide and 90 mgr/m2 iv fludarabine. Secondly, we performed a larger than usual graft infusion of mobilized peripheral blood stem cell CD34+ cell from her donor (total dose of 7.75x106 /kgr) by three consecutives collection dates. The collected cells were infused "fresh"at the evening of the same collection day. Total CD3 infused cells were 54x106/kgr. Finally we used three different human recombinants growth factors to improve the engraftment: erythropoietin 10.000 UI three times a week from one month before the AloSCT, G-CSF 300 µgr daily from d+6 to d+13 and romiplostim 5 µgr/kgr 2 doses on d+5 and d+10. Neutrophil engraftment occurred on d+13. Platelet engraftment occurred on d+14. No significant bleeding occurred (platelet nadir of 31x103/ul on d+12). She was discharged on d+14 and has remained as an outpatient uptodate. No one single blood product has been infused to the patient since her diagnosis of ALL. Currently she`s alive in CR on d+125, with mixed chimerism and on treatment for grade II graft versus host disease. Conclusion: Transfusion-free stem cell trasnplantation has been described in isolated cases in the literature. New avalaible second generations thrombopoietic growth factors like romiplostin, allows a safer approach to this procedure. We conclude that transfusion-free AloSCT is feasible.

1495

LEVOFLOXACIN FOR BK VIRUS-ASSOCIATED HEMORRHAGIC CYSTITIS IN THE POST-HEMATOPOIETIC STEM CELL TRANSPLANTATION PERIOD

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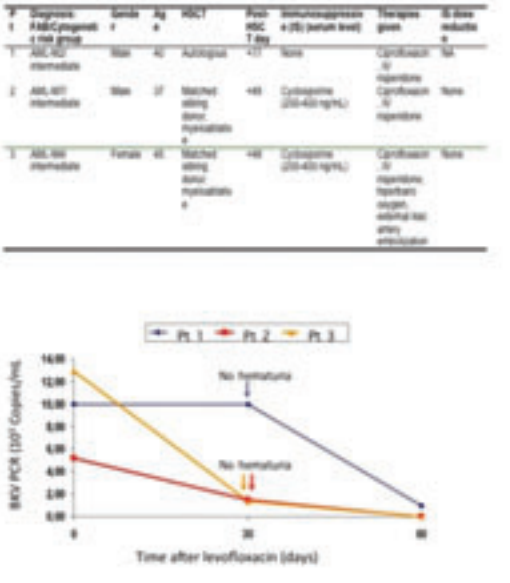
Background: BK virus (BKV) is one of the most common causative agents of hemorrhagic cystitis (HC) in the early postengraftment period of hematopoietic stem cell transplantation (HSCT). Cidofovir may be a potentially effective treatment option with a 67% complete response rate. Unfortunately, it is not available in most European countries. High cost rate and possible renal side effects are the other important disadvantages of Cidofovir. In vitro activity of fluoroquinolones has long been known. But their in vivo effects are limited. There are no data about the efficacy of respiratory fluoroquinolones such as levofloxacin. Aim: We present here the clinical and molecular activity of levofloxacin in three patients with BKV-related HC experienced during postengraftment period following HSCT. *Methods:* Patients' chart records were reviewed retrospectively.



Results: Data about the three patients who experienced severe HC within the first 100 days of HSCT were reviewed (Table). All patients needed continuous intravesical irrigation because of gross hematuria and urinary obstruction caused by blood clots. Routine microscopic analyses and bacterial cultures revealed no specific pathogenic organism. Analyses for adenovirus, cytomegalovirus, mycoplasma, and ureaplas-

ma were all negative. Patients were receiving immunosuppressive agents in appropriate doses. None of the patients had any clinical sign of graft versus host disease. But BKV was detected in the urine of patients in varying degrees. All patients received ciprofloxacin at least two weeks and intravesical risperidone. Additionally, hyperbaric oxygen and subsequently external iliac artery embolization were performed in patient 3. But no improvement was observed. Patient 1 received levofloxacin, 500 mg/d, and his HC resolved completely in one week. The other two patients also received the same treatment and complete clinical response was achieved in both. Levofloxacin treatment was given for 8 weeks and urine BKV copies were monitored. BKV copies in urine decreased more than 90% in all (Figure). These patients are still disease- and GVHD-free and alive. Levofloxacin was given to another patient who had HC not related to BK within the 100 days of post-HSCT period. But no response was observed. *Conclusions*: Levofloxacin may be an effective treatment option in patients with refractory BKV-related HC experienced during post-HSCT period. Efficacy of levofloxacin may be associated with the inhibition of DNA topoisomerase IV and DNA gyrase. Some other factors specific to levofloxacin may have an effect on BKV.

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LARGE VOLUME LEUCAPHERESIS (LVL). FOR PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) COLLECTION IN HEALTHY DONORS FOR ALLOGENEIC TRANSPLANTATION: EXPERIENCE OF A SINGLE CENTER

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Introduction: There are no defined guidelines to improve the efficiency of collections of peripheral blood cells in allogeneic transplant programmes. We present our experience using Large Volume Leucapheresis (defined as more than 3 times the total blood volume) for collection of PBPC in healthy donors, applied in our centre with the aim of simplification of procedure. *Methods and Donors:* 124 healthy donors (66 males and 58 females; median age 48, 19-73) were included in this study since November 2001 to December 2010. All donors received 12-14 g/kg/day s.c of rhG-CSF (filgrastrim®, Amgen, Thousand Oaks, CA, USA) in two doses during 4 days for mobilization. Our main objective was to yield at least 3x10e6/kg receptor weigth of CD 34+ cells. Leucapheresis was started on the fifth day after the administration of rhG-CSF. Large Volume Leucapheresis (LVL) was programmed and cells were collected through peripheral vein access in most cases, using a Cobe Spectra separator (COBE SPECTRA, Gambro BCT, Lakewood, CO, USA). Intraprocess controls were performed to finish the procedure according the objectives and results. *Results:* The median number of patient's blood volumes processed was 3 L (1-4). 113 donors (92%) required only one session to achieved the CD34+ cells objective. PBPC collection yield a median of 6.02x10e6/kg CD 34+ cells (2.85-13.57). No mobilization failure was observed. All products were transplanted with rapid and sustained engraftment in all cases. No serious adverse effects were observed and minor morbidity related to the PBPC collection was scarce and reversible. *Conclusions.* In our experience Large Volume Leucapheresis (LVL) allows an adequate PBPC collection for transplantation with the simplification of a single harvesting procedure valid for prompt hematological engraftment.

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METHYLATION STATUS OF RUNX2, OSX, DLX5 AND BSP GENE P ROMOTERS IN OSTEOBLASTIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS (MSCS)

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Background. Epigenetic mechanisms, including DNA methylation, histone modifications, and non-coding RNA-mediated regulatory events, are essential to controlling the heritable cellular memory of gene expression during differentiation. DNA methylation is modulating gene expression by addition of a methyl moiety to the cytosine-5 position in CpG islands. Two specific osteoblast transcription factors and one non-specific transcription factor i.e. RUNX2, OSX and DLX5 are important in regulating osteoblast related genes as well as osteocalcin, BSP, OPN and collagen type Iα1. *Aims*. In this research we speculated whether epigenetic regulation of these genes plays any role in osteoblastic differentiation of mesenchymal stem cells. Therefore, we evaluated methylation status of RUNX2, OSX, DLX5 and BSP promoters in osteoblastic differentiation of MSCs. *Materials and Methods:* MSCs were isolated from human bone marrow (a written informed consent was obtained from all participants). Osteogenic differentiation was done under the influence of the synthetic glucocorticoid dexamethasone, beta glycerol phosphate, and ascorbate in the presence of 10% FCS. DNA and RNA extraction were carried out after the first, second and third weeks of culture and also from undifferentiated MSCs. After DNA extraction and bisulfate treatment, gene specific methylation analysis for RUNX2, OSX, DLX5 and BSP was carried out using methylation specific PCR (MSP). Moreover, qualitative RT-PCR was used to study the gene expression. Results: MSP analysis revealed that promoter methylation status didn't change in RUNX2, DLX5 and BSP promoters during osteoblastic differentiation of MSCs. In contrast, OSX promoter showed a dynamic change of methylation pattern while MSCs were gradually differentiated to osteoblasts. *Conclusions:* RUNX2, OSX, DLX5 and BSP promoter regions showed 3 different methylation patterns during MSCs differentiation. This study shed light on the osteoblastic differentiation of MSCs by showing dynamism in methylation change during this process.

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HEMATOLOGIC ABNORMALITIES AND ETIOLOGICAL FACTORS OF OCCUPATIONAL TOXIC CYTOPENIAS, ABOUT 138 CASES

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Background: innovations in the fields of industry and research and the proliferation of applications of chemistry explain the growth of the use of chemicals to which humans are exposed both in the workplace than in domestic. Exposure to these xenobiotics may be responsible for a blood-toxicity. *Aims:* We are interested in studying abnormal peripheral blood findings of toxic origin in the work environment and factors in the workplace. Methods: This is a retrospective study over a period of 5 years (January 2004-December 2008) on cases of cytopenia collected from patients with professional activity, followed by the hematology department of university hospital Hedi Chaker, Sfax, Tunisia and professional investigation which was conducted by the department of occupational medicine at the same hospital. *Results:* A total of 5611 patient records identified the number of patients with suspected toxic cytopenia is 138. The annual recruitment of hematology department of Sfax is 77 cases of presumed toxic origin cytopenia year. The prevalence is estimated at 7%. A male predominance is noted with a sex ratio of 1.76. The average age is 40 years. Nearly a third of the population belongs to the age of 25 to 34 years. A monocytopenia is found in 70% of cases with leukopenia 40% of cases, thrombocytopenia 27% and 3% of anemia cases. The bone marrow examination was performed in only 34 patients. This is normal marrow in 33 cases, poor in 6 cases and dysplastic in 4 cases Our patients perform in diverse industries, manufacturing is the leader with a rate of 49% followed by the services sector with a rate of 32% then 10% agriculture, building industry and public works 5% , 4% heavy industry. Three quarters of patients are workers. Professionals in agriculture are the most represented .Organic solvents, mainly benzene are the class of nuisance most reported in our series. *Conclusions:* In our series and in literature, the main industries using benzene are the chemical industries of plastic, mechanical petrochemicals. in the literature, very few data are available about the incidence of cytopenias toxic. Male predominance and young age of the patients are found both in our work in literature.

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SELF-RENEWAL ABILITY OF MARKED BY LENTIVIRAL VECTOR MESENCHYMAL STEM CELLS

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Background. Stem cells have to be able to differentiate and self-renew. Mesenchymal stem cells (MSCs) form hematopoietic microenvironment in vitro in long-term bone marrow culture (LTBMC), while in vivo this ability could be analyzed by implantation of femur bone marrow plug or adherent cell layer (ACL) from LTBMC under renal capsule of syngeneic recipients. Stromal cells in such foci are derived from donor MSCs while hematopoietic cells have recipient's origin. The size of the foci formed is proportional to femur equivalent transplanted and can be used for semi-quantitative determination of MSC number. The golden stan-

dard for stem cells is their ability to self-renew which could be proved by retransplantation. After the retransplantation all differentiated stromal cells in the hematopoietic foci are lost and the microenvironment in newly formed foci develops from transplanted MSCs completely de novo. Aims. The aim of the study was to investigate the self-renewal ability of marked murine MSCs able to transfer hematopoietic microenvironment. Methods. ACLs of 2-week-old LTBMCs were infected with 100 mkl of concentrated (108 viral particles/ml) self-inactivating HIV LeGO vector encoding EGFP (all plasmids and Phoenix cells were provided by Prof. Boris Fehse) in 3 ml of aMEM with 10% FCS and 8 mkg/ml polybrene for 6 hours. In 2 weeks after the infection scrapped by rubber policemen ACLs were implanted under the renal capsule of syngeneic mice. Colony-forming units fibroblast (CFU-Fs) were analyzed by plating 20000 nucleated cells from the foci per well of 96-well plate in aMEM supplemented with 20% FCS and 5 ng/ml FGF2. In 6 weeks the number of marked CFU-Fs was measured in 4 foci formed and other foci were retransplanted under the renal capsule of secondary recipients. The procedures were repeated 4 times and the numbers of marked CFU-Fs were measured in 4 foci after each retransplantation. Results. The proportion of marked cells in ACLs before transplantation under the renal capsule was 5.3 ± 0.6 %. The size of the foci formed was stable throughout all retrasplantations, the marked CFU-Fs were revealed in all foci (table). All ossicles in the foci formed were EGFP positive.

Table. The size of the ectopic foci and the proportion of marked CFUs derived from the foci.

The transplantation number	The size of the foci formed, x10^6	The proportion of marked CFU-Fs, %
1	3.8 ± 0.6	3.8 ± 2.4
2	3.1 ± 0.7	5.6 ± 2.2
3	4.1 ± 0.9	6.3 ± 2.9
4	3.4 ± 0.7	8.1 ± 3.4

Conclusions. As CFU-Fs are the progeny of MSCs, the availability of genetically marked CFU-Fs in the foci points to the presence of marked MSCs. Obviously marked MSCs are able to transfer hematopoietic microenvironment at least 5 times and keep the ability to produce the considerable number of marked progeny, so their ability to self-renew is proved. Thus, all crucial MSCs features (prolonged lifespan, ability to self-renew and differentiate) are not affected by stable integration of the vector containing gene of interest meaning that these cells are preferable targets for gene therapy.

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INCREASED LEVELS OF BONE MARROW ENDOTHELIAL CELLS, PROGENITOR AND MATURE, IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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The role of endothelial progenitors (EPCs) and mature cells (ECs) in angiogenesis has attained the scientific interest of many recent studies. *Aim:* The immunophenotypic analysis of EC subpopulations in the bone marrow (BM) and the study of their potential role in the pathogenesis of various malignant and autoimmune diseases. Methods: Bone marrow cells from children with acute lymphoblastic leukemia at diagnosis (ALLd, n=8), at day 15 (ALL15d, n=5), and day 33 of treatment when remission is achieved (ALL33d, n=7), ALL under consolidation therapy (ALLct, n=10), ALL following the end of treatment (ALLet, n=10), solid tumors without BM involvement at diagnosis (ST, n=9), idiopathic thrombocytopenic purpura (ITP, n=4) and autoimmune diseases (AI, n=5) were studied. The putative antigenic phenotypes of EPCs and ECs were assessed using a 4-color flow cytometry procedure in both the CD45neg and CD45neg and dim cell subpopulation. *Results:* The highest levels of CD45negCD133+CD34+VEGFR-2+ EPCs were estimated in ALLd with statistically significant differences compared with ALLet (0.015±0.112 vs 0.0059±0.0025, p=0.016), and also with ITP (0.015±0.112 vs 0.00, p=0.034) and AI (0.015±0.112 vs 0.0013±0.0013, p=0.05). The levels of CD45negCD146+CD34+VEGFR-2+ are high following the end of treatment in the ALL group compared to the group of ST (p=0.036). CD45negCD31+CD34+VEGFR-2+ subpopulation is estimated to be equally high in ST, ALLd and ALL15d. Statistical analysis revealed significant differences between ALLd vs ALL33d (p=0.024), ALL15d vs ALL33d (p=0.003), ALL15d vs AI (p=0.046) and ALL33d vs ALLet (p=0.035). The highest levels of CD45negCD31+VEGFR-2+, CD45negCD34+VEGFR-2+ and CD45negCD133+VEGFR-2+, were determined in ALLd. The comparison of ALLd with ALL33d revealed statistically significant differences for all the three different immunophenotypic combinations (p=0.07, p=0.019, p=0.012 respectively) as well as with ALLet (p-0.016, p=0.009, p=0.007 respectively). ALLd compared with ALLct showed statistical differences for CD45negCD31+VEGFR-2+ (p=0.005) and for CD45negCD133+VEGFR-2+ (p=0.027). CD45neg CD146+VEGFR-2+ levels were significantly higher in ALLd vs ALL15d (p=0.048), ALLd vs ALL33d (p=0.004) and ALLd vs ALLct (p=0.032). The same tendency occurred for the comparison between ALLd and other childhood diseases of immunological origin studied (ITP, AI). In the CD45neg/dim cell subpopulation the CD133+CD34+VEGFR-2+ phenotype was significantly higher in ALLd vs ALL33d (p=0.006) and ALLd vs ST (p=0.038). In the above CD45neg/dim subpopulation the CD31+CD34+VEGFR-2+ cells were higher in ST and following the end of treatment in ALL(ALLet). *Conclusions.* The levels of both progenitor and more mature EC subpopulations seem to be highest in diagnosis of ALL among all the group of blood and autoimmune diseases studied. The more mature EC subpopulation tend to be higher following the end of treatment in ALL and solid tumors without BM involvement. The precise role of these findings warrant further investigation.

1501

PHENOTYPICAL AND FUNCTIONAL CHARACTERIZATION OF MESENCHYMAL STEM CELLS DERIVED FROM PATIENTS AFFECTED BY SCHWACHMAN-DIAMOND SYNDROME

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Background. Shwachman-Diamond Syndrome (SDS) is an inherited marrow failure disorder characterized by varying cytopenias, pancreatic dysfunction, and metaphyseal dysostosis. Neutropenia plays a crucial role in the occurrence of recurrent and severe infectious complications representing one of the major causes of death in SDS patients. Aims: the aim of our study is to better comprehend the marrow dysfunction occurring in SDS patients, by analyzing the functional properties of bone marrow (BM)-derived mesenchymal stem cells (MSCs). Methods: after informed consent, BM cells obtained from 20 SDS patients were plated in sterile tissue culture flasks. At the third passage of the culture, cells were tested for the expression of specific surface markers, their ability to differentiate into mesengenic lineages, their capability to abrogate T cell proliferation and their capacity to prevent neutrophil apoptosis. *Results*. MSCs derived from SDS patients (SDS-MSCs) displayed typical fibroblastoid morphology; they were consistently devoid of contaminating hematopoietic cells, being negative for CD34, CD45, HLA-DR, CD11b, CD19, and CD14, but expressed common MSC markers including CD90, CD73, CD105 and HLA-ABC. Similarly to MSCs obtained from healthy donors (HD-MSCs), these cells were able to differentiate into adipocytes and osteoblasts. In addition, SDS-MSCs drastically decreased the mitogen-induced lymphocyte proliferation, in a dose dependent manner. We also cultured neutrophils obtained from HD in presence or absence of MSCs at different time points. We demonstrated that SDS-MSCs were comparable to HD-MSCs in supporting the viability of neutrophils. Importantly, SDS-MSC were able to produce high amount of IL-6 (mean= 2658 pg/ml, range= 2086-3229 pg/ml), a crucial cytokine involved in the protection of neutrophils from apoptosis. Conclusions: we successfully isolated and characterized MSCs from SDS patients. These cells did not show any significant differences from HD-MSCs. Further studies are needed to better comprehend the functional and molecular features of SDS-MSCs, which are potentially involved in the hematological abnormalities typical of SDS patients.

1502

EXPRESSION CLONING OF A REPROGRAMMING ACTIVITY THAT INDUCES BONE MARROW ADHERENT MYOFIBROBLASTS TOWARD HEMATOPOIETIC STEM CELLS: INTERLEUKIN 1? PROMOTES THE EXPRESSION OF CD34 AND CYTOKINE RECEPTORS

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Background and Aims: We recently reported that acute myelogenous leukemia blasts and chronic myelogenous leukemia cells can convert to stromal myofibroblasts to create an environment for the proliferation of leukemic cells in vitro and also in vivo in the immunodeficiency murine bone marrow. In normal hematopoiesis, hematopoietic cells are also speculated to contribute to the formation of the hematopoietic stromal tissue. And we also hypothesized that bone marrow stromal myofibroblasts may convert to hematopoietic cells. To make clear this issue, bone marrow stromal myofibroblasts from informed healthy volunteers were cultured with phytohemagglutinin (PHA-P)-stimulated lymphocyte conditioned medium in vitro. After one week morphological changes were observed microscopically, in which bubbling from the spindle-shaped myofibroblast was demonstrated, and a few cells were detached from the dishes and were floating. To determine the mechanism of this biological finding, expression cloning was performed. *Materials and method*s. Blood was collected from the informed healthy volunteers, and lymphocyte-rich fraction was separated with gravity sedimentation method. PHA-P was added in the cultures, and after 48 hours lymphocytes were collected, with which cDNA library was constructed after ligation to the mammalian expression vector. Electroporation to Ecoli DH10β cells made 5x105 independent colonies, which were divided into sub-pools (1000 independent colonies/pool). Plasmids were prepared from each pool and transfected to COS7 cells with DEAE-Dextran method. After 3 days of culture supernatants were collected, and were cryopreserved until use. Bone marrow cells were aspirated from informed healthy individuals, and were separated to mononuclear cells. Adherent cells were prepared after 2-hour cultures in the coated dishes. Cells were further cultured long term with splitting using trypsin/EDTA once a week. After one month, bone marrow-derived STRO-1(+) and smooth muscle actin (+) myofibroblasts were prepared, which were used as a target for the expression cloning. The transfected COS7 supernatants were added in the myofibroblast-cultures (final 10%), and cells were further cultured for one week. RNA was extracted from the cultured cells, and cDNA was synthesized. Positive clones were selected with reverse transcription-polymerase chain reaction using human CD34 primers, and further selected to be a single clone. *Results and discussion:* Isolated single clone was revealed to be human interleukin 1β. When the purified interleukin 1β protein was added in the myofibroblast cultures, cell growth was increased, and up-regulation of the expression of several hematopoietic cytokine receptors including c-kit was observed. Now we determine the precise actions of human interleukin 1β on the reprogramming of bone marrow stroma-derived myofibroblasts toward hematopoietic stem cells.

1503

BONE MARROW MESENCHYMAL STROMAL CELLS PROMOTE PROLIFERATION OF LYMPHOMA CELLS AND GASTRIC CANCER CELLS VIA CELL-TYPE SPECIFIC MANNER

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Background: Bone-marrow derived mesenchymal stromal cell (bmMSC), one of major sources of MSC, has been known to participate for reconstituting microenvironment. Cancer cells, as like normal cells, have been known to need microenvironmental support for their survival and growth. Studies show that MSCs were recruited and integrated into tumors, however, what is the precise effect of MSC on tumor cells still remains unclear. Aims: To address this, investigators employed co-culture and mice co-transplantation assay with hematopoietic tissue-derived bmMSC and adipose tissue-derived MSC (ADSC), and hematologic and malignancies (lymphoma cells and gastric cancer cells). Methods: Lymphoma cells (Pfeiffer and Raji) and gastric cancer cells (SNU5FU and SNUADR) were cultured in RPMI 1640 with basic supplements. BmMSCs and ADSCs were cultured in DMEM with basic supplements. Transwell system (BD falcon, USA, 0.4um pore) was used for indirect contact co-culture. To assay apoptosis-protection effect of MSCs, 5-fluorouracil (5ug/ml) and doxorubicin (5ug/ml) were treated for lymphoma cells and gastric cancer cells, respectively. Viable cells were assayed by trypan blue method or flowcytometry with propidium iodide and Annexin V. Cancer cells (2x105) only or mixed with MSC (5x104) were transplanted into NOG/SCID mice (13-15 week-old) subcutaneously. Results: BmMSC increased the proliferation of Pfeiffer cells by direct contact (x3.5), but not by indirect contact compared with Pfeiffer alone at day 4. BmMSC also promoted the proliferation of SNU5FU cells by both direct (x1.9) and indirect (x1.6) contact at day 6. Whereas, Raji cells and SNUADR cells were minimally influenced. BmMSC-cocultured Pfeiffer showed increased cell viability (16.7%) compared with Pfeiffer alone (9.8%) under the doxorubicin treatment. For the SNU5FU cell under 5-fluorouracil treatment, two different bmMSCs didn't show chemoprotective effect, but ADSC-cocultured cells showed increased chemoresistance (84.1%) compared with SNU5FU alone (46.7%). In NOG/SCID mice cotransplantation assay, SNU5FU alone, SNU5FU-bmMSC and SNU5FU-ADSC cotransplanted mice showed 43%(3/7), 33%(1/3) and 75%(3/4) tumor formation, respectively. SNU5FU alone induced tumor and SNU5FU-bmMSC induced tumor showed compact tumor mass without desmoplasia, whereas SNU5FU-ADSC tumor showed marked stromal reaction, infiltrative growth and lung metastasis. Summary/Conclusions: BmMSC showed growth promoting effect on Pfeiffer and SNU5FU, but not on Raji. BmMSC showed chemoprotective effect on Pfeiffer cells, but not on SNU5FU cells. ADSC, but not bmMSC, showed in-vitro chemoprotection and in-vivo tumor promoting effect on SNU5FU. These findings suggest that the MSC type, the targeted tumor cell type and environmental condition should be considered for understanding the effect of MSC on tumor cells.

1504

POOR CLINICAL OUTCOMES IN NON TRANSFUSED PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

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Introduction. PNH is a debilitating and life-threatening hematopoietic stem cell disorder characterized by chronic hemolysis leading to significant morbidities, end organ damage and mortality in PNH patients. Historically, PNH was considered an anemic disease with management focused on improving hemoglobin (Hb) levels through transfusions. This has led to the perception by some that absence of transfusion requirements or mild anemia as a measure of mild or stable disease.Aims. To understand the impact of transfusions on the risk factors associated with poor patient outcome, we retrospectively analyzed medical charts of 123 PNH patients with no documentation of transfusions from national data registry in South Korea over the last 41 years. Results Patient ages ranged from 17 to 88 years (median 37 years), median PNH duration was 6.8 years (14 days to 27 years), and median PNH granulocyte clone size was 40% and median LDH at diagnosis was 4 fold above normal. Evaluating hemolytic symptoms associated with end organ damage, we found that approximately 12% of patients with no documentation of transfusions had late stage renal dysfunction (defined as history of renal failure or GFR <60 ml/min/1.73*m^2), a predictor of early mortality. Gastrointestinal pain, a symptom predictive of thrombosis and poor quality of life, was reported in 38% of non-transfused patients. Dyspnea, a symptom of pulmonary hypertension and predictive of thrombosis was reported in 28% of patients. Elevated hemolysis at diagnosis (as measured by LDH ≥1.5 above normal) is predictive of early mortality. We evaluated non-transfused patients with LDH ≥1.5 above normal at diagnosis (n=70) or LDH < 1.5 of normal (n=20) for patient death. There were 6 patient deaths reported (median 7.3 yrs disease duration) in the hemolytic non-transfused group compared to no reported deaths (median 4.6 yrs disease duration) in non-transfused patients with no elevated hemolysis at diagnosis. Conclusion: Our data demonstrates that patients who are not transfused suffer from significant disease burden. Elevated hemolysis leads to poor outcomes in PNH patients and should be the target for PNH management.

1505

TWO CASES OF LANGERHANS HISTOCYTOSIS X AND THEIR THERAPEUTIC APPROACH

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Background. Langerhans cell histiocytosis results from the abnormal accumulation of a class of dendritic cells normally found in the skin, which proliferate in many organ systems along with lymphocytes, macrophages and eosinophils. There is multisystem disease involvement described with lytic skull lesions, involvement of the skin and lymph nodes. Aims:To present the rare disease of Langerhans histiocytosis. Methods:Two clinical cases of the rare histiocytosis X cases are reported along with the therapeutic management. Results:A 24-year old woman presented with a 3-month history of headache localised to the right posterior parietal area. The patient reported concurrent migraines and vomiting. A brain MRI revealed a non-enhancing semicircular mass 1,3x0,9 cm of parietal bone with no pressure effects on the nearby structures, meninges and soft tissues. A whole body bone scan was free of disease. Biopsy of the osteolytic lesion revealed lesion similar to Langerhans histiocytosis with eosinophilic granulomatous origin based on positive immunochemistry for S-100/Langerin and CD1a (-/+). The histiocytes had medium size with nuclear grooves. The patient had normal lung function, and a negative for disease thorax CT. The abdomen CT was normal with no lymph nodes enlarged. Therapy included complete surgical excision and replacement of the area with bone transplant. She received prednisone 1mg/kg/day for 4 weeks and tapering over a 2-week period followed with a weekly infusion of vinblastine 6mg/m2 for 5 weeks. Then, the same dose of prednisone was administered (5 days/week) followed by vinblastine once every three weeks to complete a six month period. 6-mercaptopurine 30mg/m2 was not administered due to desire of future pregnancy. She was previously diagnosed with systemic lupus erythematosus under medication with hydroxychloroquinone 200 mg/day from June to September every year. The second patient a 45-year old female reported to our clinic after a painless palpable mass of the right submandibular area. An ultrasound at the area revealed a multilobular, compact mass 1,9X1,5 cm. The neck CT confirmed the mass as bilobular with central erosion. Later, it was totally excised and sent for biopsy. The biopsy was positive Langerhans cells (S-100+,Langerin+,KP1+,CD30+,CD20-,CD3). Near the atypic cells eosinophils were present with nuclear grooves. Last there was a great number of mast cells (mast-cell-tryptase+). The bone marrow biopsy was negative. An abdomen ultrasound showed increased spleen(10cm) and a liver mass. The thorax CT showed a few lymph nodes(<1 cm), an abdomen CT revealed a benign liver mass, negative for disease after biopsy. An abdomen MRI showed the liver mass sub-diaphragmatically 4,6X3,8cm with differential diagnosis problem arising between focal hyperplasia and adenoma. A bone-scan was negative for disease. Both patients had normal thyroid function tests and negative testing for thyroid autoimmune abnormalities. No treatment was followed after excision of primary site and she was put under follow-up. The 45 year-old woman had positive serum antithyroglobulin antibodies. Virology for Herpes Simplex Viruses-1,-2 were negative(IgM-). Conclusion:These two cases of Langerhans cell histiocytosis highlight the difficulty of disease diagnosis and its multifocal presentation. A different approach in the management of the disease is described according to patient profile.

1506

HUMAN CHRONIC MYELOID LEUKEMIA HEMATOPOIETIC CELLS DO NOT TRANSDIFFERENTIATE INTO NEURAL LINEAGES

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Introduction. Adult hematopoietic stem cells (HSC) are able to survive in the CNS of animal models and to *transdifferentiate* into neural cells upon exposure to signals generated by CNS tissue injury (*Bonilla et al, 2002*). This fact has been difficult to prove when studying human HSC in animal models due to the lack of a good marker to follow the fate of the transplanted cells. Chronic myeloid leukemia (CML) is a stem cell disorder characterized by immune-scape and resistance to apoptosis due to an acquired chromosomal translocation, the t(9;22). This translocation generate a specific BCR-ABL chimeric gene that may be identified by Fluorescence in situ hybridization (FISH), and could facilitate tracking the fate of CML human cells in xenogeneic transplant experiments. *Methods:* We isolated CD34+ positive cells from two untreated chronic phase CML patients by Macs immunomagnetic selection. These cells were infused in the white matter of the parietal hemisphere of six adult Swiss mice, six neonates and six NOD.Cg-Prkdc scid Il2rgtm 1Wjl/SzJ immunosuppressed mice. Mice were sacrificed at 7, 15 and 30 days. LSI t(9;22) BCR/ABL Dual Fusion Dual Color Translocation Probe (Vysis) was used to perform the FISH. Neural differentiation was checked by colocalization of the FISH signal with antibetaIII-tubulin and antiGFAP by immunofluorescence. *Results:* The mice received 1 to 2x105 CD34 selected cells. No cells with the bcr-abl translocation could be observed at 7 day post-transplant in either the Swiss or the NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ mice. In the 6 neonatal transplanted mice scarce bcr-abl positive cells could be observed at day seven, but not thereafter. We did not observe colocalization of the bcr-abl signal with betaIII-tubulin or antiGFAP. *Conclusions*: Human CML hematopoietic stem cells are not able to survive in the CNS of either normal mice or naturally or artificially immunosuppressed mice. CML cells are not a good candidate to study the HSC neural transdifferentiation potential.

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1507

This abstract has been withdrawn.

1508

FAS AND FAS LIGAND EXPRESSION IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA, BENIGN HEMATOLOGICAL DISEASES AND SOLID TUMORS

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Fas/FasL is a key pathway of cellular apoptosis. Fas receptor is expressed on membranes of both normal and neoplastic cells while, Fas ligand (FasL) mainly on activated T-lymphocytes. Fas-FasL abnormalities have been detected in malignancies and autoimmune diseases and implicated in resistance to treatment. The aim of the study was the determination of the levels of Fas, FasL and their coexpression in bone marrow cells of children with acute lymphoblastic leukaemia (ALL) at diagnosis, during the course of treatment and the comparison with relevant levels in other hematological and neoplastic diseases. Methods: Expression levels of Fas and FasL were determined with flow cytometry in children with ALL at diagnosis (ALLd, n=13), on day 15 of treatment (d15, n=6), on day33 (ALLd33, n=6), during consolidation (ALLhr, n=12) and at the end of therapy (ALLet, n=7) as well as in children with Langerhans Histiocytosis (LCH, n=4), cytopenias (Cytp, n=8) and solid tumors without bone marrow involvement at diagnosis (STd, n=5) and on therapy (STther, n=6). Results: The lowest levels of Fas expression were detected at diagnosis of ALL and were gradually statistical significantly increased until remission of day 33 (ALLd vs ALLd33: 8.02±1.94 vs 24.04±6.11, p=0.035). At consolidation Fas levels were found to be decreased compared to day33 (16.1±4.18) and were again increased at the end of therapy (ALLd vs ALLet: 8.02±1.94 vs 24.96±7.95, p=0.024). On the contrary, FasL levels were gradually decreased and finally increased to similar to diagnosis levels at the end of treatment (ALLd: 4.59±1.41, ALLet: 5.89±1.99). In solid tumors at diagnosis Fas levels were similar to the ones while on chemotherapy (STd vs STth:16.04±2.2 vs 15.19±6.4). The highest FasL levels were detected in the group of STd with the relevant levels on treatment being lower in comparison (STd vs STther: 10.91±3.32 vs 2.92±0.79, p=0.052). In LCH both Fas and FasL levels were found to be as low as at ALL diagnosis. In cytopenias no significant difference was observed between groups for either Fas (11.05±4.49) or FasL (3.01±0.62). As for Fas+FasL+ coexpression no difference was evident between ALLd, ALLet and STd or STther [ALLd (0.73±0.38), ALLet (0.64±0.17), STd (0.68±0.095), STther(0.66±0.38)]. The lowest coexpreesion levels were observed in the group of cytopenias with statistical significant difference compared to STd (0.68±0.095 vs 0.26±0.08, p=0.031). In conclusion, at diagnosis of ALL Fas levels were expressed in lowest levels that were found to be gradually increased at remission and at the end of treatment. This finding probably correlates with the apoptotic process of the leukemia clone and possibly with response to treatment.

1509

THE ONCOGENE EVI1 ENHANCES TRANSCRIPTIONAL AND PROLIFERATIVE RESPONSES TO ATRA

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Background. EVI1 is an oncogene whose overexpression is associated with a poor prognosis in myeloid leukemias and certain solid tumors. Even though its protein product exhibits properties of a transcription factor, only few direct target genes have been identified. Several reports suggest that EVI1 may elicit its biological effects through modulation of the activity of other transcription factors. In this context we have recently shown that EVI1 affected all-trans retinoic acid (ATRA) dependent gene regulation in that it enhanced the ATRA induction of the RARbeta gene and counteracted its own upregulation by ATRA. Aims: In the present work we asked whether EVI1 would modulate the ATRA regulation of additional genes and whether it would also affect biological responses elicited by ATRA. *Methods*. Triplicate cultures of U937 cells with or without ectopic expression of EVI1 were incubated with ATRA or solvent for 24 h. RNA was extracted and subjected to microarray analyses (Affymetrix HG-U133 Plus 2.0). Candidate genes were confirmed by qRT-PCR. Cell cycle analysis was performed by FACS after propidium iodide staining. Results: Array analyses revealed that the transcriptional response to ATRA of 44 unique genes was modulated by EVI1 at least twofold and in a statistically significant manner. The ATRA induction of 34 and the ATRA repression of seven genes was enhanced by EVI1, while ATRA repression of three genes was counteracted by EVI1. Eight genes were selected for confirmation by qRT-PCR. The regulatory pattern observed in the array experiments was confirmed in all cases. To determine whether EVI1 would also enhance biological responses to ATRA, cell cycle analyses were performed. Upon treatment with ATRA a higher percentage of EVI1 overexpressing cells than of empty vector containing cells accumulated in the G0/G1 phase of the cell cycle, demonstrating that indeed EVI1 enhances biological responses elicited by ATRA. *Summary/Conclusions*. EVI1 enhances the transcriptional and growth inhibitory responses of ATRA in human myeloid cells. It will be interesting to investigate whether AML patients overexpressing EVI1 are responsive to differentiation therapy.

1510

PIPKII-ALFA REGULATES THE ALFA AND GAMA GLOBINS EXPRESSION IN HEMATOPOETIC-DERIVED CELLS

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Background. Phosphatidylinositol phosphate kinases (PIPK) belong to a family of enzymes that generate various lipid messengers. The PIPK subfamilies are divided into type I (α, β and γ), type II (α, β and γ) and type III. In a recent study in our laboratory, the PIPKIIα gene was differentially expressed in reticulocytes from 2 siblings with hemoglobin (Hb) H disease. Expressions of both the PIPKIIα and β-globin genes were higher in the patient with the higher Hb H level, suggesting a possible relationship between PIPKIIα and the production of globins. However, the role of PIPK proteins in hematopoietic process has been rarely directly addressed. Aims: To evaluate the PIPKIIα expression in hematopoetic-devided cells lines and to investigate the effects of PIPKIIα silencing in K562 cells on alfa and gama globin expression and on proliferation, cell cycle, differentiation and apoptosis. Methods: K562, KG1, NB4, HL60 and P39 cell lines were used. Specific siRNA-expressing vector targeting the PIPKIIα gene or no specific sequence were electroporated in the K562 cell line. Cells expressing no specific sequence or parental cells were used such as control. Quantitative PCR (qPCR) and Western blot analysis were performed to determine the expression of PIPKIIα, alfa and gama globin. β-actin and GAPDH were used as control in qPCR and actin in Western blot. After 48 hours of culture, proliferation was analyzed by MTT assays, apoptosis by Anexin-V and propidium iodide (PI), cell cycle by incubation with PI and RNase A buffer and flow cytometry. After 15 days of silencing the differentiation was evaluated by glycophorin A and transferin fluorescence intensity and percentage of double positive cells. Imatinib was used as control in the arrest of proliferation and inductor of apoptosis in K562 cells. Results: qPCR and Wester blot showed that PIPKIIα was express in all the cell lines tested and was observed a slight increase of PIPKIIα expression in K562 cells when compared with other cells included in this study. The levels of PIPKIIα mRNA and PIPKIIα protein in knockdown cells were significantly reduced by 80% and 75%, respectively ($P<0.05$). MTT assays showed that the proliferation was slight reduced by 10% in PIPKIIα knockdown cells when compared with control cells ($P<0.05$). Cell cycle, apoptosis and differentiation analysis showed no difference in PIPKIIα knockdown when compared with control cells. Interestingly, PIPKIIα silencing resulted in a significant increase in alfa and gama globin expression compared to control cells, as observed by qPCR and Western blot ($P>0.05$). Conclusions: PIPKIIα is express in hematopoetic-derived cells and PIPKIIα silencing results in slight decrease in proliferation and an increase of alfa and gama expression. Our findings show that in K562 cells there may be, at least in vitro, a regulatory mechanism that acts on α and γ genes in response to the reduction in PIPKIIα gene expression.

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1511

MULTIVARIATE ANALYSIS REVEALS RELATIONSHIPS AMONG PROMOTERS OF HAEMATOLOGICAL INTEREST

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Background. Human promoters tend to have a characteristic consensus sequence although variation about the consensus sequence has received little attention. We have used multivariate analysis to define groups of promoters with similar sequences, taking account of the correlations amongst them. Correlations have been addressed in the region of the transcription initiation site and upstream in the region of the TATA site. *Aims.* We have sought to analyse numerically coded sequences so as to group the promoters systematically and reveal the relationships amongst them. Methods: The DNA sequences of all 1975 promoters experimentally confirmed in humans were extracted from the Eukaryotic Promoter Database; each sequence was 60 bases long. A base was coded by two binary digits: GC vs. AT and purine vs. pyrimidine. A principal component analysis of the resulting 120 variables was carried out. Most of the eigenvalues showed a regular downward trend, consistent with random sampling. However, the first five eigenvalues were greater than expected from the trend. The corresponding eigenvectors were used to calculate principal components, dimensions across which the promoters were distributed and by which they were classified. Results: The consensus sequence was found to be ggggg gg(c/g)cg (c/g)g(c/g)(c/g)g gg(c/g)gg g(g/t)aaa ggggg ggggg gc(c/g)cg ggggg cgcca ttgcg g(c/g)cgg. The first principal component distinguished promoters which were particularly GC rich. The twenty promoters with the highest values for the first principal component contained 87% GC and only 13% AT, while the twenty with the lowest principal component were 32% GC and as much as 68% AT. The promoters with the largest positive value of the first component included APLP1_1 (amyloid β A4 precursor-like protein 1), BCL2_1 (B-cell leukemia/lymphoma 2 proto-oncogene) and TGFB1I1 (transforming growth factor β 1 induced transcript 1). In contrast, the promoters with the largest negative values included those of the genes for interferon α 4, 6, 7, 16 and 17, interleukin 2 and 4, and albumin. *Conclusions*. The first principal component sorts promoters according to sequence features, and separates promoters of different functional groups. Similarly, the second, third and further principal components could be used to distinguish further groups of promoters. This multivariate approach accounts in a systematic way for the variation of the promoter sequences about the consensus sequence and the correlations amongst them.

1512

TYROSINE KINASE INHIBITORS MODULATE THE APOPTOMIRS: MIR-16, MIR-21, MIR-30E, MIR-145, MIR-142-3P, MIR-LET-7D, MIR-LET-7E AND MIR-15A EXPRESSION IN BCR-ABL-POSITIVE CELL

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Background. The oncogene bcr-abl codifies the BCR-ABL tyrosine-kinase which is responsible for cell malignant transformation and apoptosis resistance in Chronic Myeloid Leukemia (CML). CML patients are usually treated with imatinib mesylate (IM), a tyrosine kinase inhibitor (TKI). Unfortunately, resistance to TKI emerges, especially in CML patients in advanced. Thus, others molecules have been investigated as potential secondary targets for CML therapy. In this context, the present study investigates the microRNAs, which have target genes involved in apoptosis regulation in Bcr-Abl+ AIM: To quantify the microRNAs let-7d, let-7e, miR-15a, miR-16, miR-21, miR-26a, miR-30e, miR-142-3p and miR-145 in HL-60.Bcr-Abl treated with imatinib mesylate (IM), dasatinib (DAS) and nilotinib (NL). Methods. HL-60.Bcr-Abl+ was obtained by the HL-60 infection with the recombinant retrovirus (ψ2 + PA 317) containing the pSR MSV p185bcr-abl tkneo plasmid. The HL-60.Bcr-Abl

cells were cultured in complete RPMI medium with 10 mili molar of TKIs during four or eight hours. After this period, RNA from cell lines were extracted, RNA were reverse transcripted and real time PCR were performed to quantify miRNAs expression. The results of miRNAs expression were given as fold change (fc) between HL-60.Bcr-Abl+ treated with TKIs and untreated HL-60.Bcr-Abl. Results.M downregulates miR-16 (fc=0.73), miR-21(fc=0.53), miR-30e (fc=0.89) and miR-145 (fc=0.28) expression after four hours of treatment in HL-60.Bcr-Abl. In contrast, DAS upregulates miR-21 (fc=11.71) and miR-142-3p (fc= 7.12). The levels of miR-let-7d decreased (fc=0.01) while let-7e (fc=2.89), miR-15a (fc=3.97) and miR145 (fc=11.97) increased in HL-60.Bcr-Abl after eight hours of NL treatment. CONCLUSION: TKIs are capable of modulating the microRNAs miR-16, miR-21, miR-30e, miR-145, miR-142-3p, miR-let-7d, miR-let-7e and miR-15a, which are involved in apoptotic machinery regulation in BCR-ABL+ cells. Thus, it seems that miRNAs expression profile may contribute to TKI response and suggest the potential of miRNAs as a new marker of CML prognosis.

1513

RELATIONSHIP BETWEEN MCV/MCH AND SEVERITY OF βGLOBIN GENE MUTATIONS IN β-THALASSEMIA CARRIERS

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Thalassemia as a heterogeneous disease is one of the most common single gene disorder with a worldwide distribution. The aim of this study was finding a relationship between blood indexes and severity of beta globins gene mutations in beta-thalassemia carriers. In this cross-sectional study, we were determined 30 beta goblin gene mutations in 1206 unrelated beta thalassemia carriers. Furthermore their blood indexes, including CBC and electrophoresis were also prepared. Then, by using SPSS software and t-test, the relationship between genetic findings and the results of their blood parameters were analyzed. In this study, the relationship between the severity of beta goblin gene (β+/β○), in beta thalassemia carriers, and their average blood indexes, were evaluated. The results indicated that β+ thalassemia in comparison with β○ thalassemia had a lower mean MCV and MCH value. That means with less time and expense it could be possible to find a statistically significant relationship between a specific ranges of blood indexes and type of mutations in beta thalassemia carriers. The results confirmed a significant correlation with blood indexes and certain type of mutations in beta thalassemia carriers.

1514

HEMATOLOGIC DISORDERS CAUSING DIAGNOSTIC PROBLEMS IN DIABETES MELLITUS

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Aims. Investigation of diagnostic problems caused by hemoglobinopathies in diabetes mellitus, comparision of different chromatographic methods to determine glycated hemoglobin (HbA1c) in certain types of diabetic patients, following blood samples after in vitro glycation. *Methods*. Glycated haemoglobin was determined by 5 chromatographic methods, including HPLC. The subjects were 2480 patients from Romania and 150 from Canada (including 27 persons with hemoglobinopathies). Statistical analysis of the experimental data was performed using the GraphPad InStat program. *Results*. The results of different chromatographic methods showed positive correlation (r>0,9, p<0,05), but elevated HbF was found in 1,8% of the patients from Romania, and HbF can be co-eluted with the HbA1c fraction in case of some chromatographic methods. Higher HbA1c values could be observed in type 1 diabetic patients compared to type 2 (p<0,001), and in subjects from rural areas compared to those from urban environment (p<0,05). Following in vitro glycation in blood samples from patients presenting HbS, Hb E, HbD, Hb Camden and Hb G Coushatta reveals new peaks on the chromatogram that could represent glycated fractions of these pathological hemoglobin variants. Conclusions: The presence of hemoglobinopathies could disturb the proper diagnosis and following of diabetic patients because of their interference with the HbA1c fraction. In some homozygotes HbA1c can be absent on the chromatogram, and another problem is caused by the glycated fractions of pathologic hemoglobin variants which can appear as unknown peaks on the chromatogram. Special attention is required in case of type 1 diabetic teenagers especially from rural areas, to prevent complications of the disease.

1515

DERIVATIVE (6)T(1;6)(Q21;P21): A RECURRENT CYTOGENETIC ABNORMALITY IN TWO CASES OF PEDIATRIC THERAPY-RELATED MYELODYSPLASTIC SYNDROME

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Childhood myelodysplastic syndromes (MDSs) are a rare group of clonal hematopoietic stem cell disorders occurring de novo or secondary to cytotoxic chemotherapy and/or radiotherapy for a previous malignancy. Cytogenetic aberrations have been detected in about 50% of de novo MDS and in almost all therapy-related MDS (t-MDS) patients. The most common abnormalities are complete or partial loss of chromosome 7 and trisomy 8. Unbalanced rearrangements involving the long arm of chromosome 1 and leading to its trisomy, usually as t(1;7), are also recurrent. We report two cases of pediatric t-MDS with der(6)t(1;6)(q21;p21). This unbalanced translocation has been reported in the literature associated with adult chronic myeloproliferative disorders. The first patient developed refractory cytopenia 9 years after suspension of chemo- and radiotherapy for a medulloblastoma diagnosed at the age of 7 months. The second patient, a 6-year-old boy, is slowly developing MDS features after a diagnosis of neuroblastoma 4S at age 1 month and of anaplastic lymphoma 10 months later. Cytogenetic analysis of peripheral blood and bone marrow blasts of both patients revealed der(6)t(1;6)(q21;p21). FISH for the painting of chromosomes 1 and 6 confirmed the rearrangement. Further array-CGH analysis performed on the bone marrow of the second patient, using 5500 BAC clones, showed gain of material of chromosome 1 (q21.1[ARROWRIGHT]q44) and chromosome 6 (p22.1[ARROWRIGHT]p12.1) and loss of material of chromosome 6 (p25.3[ARROWRIGHT]p22.1). Array-CGH analysis is being carried out in the other patient to verify whether the imbalance is identical.

1516

CYTOGENETIC FINDINGS IN *DE NOVO* ACUTE MYELOID LEUKEMIA: A STUDY BASED ON 553 PATIENTS IN A SINGLE INSTITUTION OF GREECE

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Background. Acute myeloid leukemia (AML) is a heterogeneous disease with regard to clinical features and acquired genetic alterations. Currently, cytogenetic aberrations detected at diagnosis constitute the most common basis for predicting clinical outcome. *Aims*. We performed a conventional cytogenetic study of 553 *de novo* AML patients in order to define the chromosomal abnormalities and their frequencies as well as the frequencies of AML subtypes according to FAB classification. *Methods*. Chromosome studies were performed on unstimulated bone marrow cells, derived from 553 AML patients, aged ≥16 years at the time of diagnosis, between 2006 and 2010. Patients were classified according to FAB classification. *Results*. Three hundred ten patients were male and 243 were female. The median age of patients was 55,1 years (range 16-88). FAB classification was available in 294 patients with M0 in 5.4%, M1 in 7.8%, M2 in 21.4%, M3 in 26.9%, M4 in 23.1%, M5 in 11.2%, M6 in 3.4% and M7 in 0.7%. Karyotypic analysis was successful in 529 patients (95.7%). Normal karyotypes were found in 208 patients (39.3%) and abnormal in 321 (60.7%). Among the abnormal karyotypes 32.7% were complex (≥3 chromosome aberrations) and 48.6% had only one aberration. Chromosome aberrations found in abnormal karyotypes were: +8 in 20.2%, t(15;17)(q22;q11-12) in 12.1%, -7 in 9.7%, -Y in 7.5%, del(7q) in 6.9%, -17 in 6.9%, inv(16)(p13q22) in 6.2%, del(5q) in 5.6%, -5, t(8;21)(q22;q22), translocations of 11q and -18 in 5% each, +21 in 4.1%, +22 in 3.7%, del(9q) in 3.1%, del(11q) in 3.1%, abnormalities in 12p in 3.1%, -X in 3.1%, t(9;22) in 2.8%, +11 in 2.8%, +13 in 2.8% and chromosome-markers in 14.3%. The most frequent sole abnormalities found in karyotypes with only one chromosome change were t(15;17)(q22;q11-12) in 18.6%, +8 in 15.4%, inv(16) in 6.4%, -7 in 3.8%, translocations of 11q in 3.8%, t(9;22) in 3.2%, -Y in 3.2%, del(7q) in 2.6%, t(8;21) in 2.6%, del(11q) in 2.6%, +21 in 1.9%, del(20q) in 1.3% and tetrasomy 8 in 1.3%. The most common abnormalities found in complex karyotypes were +8

in 23%, -7 in 21%, -17 in 19%, del(5q) in 18.1%, -5 in 15.2%, -18 in 15.2%, del(7q) in 13.3%, -16 in 10.5%, +22 in 10.5%, -13 in 9.5%, -15 in 9.5%, -21 in 9.5%, -Y in 8.6%, t(8;21) in 7.6%, -20 in 6.7%, +20 in 6.7%, +21 in 6.7%, del(11q) in 5.7%, inv(16) in 5.7%, +11 in 4.8%, t(15;17) in 3.8% and chromosome-markers in 45.7%. Summary/Conclusions: AML was slightly more common in men than women. Interestingly, the most common chromosome abnormality in total abnormal karyotypes and complex karyotypes was +8, with a median age of 59.4 years, while t(15;17) was the most common sole aberration. Marker chromosomes, del(5q) and monosomies are mainly restricted to complex karyotypes with the exception of monosomy 7. The main FAB subtypes showed a distribution different to that reported in the literature, with a higher incidence of M3 followed by M4 and M2.

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A CONVENTIONAL AND MOLECULAR CYTOGENETIC STUDY IN A LARGE SERIES OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: In Chronic lymphocytic leukemia (CLL) karyotypic data have been scarce due to the low mitotic activity of B-CLL cells in vitro. Nowadays, the improvement of cultivation techniques and fluorescence in situ hybridization (FISH) studies give an opportunity to a much more heterogeneous pattern of genetic abnormalities to be revealed. The prognostic significance of these aberrations may be used for a novel, more accurate classification system for CLL patients. AIM: The aim of this study was to define the chromosomal abnormalities and their frequencies among Greek CLL patients, between 1998 and 2010, using conventional and molecular cytogenetic methods. Methods: Conventional cytogenetics were performed on unstimulated and stimulated initially with tetradecanoyl phorbol acetate (TPA) and more recently with the oligonucleotide DSP30 plus IL-2 bone marrow cells, derived from 505 patients, aged 16-88 years. Fluorescence in situ hybridization (FISH) studies were accomplished in 60 patients using the commercial CLL set probes LSI p53/LSI ATM και LSI D13S319/LSI 13q34/CEP12 Multi-Color Probe Sets. Results: Three hundred and thirty seven patients were male and 168 were female. The median age was 64.75 years. Karyotypic analysis was successful in 473 patients (93.7%). Normal karyotypes were found in 58% and abnormal in 42% of patients. Among the abnormal karyotypes 27.3% exhibited complex karyotypes and 51.5% carried only one aberration. The frequencies of chromosome aberrations in abnormal karyotypes were the following: +12 in 29.3%, -Y in 15.7%, abnormal (abn) 17 in 13.1%, abn14q in 10.6%, del(13q) in 9.6%, del(6q) in 6.6%, -X in 5.6 %, numerical abnormalities of chromosome 8 in 5.6 %, abn18 in 5.6%, del(11q) in 5%, translocations of chromosome 11 other than t(11;14) in 4.5%, del(7q), translocations of chromosome 18 and abn20 in 3.5% each, t(11;14) in 2%, and chromosome markers in 13.1%. The most common abnormalities found in karyotypes as sole aberrations were +12 in 27.7%, -Y in 16.8%, del(13q) in 5.9%, add(14q) in 4.9%, del(11q) in 4%, del(6q) and -X in 3.3%. In complex karyotypes, chromosome markers were found in 42.6%, abnX in 20.4%, -17 and del(6q) in 16.7% each. FISH analysis using the specific probes mentioned above was successful in all 60 patients while genetic aberrations were detected in 70% of them. The frequencies of these aberrations were: del(13)(q14.3) in 46.7%, del(13)(q34.3) in 3.3%, del(17)(p13.1) (p53) in 16.7%, trisomy 12 in 15% and del(11)(q22.3) (ATM) in 10% of patients. Summary/Conclusions: The sex ratio was 2M/1F. The combination of DSP-30 plus IL-2 was able to identify more clonal abnormalities than TPA. Trisomy 12 was the most common abnormality found in karyotypes (29.3%) followed by -Y (15.7%), while del(13)(q14) was the commonest aberration (46.7%) detected by FISH. Abnormalities of chromosome X, monosomy 17 and del(6q) were mainly found in complex karyotypes with high incidence. The different frequencies of chromosomal aberrations found in conventional and molecular cytogenetics, mainly due to the restricted number of probes that can be used in FISH and the disability of karyotype to identify submicroscopic rearrangements, depict the necessity of their parallel application for CLL investigation.

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ATYPICAL DELETED SEGMENTS IN THREE PATIENTS WITH MYELOID MALIGNANCIES AND DELETION 5Q

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Background. Deletion of the long arm of chromosome 5 is a recurrent cytogenetic abnormality frequently found in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Patients with isolated del(5q) have a more favorable prognosis than those with additional karyotypic defects. Several groups of investigators have defined common deleted regions (CDRs), predicted to contain tumor suppressor genes. The proximal CDR in 5q31.2 (970 kb) was localized both in MDS, AML and therapy-related MDS/AML, while the distal CDR in 5q32-q33.1 (1.5 Mb) is involved in the pathogenesis of 5q- syndrome. Aims. The aim of this study was to exactly determine the breakpoints and size of unusual deleted segments in three MDS/AML patients with 5q deletion and to evaluate the role of genes localized in this region for the initiation or progression of the disease. Methods: During the years 1993 - 2010 we found deletion 5q as a sole aberration in bone marrow of 126 patients with MDS/AML (sex ratio M/F was 28/98, median 68 years). FISH with locus probe Vysis LSI EGR1/D5S23, D5S721 Dual Color Probe (Abbott Molecular) and ON MDS 5q- (5q31, 5q33) / hTERT (5p15) TC (Kreatech Diagnostics) were done in all of them. To precisely determine the breakpoints, FISH with BAC clones (BlueGnome, 5q14.1-5q35.1 regions), multicolor banding (mBAND) for chromosome 5 (XCyte 5, MetaSystems) and array comparative genomic hybridization (aCGH, CytoChip Focus Haematology, BlueGnome) were performed. Results: In 119 patients (94.5%) deletion 5q was large involving both proximal and distal CDRs, in four patients (3.0%) deletion of only distal CDR was confirmed. However, in three patients (2.5%) loss of the material on the long arm of chromosome 5 was outside of both CDRs - results of FISH with LSI probes for 5q31/5q33 regions did not prove the deletion. In patient No.1, a 61-year-old female with diagnosis AML, deletion 5q14q21 was confirmed in a small clone (4 mitoses out of 30 examined). In patient No. 2, a 66-year-old female with MDS-MPS, deletion del(5)(q14q23.3) was found in all examined mitoses. The same range of the deletion, del(5)(q14q23.3) was determined in patient No. 3, a 73-year-old male with RCMD. The exact breakpoints of deletions were confirmed by combination of molecular cytogenetic techniques. Patient No. 2 died of acute heart failure 18 month after diagnosis, patients No. 1 and No. 3 live in complete remission. Conclusions: Out of 126 patients with myeloid malignancies and deletion 5q, we proved atypical extent of the deletion in three of them: proximal breakpoints were localized in 5q14 in all three patients, distal in 5q21 (patient No. 1) and 5q23.2, respectively (patients No. 2 and No. 3) with retained 5q31.2 and 5q32-q33.1 bands. To our knowledge, deletion of 5q14-5q23.2 as a sole aberration has not been described so far. Deleted region encompasses approximately 140 genes (*http://www.ncbi.nlm.nih.gov/mapview/*) and we assume that not only genes harbored in conventional CDRs but also genes localized in 5q14-5q23.2 region may contribute to malignant evolution of myeloid diseases.

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INTERPHASE FISH ON PURIFIED PLASMA CELLS IS SUPERIOR TO FISH ON CULTURED BONE MARROW CELLS IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Multiple myeloma 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). In multiple myeloma karyotyping is hampered by the low proliferative index of plasma cells and, therefore, in only about 30% of the patients an abnormal karyotype is found. Interphase fluorescence in situ hybridization (FISH) can circumvent this problem, but its resolving power is hampered by the overall low percentage of plasma cells present in bone marrow samples. Aims. To evaluate the diagnostic potential of FISH on purified plasma cells as compared to FISH on cultured bone marrow cells. Methods. We have developed and optimized a protocol for the purification of CD138+ cells (plasma cells) from bone marrow samples for FISH applications. Interphase FISH results obtained from such purified plasma cells were compared to those obtained from cultured bone marrow cells using an extended probe panel according to the recommendations of the Dutch Working group on Hemato-oncologic Genome Diagnostics (WHGD). This panel allows the detection of IGH rearrangements, including t(4;14),

t(11;14) and t(14;16), 1p loss, 1q gain, 13q loss, 17p loss, and hyperdiploidy. Results. Our pilot study on 51 patient samples showed that clonal genetic abnormalities can be detected in 98% of the patients when purified plasma cells were used. In 34% of these patients the genetic abnormalities were not identified when FISH was applied on cultured bone marrow cultures. Furthermore, FISH on purified plasma cells resulted in the identification of higher percentages of genetic abnormal cells, thus allowing the detection of genetic sub-clones. Summary/Conclusions. We conclude that interphase FISH on purified plasma cells is superior to interphase FISH on cultured bone marrow cells for the detection of prognostic relevant genetic abnormalities in multiple myeloma.

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TRUE MONOSOMY OF CHROMOSOME 5 IS PRESUMABLY NOT AN ISOLATED CYTOGENETIC ENTITY IN MDS

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Background. Various aberrations of chromosome 5 are common cytogenetic findings in bone marrow cells of patients with MDS. The most frequent is interstitial deletion del(5q) occurring as sole chromosome abnormality or in combination with additional chromosomal changes. The size and breakpoints of deleted segment differs among patients, however the region 5q31 is commonly deleted in most of them. Two CDRs have been identified in 5q, indicating the presence of critical genes within these loci. Monosomy 5 is found by conventional cytogenetic methods in about 3-8% MDS cases only, usually in combination with other aberrations. *Aims*. The aim of this study was to perform detailed genome wide analysis in series of newly diagnosed patients with MDS and monosomy 5 detected by conventional cytogenetics, and to assess real frequency of true monosomy 5 in primary MDS. Methods: Fixed bone marrow cells of 46 MDS patients with suspected monosomy 5 found by G-banding (32 males, 14 females; median age 63 years) were retrospectively analyzed. To detect deletion 5q31 interphase FISH (I-FISH) with Vysis LSI EGR1/D5S23, D5S721 Dual Color probe (Abbott Molecular) was used. Complex aberrations were analyzed by mFISH/mBAND (MetaSystems). For identification of genomic imbalances BAC-based arrays (CytoChip Focus Hematology, BlueGnome) and/or SNP arrays (HumanCytoSNP-12 BeadChips, Illumina) were applied. Results: All 46 patients with suspected monosomy 5 presented a complex karyotype (≥3 chromosomal aberrations). Deletion of 5q31 was detected in 45 of them (97,8%) and loss of both regions (5p15 and 5q31) just in one by I-FISH. Whole genome molecular cytogenetic analyses confirmed that in all cases, parts of chromosome 5 material remains retained. No patient with true monosomy 5 was identified in this study. Common region conserved in all patients was established at 5p11.1-p14.2 [22.31Mb]. mFISH/mBAND revealed cryptic translocations and insertions of chromosome 5 material to several chromosomal partners (chromosomes 17, 3, 7 and 12 as the most frequent ones). Finding of complex karyotypes involving deleted chromosome 5 at diagnosis was connected with particularly poor overall survival (median 3 months). *Summary/Conclusions*. In all patients with suspected monosomy, the parts of the deleted chromosome 5 have been shown to be retained elsewhere in the karyotype. Therefore, we believe that the true monosomy 5, quoted in the literature, in bone marrow cells of MDS patients probably does not exist. We assume that 5q deletion is arising in myeloid precursor cells as the primary event and subsequently leads to chromosome instability and higher susceptibility to the rearrangements. This process is resulting in increased genomic damage and fast disease progression. In this study patients with deleted chromosome 5 involved into complex aberrations had an extremely poor prognosis.

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PATIENT-SPECIFIC MICRORNA EXPRESSION PROFILES IN CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA AS A PLATFORM FOR MINIMAL RESIDUAL DISEASE DETECTION

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Background. MicroRNAs are a class of small noncoding RNAs playing a crucial role in the fine tuning of mRNA expression under physiological and pathological conditions. Recently, a number of reports demonstrated the applicability of genome-wide microRNA expression profiling in Acute myeloid leukemia (AML) for disease subclassification and prognostic stratification and suggested microRNAs as a novel clinically useful class of biomarkers. On the other hand, AML cases with normal cytoenetics (NC-AML) usually lack suitable molecular markers for minimal residual disease detection. It is, therefore, tempting to explore the utility of microRNA expression profiling for minimal residual disease detection. *Aims*. In this pilot study we aimed at examining the feasibility in clinical settings of identification of patient-specific profiles of overexpressed microRNAs in cases of NC-AML. Methods: We used a custom Flexmir v.2 (Luminex Corp., USA) bead-based liquid assay for single-tube detection of 50 selected microRNAs. A total of 36 newly diagnosed cases of NC-AML cases were included in the study and 1 commercially available normal bone marrow RNA sample was included as a normalization control. Mean expression value for each microRNA in each sample was background corrected, normalized to the normal control and log2 transformed. MicroRNAs were considered significantly overexpressed if the log2 value was ≥1.5. Results: Thirty (83.3%) cases had at least one significantly overexpressed microRNA. The number of overexpressed miRNAs varied between samples - 11 cases had 1-3 overexpressed microRNAs, 11 cases had 4-7 miRNAs and 8 cases had a profile with more than 8 overexpressed micoRNAs. The most frequently overexpressed microRNAs were as follows: miR-19a (in 21 cases), miR-181a (in 20 cases), miR-19b (in 18 cases) and miR-17 (in 17 cases) whereas a total of 15 miRNAs were not found to be overexpressed in either of the cases. *Conclusions*. We showed that it is possible to identify a patient-specifc profile of overexpressed microRNAs in more than 80% of the cases of NC-AML cases. Besides, some of the overexpressed miRNAs were present in most of the cases, which makes it possible to use custom assays for just a few microRNAs (bead-based liquid assay, qPCR) for screening of overexpressed microRNAs in clinical settings.

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THE BIOINFORMATIC ANALYSIS OF ALU-REPEAT IN 5'-AREA OF MDR1 GENE PROMOTER REGION

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Alu repeats have obtained their name from the fact that most of them contain nucleotide AGCT, a cleavage site for the Alu I restriction endonuclease. A high concentration of Alu elements in the chromosome regions which contain a lot of genes allows duplication or elimination of genome fragments located between two Alu copies, as well as chromosome rearrangements. Alu repeats can affect the composition, organization and expression of the genome. Owing to their own promoter or enhancer activity, Alu repeats may enhance transcription of the adjacent locus. Transcriptional suppression is also possible, as Alu elements may expedite nucleosome assembly on the adjacent region. In addition, Alu repeats seem expedite methylation of neighboring loci, contributing to gene expression regulation. While methylation commonly suppresses transcription, cases are known when methylation of Alu repeats increases the transcriptional activity of the neighboring locus. Our aim was to investigate the interrupted ALU-repeat in 5'-area of multidrug-resistance gene (MDR1) promoter region. The investigation was performed on the DNA of lymphocyte cultures of healthy volunteers and on the DNA of leukemic cells of patients with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Totally 34 DNA samples (14 ALL, 10 AML and 10 of normal blood donors) obtained from mononu-

clear cells of peripheral blood or bone marrow were involved in the study. Almost all cases of the disease were untreated patients. Both peripheral blood and bone marrow samples were investigated. The methyl- sensitive (HpaII) and methyl-insensitive (MspI) restriction endonucleases were used for the digestion of the DNA-samples. The bisulfite sequencing method war applied in the study. The bioinformatic analysis we run had revealed the presence of interrupted ALU-repeat in 5'-area of MDR1 gene promoter region. This particular fragment does represent one of numerous subtypes of ALU-repeats and is enriched with CCWGG sites and deplete with CCGG sites being compared with "canonical"ALU sequence. Using bisulfite sequencing we revealed non-symmetrical cytosine methylation in the MDR1 gene promoter region. Comparative analysis of MDR1 promoter methylation using methylation-sensitive PCR showed distinctive differences of methylation pattern between myeloid and lymphoid kinds of acute leukemia with persistent reciprocal relationships. Namely, we observed demethylation of CG's and hypermethylation of CNG's for acute myeloid leukemia and the opposite relations for acute lymphoid leukemia: hypermethylation of CG's attended by CNG's demethylation. Overexpression of the MDR1 gene is well known marker of poor prognosis. But the genetic and epigenetic base of MDR1 'behavior' in various hematology malignancies still remains unknown. Our preliminary results suggest that epigenetic changes and thus functional activity of the MDR1 gene are not even in various hematological tumors and depends not only on cytotoxic influence, but also on the malignancy origin. This also highlights the interest to the enigma of epigenetic changes during the hematopoietic cells differentiation

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POST-SCT CHIMERISM: THE MORE MARKERS THE BETTER?

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Chimerism testing is a routine clinical decision measure of donor engraftment level in the recipient following allogeneic stem cell transplantation (SCT), with small percentage reductions used in the determination of therapy. Data from the last 5 samples issued (a total of 337 results) to 78 international laboratories participating in the UK NEQAS Post-SCT Chimerism External Quality Assessment programme were examined to determine if the number of Short Tandem Repeat (STR) markers used impacted upon the results returned. To allow data comparison the consensus median for each sample and the delta values from the relevant consensus median were calculated for each individual result. The larger the delta value, the further that data point was from the consensus median. Data was then grouped by the number of markers used in the percentage donor calculation, and this grouped data further separated by methodology (namely in-house or commercial kit); Kruskal-Wallis one way ANOVA was used to test for significance. Additionally, data grouped by methodology, irrespective of number of markers used in their calculation, was subjected to a Mann Whitney U Test. Analysis showed that number of markers used in percentage donor chimerism calculation had no significant impact on the calculated delta values (p=0.0873). Additionally, no significant difference was found when results were grouped according to number of markers used, with or without further method separation (p=0.116 and p=0.0924). However when the methodologies were compared, (irrespective of the number of markers used in the calculation), the in-house median delta was significantly higher than the commercial kits median delta (p=0.003). Statistical comparison of the number of markers used in the calculation by in-house compared to kit users is difficult as there is little overlap between the two sets of data (in-house users mode=2 markers compared to kit mode=8 markers). However, when comparing in-house users to kit users: on average they have smaller panels available (12 vs.14); analyse a smaller percentage of these available markers (74% vs. 98%); define a smaller percentage of these analysed markers to be informative (63% vs. 81%) and; use a smaller percentage of these informative markers in their final donor calculation (60% vs. 68%). In conclusion, our data has shown that statistically, results generated by in-house methodology have a larger delta value (further from the median) than those generated by a commercial kit. This difference may be due, in part, to the number of markers used in the calculation. However, this study has highlighted the urgent need for guidance and standardization in clinically significant post-SCT chimerism testing.

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ANALYSIS OF 6Q DELETION IN B CELL CHRONIC LYMPHOCYTIC LEUKEMIA BY FLUORESCENCE *IN SITU* HYBRIDIZATION USING BACTERIAL ARTIFICIAL CHROMOSOME

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Deletions of the long arm of chromosome 6 (6q) are known to occur at relatively low frequency (3-6%) in B cell chronic lymphocytic leukaemia (B-CLL), and are more frequently observed at 6q21. Patients with B-CLL carrying a 6q21 deletion show clinicobiologic features, and their outcome should be allocated in an intermediate-risk category. Few data have been reported about other band at 6q with cytogenetic alterations in B-CLL. In the current study, we used Bacterial Artificial Chromosome (BAC) clones as probes for fluorescence in situ hybridization (FISH) to analyze the incidence and localization of 6q aberrations in 110 cases of B-CLL at diagnosis. In this study, we have four BAC clones mapping regions in bands 6q16 (RP11313A17), 6q23.3 (RP11323N12), 6q25.2 (RP11589G2), 6q27 (RP1137D8). The FISH study was performed on nuclei and metaphases after stimulation with a combination of CpG-oligonucleotide DSP30 and interleukin 2 (IL-2) as previously described. Of 110 samples studied with our set of BAC clones probes, 94% could be successfully analyzed by interphase-metaphase FISH. We identified 11 cases (10%) with 6q deletion, the percentage of cells containing 6q deletions ranged from 7% to 89%. Both trisomy for chromosome 12 and homozygous deletion of 13q14 as well as no anomaly were found in 4 cases deleted in 6q16 using FISH. Trisomy for chromosome 12 and loss of 17p were demonstrated in 1 patient with a 6q23.3 and a 6q25.2 deletion using FISH. We also observed a 6q25.2 deletion without other aberrations in 2 cases using FISH. In one patient was found deletion by our set of BAC clones probes in all investigated bands associated with 6q21deletion in FISH. One case showed deletion of 6q16 and 6q25.2 coupled with trisomy 12. In one sample 6q27 deletion was associated with homozygous deletion of 13q and loss of 17p13. The last case deleted in 6q25.2 and 6q27 showed no other anomalies in FISH. In our study, of 11 cases with 6q deletion, 6 have the data of chromosome banding analysis (CBA) available. In 4 cases the abnormalities observed in addition to the 6q deletion were a trisomy 12, a loss of 13q, 17p, 14q, a gain of 7p or presence of marker chromosome. In addition 2 cases with deletion 6q show a complex aberrant karyotype. Moreover six results showed borderline value which should be further investigated. Previous cytogenetic studies suggest that tumor suppressor genes that are involved in the pathogenesis of malignant lymphoid diseases have been localised in 6q site. Using our panel of BAC probes FISH procedure, we detected deletion in the region 6q16, 6q23.3, 6q25.2 and 6q27, and we identified 11 cases with a 6q deletion, but only one showed a deletion of at 6q21, which is recognized by the commercially available probe. We are correlating cytogenetic data obtained with clinical course to evaluate the prognostic value of 6q deletions. Our data revealed that the 6q deletion is characterized by increased genomic instability and suggested that these deletions represent a secondary event in tumorigenesis of CLL.

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EVALUATION OF THE ASURAGEN® BCR/ABL1 QUANT TEST (CE IVD) FOR MONITORING THE BCR/ABL1 TO ABL1 RATIO BY RT-QPCR IN PH-POSITIVE CML PATIENTS EXPRESSING B2A2, B3A2, OR THE E1A2 FUSION TRANSCRIPT

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Background. Real-time quantitative polymerase chain reaction (qPCR) of BCR-ABL hybrid transcripts is the standard method for monitoring the response to treatment in patients with chronic myeloid leukemia (CML) or Ph-positive-acute lymphoblastic leukemia (ALL). Recently, various methods based on RQ-PCR have been adopted to monitor residual dis-

ease in clinical studies. This has underlined an increased need for standardization and the adoption of CE-IVD regulated devices for BCR-ABL monitoring. The Asuragen® BCR/ABL1 Quant® Test is a quantitative in vitro diagnostic device to monitor the BCR/ABL1 to ABL1 ratio by RQ-PCR on whole blood or bone marrow of diagnosed Ph-positive CML patients expressing b2a2, b3a2, or e1a2 fusion transcripts. The kit includes a calibrator set to generate three calibration curves and an exogenous spike in control to assess process efficiency, both built with Armored RNA Quant® (ARQ) technology. Aims. The aim of this study is to evaluate the Asuragen® BCR/ABL1 Quant® for monitoring the BCR/ABL1 to ABL1 ratio by qPCR in Ph-positive CML patients express¬ing b2a2, b3a2, or e1a2 fusion transcripts by performing a comparative study on clinical samples with known percentages of tumour cells using the Asuragen® BCR/ABL1 Quant® Test, the Europe Against Cancer (EAC) method and the IPSOGEN FusionQuant® Kit. *Methods*. 45 clinical samples with known percentages of tumor cells were selected to show different methods' abilities to detect BCR-ABL transcripts at varying levels and to attest the reproducibility of the BCR/ABL1 Quant Test.Total RNA was extracted from the leukocytes using TRIZOL® Reagent and processed using the EAC method, the IPSOGEN FusionQuant® Kits and BCR/ABL1 Quant® Test. Results. Visual inspection of the data showed that all measurements closely followed the equality line. The results from the EAC protocol were converted to the International Scale (IS) for the p210 group and using the method comparison procedure of Bland and Altman, the bias between the BCR/ABL1 Quant® Test, IPSOGEN FusionQuant® Kits and the EAC method was determined. The BCR/ABL1 Quant® Test gives the comparable results to the EAC protocol and Ipsogen FusionQuant® kit. The best results observed in the p210 group indicate that the EAC protocol results were only calculated according to IS for the p210. *Conclusions*. Considering the increasing number of laboratories adopting methods for molecular monitoring in CML, concordance of results between different methodologies is essential to assure harmonization of results irrespective of the method used. In this study we demonstrated the concordance between the Asuragen® BCR/ABL1 Quant® Test, the Europe Against Cancer (EAC) program method, and the IPSOGEN FusionQuant® Kit. The multiplex format of the Asuragen® BCR/ABL1 Quant® Test enables the simultaneous detection and quantification of the three predominant BCR/ABL1 fusion transcripts and ABL1 endogenous control in a single reaction, providing the possibility to use less cDNA, less reagents and to invest less time in performing the analysis. The kit format eliminates much of the variability seen across different laboratories due to individual primer and probe preparation protocols. Using the standardized and high quality controlled Asuragen® BCR/ABL1 Quant® Test would help in saving time and providing more reproducibility in results.

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DOES ADDITIONAL ABERRATIONS DETECTED BY METAPHASE CYTOGENETICS CHANGE THE FAVORABLE PROGNOSIS OF SOLE 13q14 DELETIONS DETECTED BY FISH ? - PRELIMINARY RESULTS

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Background. Loss of genetic material from the long arm of chromosome 13 is the most frequent chromosomal aberration in CLL reported in over 50% of CLL cases and associated with a favorable prognosis when present as the sole genomic abnormality. However FISH analysis as a routine clinical test is usually performed only for the detection of 13q14, ATM and TP53 deletions, and trisomy 12. *Aims*. The aim of the study was to estimate the type and frequency of additional chromosomal aberrations detected by metaphase cytogenetics (MC) in CLL patients with sole del(13)(q14) and to answer the question if they are of clinical significance. Patients and methods. Twenty-four previously untreated CLL patients with sole del(13)(q14) were included into the study. Sole del(13)(q14) was defined as presence of del(13)(q14.3) and absence of: del(11)(q22.3), del(17)(p13.1) and trisomy 12. The patients were divided into two groups: with sole del(13)(q14) only (group A) and with additional aberrations detectd by MC (group B). Submicroscopic character of del(13)(q14) in 21 patients was confirmed by metaphase FISH. The diagnoses were based on the IWCLL criteria. The laboratory and clinical assessments included: age, absolute lymphocyte count (ALC), serum LDH activity, serum beta2microglobulin level, CD38 expression and Rai staging. Seventeen patients were treated with purine analogues and/or alkylating agents. The follow-up range as the time from the cytogenetic analysis to the last observation was from 20 to 42 months. Overall response rate (OR) for the patients was established. Chromosome banding analysis was performed after 72h culture with the CpG-oligonucleotide DSP30 according to standard protocol. FISH analysis was performed on peripheral blood smears using D13S319(13q14.3)/13q34/CEP12 and TP53(17p13.1)/ATM(11q22.3) probes. Mann-Whitney U test and Fisher's exact test were performed with $P<0.05$ as the threshold of statistical significance. Results. Group A consists of 11 patients (45.8%) and group B of 13 patients (54.2%). In group A karyotype was normal (n=9) or del(13)(q14) as the only aberration (n=2) was present. In group B additional aberrations were detected: in 5/13 (38.5%) cases karyotype was complex, only in 2/13 (15.4%) cases aberrations were balanced. The most recurrent regions involved in aberrations (especially translocations) included: 13q14 (n=3), 14q32 (n=2), 2p11-13 (n=2), 3p26 (n=2) and 6q13-26 (n=3, deletions). No significant differences between the two studied groups were observed with respect to age, ALC, LDH, beta2microglobulin and CD38, or to the stage of the disease: RAI stage 3 and 4 was established in 5/11 (45.5%) and in 7/13 (53.9%) patients in group A and B, respectively. OR was obtained in 5/7 (71.4%) patients of group A and in 7/10 (70.0%) patients of group B, and the difference was also not statistically significant. *Conclusions*. Patients with sole del(13)(q14) as determined by FISH do not represent a cytogenetically homogenous group. No differences were observed in laboratory and clinical data among patients with and without additional aberrations. However, the time of follow-up was too short to determine definitively if these additional aberrations have clinical significance, so further studies are continued.

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DELINEATION OF REARRANGEMENT BETWEEN THE RUNX1 AND THE HOXA GENE CLUSTER; A POSSIBLE NOVEL MECHANISM FOR LEUKEMOGENESIS

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Background. Translocation involving the Runt-related transcription factor 1 (RUNX1) gene commonly occurs in hematologic malignancies. The HOX family consists of 39 HOX genes arranged in four clusters (A-D) that are located on chromosome 7, 17, 12 and 2, respectively. Studies have shown that some of the HOX gene family are expressed in human leukemia and play an important role in development of leukemia by fusion with NUP98. But the translocation between RUNX1 and HOX gene cluster has not been addressed so far. Currently we experienced acute myeloid leukemia with t(7;21) on G-banding implying the involvement of RUNX1 and HOXA genes. Aims: To address a possible role of RUNX1/HOXA gene fusion as a novel leukemogenic mechanism, we performed FISH analysis on human acute myeloid leukemia case and explored the breakpoint responsible for RUNX1/HOXA translocation. Methods: G-banding was performed on bone marrow cells of AML patient according to standard unstimulated 24-hour culture method. FISH using a commercial LSI AML1/ETO dual color, dual fusion probe (Vysis, Downers Grove, IL) was performed to assess and exclude the cryptic translocation of RUNX1 and RUNX1T1. And next, to explore the breakpoint on the short arm of chromosome 7 involved in the t(7;21), we referred to the human genome browser database and designed 5 paired-sets of dual color (SpectrumOrange and SpectrumGreen) break-apart probes with using relevant eight bacterial artificial chromosome (BAC) clones targeting the HOXA gene cluster. Results: G-banded karyotyping demonstrated 46,XX,t(7;21)(p15;q22)[5]. Metaphase fluorescence in situ hybridization (FISH) analysis using the AML1/ETO dual-color, dual-fusion probes showed three (one large and two small) green signals for AML1(RUNX1) implicating some aberration in one RUNX1 gene. The signals for the ETO(RUNX1T1) gene remained intact, which excluded the possibility that AML1/ETO gene fusion might involved in the leukemogenesis of this case. The results of FISH with 5 paired probe sets for the HOXA genes narrowed the candidate region for breakpoint. Metaphase FISH using RP11-163M21 labeled SpectrumOrange, HOXA1-7 spanning probe, and RP11-1148E13 labeled SpectrumGreen, HOX13 flanking probe on centromere side showed one fusion signal on normal chromosome 7, one red signal on der(21) and one green signal on der(7). These findings indicate that the region including HOXA1-7 is

translocated to der(21). Metaphase FISH using RP11-838G2 labeled SpecrumOrange, HOXA gene flanking probe on telomere side, and RP11-1025G19 labeled SpectrumGreen, HOXA7-13 spanning probe showed one fusion signal on normal chromosome 7, one small fusion signal on der(21) and small green signal on der(7). This finding indicates that the region including HOXA7-13 or part of HOXA7-13 is translocated onto der(21). Conclusions. These results clearly demonstrate that the translocation of RUNX1 and HOXA gene was involved in acute myeloid leukemia and suggest that juxtapositioning of the RUNX1 and HOXA genes would be a novel mechanism for leukemogenesis.

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EVALUATION OF A QUALITATIVE *IN VITRO* DIAGNOSTIC DEVICE FOR THE MULTIPLEX DETECTION OF SPECIFIC FUSION TRANSCRIPTS TO AID IN THE DIAGNOSIS OF LEUKEMIA

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Background: Multiple recurring chromosomal translocations occur in leukaemia. Sensitive detection and accurate classification of the associated gene fusions is critical for the diagnosis and prognosis of many leukaemias. At the molecular level the breakpoints can vary over a wide region within the genes involved, and it is often necessary to determine the specific fusion sub-type for the purposes of ongoing monitoring with quantitative molecular techniques. Aims: To evaluate a new commercially available CE-marked IVD test kit in a clinical setting for the simultaneous detection of leukaemia fusion transcripts in a single multiplex assay. To determine diagnostic specificity and sensitivity of the test relative to standard cytogenetic and molecular methods. Methods: Archived total RNA samples from leukaemia patients at presentation were analysed using the commercial kit. The kit can detect 12 fusion transcripts (BCR-ABL1 e13a2, BCR-ABL1 e14a2, BCR-ABL1 e1a2, TCF3-PBX1, ETV6-RUNX1, MLL-AF4 e9e5, MLL-AF4 e10e4, PML-RARA bcr1, PML-RARA bcr3, CBFB-MYH11 type A, CBFB-MYH11 type D, RUNX1-RUNX1T1) and includes GAPDH as an endogenous control to assess sample quality. Following multiplex RT-PCR using 100 to 400 ng RNA per test, the identity of biotin-labelled PCR products was simultaneously determined by hybridization on a liquid bead array, containing target-specific probes, and flow cytometric detection. Qualitative calls (positive or negative for each target) relative to a fixed cut off signal (350 MFI) were generated and compared to known diagnostic status. Results: To date, 70 specimens have been evaluated, 58 positive and 12 negative. One sample failed (no signal) and one positive sample generated a low signal at 294 MFI, above the average background negative signal (51 MFI), but below the fixed cut off (350 MFI). These 2 samples are being further investigated. For the 69 samples with results, a total of 759 calls were generated for the individual fusion transcripts corresponding to an overall agreement with reference methods of 99.9% (758/759; 95% CI: 99.3 to 100%). Diagnostic sensitivity was 98.2% (56/57; 95% CI: 90.7 to 99.7%) and diagnostic specificity was 100% (702/702; 95% CI: 99.5 to 100%). Summary/Conclusions: The assay is quick, simple and reliable. It detected all twelve fusion transcript types in representative clinical specimens. Analytical sensitivity was not evaluated here (1% reported by the test manufacturer) but was appropriate for the analysis of samples at initial diagnosis. Further, the inclusion of an endogenous control enables determination of poor quality or low input samples. The kit also has the advantage of typing individual fusions, and assigned these correctly in 100% of cases. This could facilitate the downstream use of specific molecular assays such as RT-qPCR for follow-up analyses and minimal residual disease monitoring. Finally, multiplex detection in a single reaction can greatly improve laboratory efficiency, which would be further enhanced by the addition of more rare fusion subtypes such as BCR-ABL1 e13a3 and e13a4 or CBFB-MYH11 type E to the kit. Overall the CE-marked IVD test kit is an attractive novel molecular method that is compatible with the clinical laboratory workflow and complements standard cytogenetic methods.

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SMALL B-CELL LYMPHOPROLIFERATIVE DISORDERS DISPLAYING CLL LIKE FEATURES AND HARBORING TRISOMY 3

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Trisomy 3 was first reported as a sole abnormality in adult T cell lymphoma/leukaemia and subsequently in other subtypes of T-non Hodgkin lymphoma (NHL), particularly lymphoepithelioid and angioimmunoblastic. Later, it was identified as the most frequent abnormality in marginal zone B-cell lymphoma. In other B cell NHL it is relatively rare and is reported only sporadically in chronic lymphoproliferative disorders (CLPD). Although it is not a recognised recurrent abnormality associated with chronic lymphocytic leukemia (CLL), it is detected in 1.5%-2.8% of karyotypically abnormal cases and in around 3% of all cases by FISH. Given the importance of recognising new cytogenetic entities with differing clinical course and prognosis we set out to determine whether trisomy 3 is a rare recurrent abnormality associated with CLL or whether the presence of this abnormality in the karyotype should evoke a differential diagnosis. Twelve cases with a trisomy 3, complete or partial, for whom a diagnosis of CLL was retained by the referring institution, were retreived from the databases of two Belgian genetic centres. The sample type analysed was blood in four cases, bone marrow in two and blood and bone marrow in one. The clinical, biological and histological findings of these presumptive cases were reviewed. A diagnosis of typical CLL, or atypical-CLL, was retained for cases with either a Matutes-Catovsky score of ≥3 or histological confirmation of CLL on review. Five cases presented with Binet stage A and two with Binet stage B disease. On follow up, three had stable disease, two showed evolutive disease requiring treatment and two underwent Richter transformation. Morphological examination of all cases was consistent with CLL. Three cases displayed a typical immunophenotype with a score of 4 or 5, three had a score of 3 and one a score of 2, included after histological confirmation of typical CLL. One had a raised monoclonal IgMK and another cold agglutinins. Trisomy 3 was present as the sole abnormality in 3 cases, was associated with one other aberration in 2 cases (balanced translocation, trisomy 12), and occurred as part of a complex karyotype in 2 cases (>3 abnormalities). Interestingly, the two cases that underwent Richter transformation had a complex karyotype. To date, the clinical details associated with trisomy 3 have only been described in 5 cases of CLL (Wong 2002, Specchia 2002, Michaux 1998). For these cases a diagnosis of CLL was based on morphological criteria despite an atypical phenotype. Unlike those previously reported three of our cases had a typical CLL immunophenotype. This study brings the total number of reported cases of trisomy 3 associated CLL to twelve. Morphological criteria was consistent with CLL in all cases. Phenotype was frequently atypical, but not evocative of any other specific disease entity. Whether these atypical cases represent true CLL or a distinct subtype of CLPD with CLL like features remains to be determined. Indeed, as suggested by others, these cases may in the future correspond speculatively to a new disease entity warranting further investigation.

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ANTICIPATION IN FAMILIAL HEMATOLOGICAL MALIGNANCIES

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Background. The anticipation is the phenomenon in which a disease is diagnosed at an earlier age as it is passed on to the next generation. Whereas there are a lot of studies about it on solid neoplasms, there is not a good knowledge of it in the hematological field. *Aims*. Our purpose was to evaluate the anticipation in our experience. *Methods*. We observed thirty-four families with at least two members who presented a hematological malignancy diagnosed from 1950 to 2010. The male-to-female ratio was 42:33; they were affected by Non-Hodgkin's Lymphoma (25 pts), Chronic Lymphocytic Leukemia (11 pts), Hodgkin's Lymphoma (8 pts), Acute Myeloid Leukemia (8 pts), Chronic Myeloid Leukemia (4 pts), Myeloproliferative Neoplasms (4 pts), Multiple Myeloma (4 pts), Acute Lymphoid Leukemia (2 pts), Waldenstrom's Macroglobulinemia (1 pt) and Acute Leukemia non otherwise specified (8 pts). At this time 30 patients are dead. Two or three different generations are involved in twenty-two families including forty-nine patients. We have restricted our analysis to two generations because of the small number of cases in the previous ones. Then we studied fourteen families in which two or more

members are affected by hematologic malignancies (HMs) among the same generation. Anticipation is assessed with a logrank test for trend using GraphPad Prism software version 5.0. Results. The median age at diagnosis for patients of the first generation was 58 years (range 9-82 years) whereas the median age at diagnosis for patients of the second one was 30.5 years (range 3-61 years).

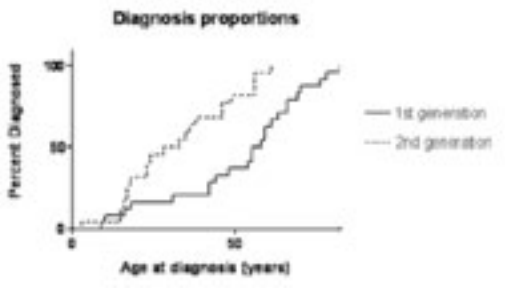

Figure 1. Age at diagnosis by generation.

We demonstrated the phenomenon of anticipation between the second and the first generation with an important and significant difference of 28.5 years (p=0.0003) (Image). The analysis on patients of the same generation showed that the median age at diagnosis for first-borns was 61 years (range 23-75 years) and for the second-borns was 53 years (range 17-68 years), with a difference of 8.0 years, which was not statistically significant (p=0.249). *Conclusions*. It is clearly recognized in the literature that there is a genetic basis for familial risk of HMs; however the causative mutation has not been identified. Future studies on these families should help to clarify genetic pathways underlying familial HMs.

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USEFULNESS OF IGH/TCR CLONALITY PCR STUDIES IN LYMPHOPROLIFERATIVE DISORDERS WITH DOUBTFUL CLONALITY BY FLOW CYTOMETRY

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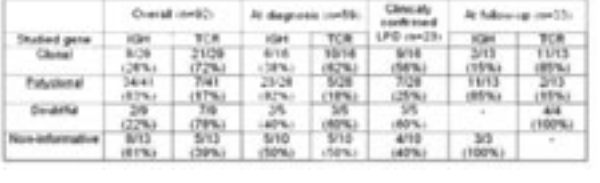
Background. The kappa/lambda index in flow cytometry (FCM) is used to check clonality in B-cell diseases. A clonal result is considered when this index is >3 or <0.5. However, in borderline results it is difficult to assess the clonality. For T-cell clonality assessment the qualitative status of CD expression (crosslineage or asyncronous expression) and/or the quantitative status (absolute number of T cells or CD4/CD8 ratio) is used in FCM. *Aims.* To assess the clonality by PCR in patients with lymphoproliferative disorders with doubtful clonality by FCM. Samples DNA was extracted from 92 samples of peripheral blood, bone marrow, lymph node aspirates or biologic fluids from patients with suspicion of lymphoproliferative disorders in whom the clonality assessment by FCM was doubtful.



Three different regions of the IGH VDJ segment and two regions of the TCRg VDJ segment were amplified by PCR using a commercial kit (Invivoscribe Technologies, San Diego, USA) and analysed by Genescan. A study was considered polyclonal when a Gaussian distribution of transcripts was obtained and clonal when the area of the highest peak was three times bigger than the area of the third highest peak. A doubtful result was a peak 2-3 times higher than the polyclonal population without reaching the limit of clonality. A non-informative result was considered when no IGH or TCRg amplification was demonstrated but the specimen controls were correct. *Results*. Table 1 shows the frequency of informative and non-informative PCR results, as well as the correlation with clinical diagnosis for samples evaluated at diagnosis. *Summary*. PCR was informative in 76% of samples from patients with lymphoproliferative disorders with doubtful clonality by FCM, of whom 32% were clonal. Hematological neoplasm was confirmed in 56% of clonal disorders assessed by PCR at diagnosis.

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MULTIPLEX LIGATION-DEPENDANT PROBE AMPLIFICATION (MLPA) A SENSITIVE TECHNIQUE FOR THE DETECTION OF TP53 DELETIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA?

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Background. Detection of a deletion of TP53 on chromosome 17p13 influences choice of treatment in previously untreated or relapsed patients with CLL. Fluorescence in-situ hybridisation (FISH) is widely accepted as the *gold standard* for detection of TP53 loss however the technique is both costly and labour intensive. Multiplex Ligation-dependent Probe Amplification (MLPA), a molecular technique able to simultaneously detect copy number aberrations (CNA) of multiple loci in a single PCR reaction, has been reported as a non-subjective, accurate, cost- effective and high-throughput alternative technique for the detection of CNA in CLL. However, whilst published correlations between FISH and MLPA in CLL are promising, the number of reported cases with deletions of TP53 are too few to reliably asses the efficacy of this technique in the detection of this important gene deletion. Aims. The aim of this study was to investigate the specificity and sensitivity of MLPA for detecting known deletions of TP53 in CLL. Methods MLPA analysis was performed on peripheral blood mononuclear cell DNA using two commercial kits (P037 and P038, MRC Holland) designed to detect recurrent CNA in CLL. Each kit contains four different probes targeting TP53. The normal range for each individual MLPA probe was established from 36 normal control samples and the mean ±2SD was used to determine the cut off limits for gain or loss. Blinded analysis was performed with one or both kits on a total of 64 CLL samples previously screened by FISH with Vysis/Abott LSI TP53/CEP 17 probes. Of the 64 samples, 44 had TP53 deletion in 5-96% (median=76 %) of cells and 20 had no detectable TP53 deletion by FISH. Samples were categorised as TP53 deleted by MPLA when ≥2/4 of the TP53 probes in a single kit, or ≥3/8 TP53 probes across the two kits, were below their defined cut off limit. *Results*. The sensitivity of MPLA for correct identification of samples showing TP53 deletion by FISH was 90% (37/41, 95ci=79-97%), 76% (28/37, 95ci=61-87%) and 88% (29/33, 95ci=74-96%) for the P037 kit, the P038 kit and combined kits, respectively. The P037, P038 and combined kits all showed 100% specificity (20/20, 95ci=87-100%) for correct identification of samples without TP53 deletion by FISH. All four samples with TP53 deletion detectable by FISH but not by the P037 kit alone, or in combination with the P038 kit, had deletion in ≤ 14% of cells. Looking across the control sample data, the standard deviations for probes from the P038 kit were significantly higher than those from the P037 kit (p=0.01), leading to a wider normal range, possibly accounting for the lower sensitivity of the P038 kit. *Conclusions*. TP53 deletions in CLL samples can be reliably detected by MPLA using the combined data from the P037 and P038 MPLA kits provided deletion is present in approximately 20% or more of the tumour cells. This is close to the clinically relevant clone size as determined by FISH and as such MPLA shows potential for adoption in a routine setting subject to further validation.

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STUDIES ON COMMON SPLICE VARIANTS OF PROGNOSTICALLY IMPORTANT FUSION ONCOGENES IN PAKISTANI LEUKEMIA PATIENTS: IMPLICATION IN LEUKEMIA BIOLOGY, DIFFERENTIAL DIAGNOSIS, PROGNOSIS AND TREATMENT

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Background. Leukemia is a heterogeneous genetic disease and a neoplasm involving many fusion oncogenes(1). These fusion oncogenes and their splice variants, resulted due to genetic abnormalities like translocations, deletions, are helpful in differential diagnosis and prognostic stratification as are directly involved in Leukemogenesis(2). As environmental factors coupled with natural genetic variations drive the genetic abnormalities and hence the fusion oncogenes, frequencies of fusion oncogenes and their splice variants can vary in different geographical regions and ethnic groups. Therefore, studying frequencies of different chromosomal abnormalities leading to fusion oncogenes in a given population and their clinical application can have a significant impact on management of Leukemia in terms of differential diagnosis, prognosis and treatment. Aims: The objective of this study was to find out the frequencies of various splice variants of different fusion oncogenes in Pakistani Leukemia patients (Group A), their comparison with western populations (Group B, taken from published literature) and to see how this data can be implicated in an oncology clinic to better diagnose, prognose and treat leukemia . *Methods*. Bone marrow samples were collected from 501 Leukemia patients. RNA was extracted by TriZol method(3). A very RT-PCR was used to study the different splice variants of the most common fusion oncogenes of prognostic and differential significance (3). Interphase-FISH was employed to confirm the results of RT-PCR wherever necessary(4). Results: No significant differences between Group A and Group B terms of frequencies of CBFB-MYH11 (inv 16 / t 16;16), AML1-ETO (t 8;21) in AML and PML-PARα (t 15;17) in APL/AML M3 while significant difference was found between two group in case of CML BCR-ABL splice variants b2a2 and b3a2 (Table 1, p=0.01). In ALL patients, significant differences were found between Pakistani and western populations with respect to frequencies of SIL-TAL1 (del 1),TEL-AML1 (t 12;21) oncogenes in adult ALL, BCR-ABL (t 22;9), E2A-PBX1 (t 1;19), TEL-AML1 (t 12;21), MLL-AF4 (t 4;11) in paediatric ALL (Table 1, Figure 1-3, p=0.01). This shows that cumulative frequencies of fusion oncogenes related with poor prognosis (BCR-ABL (t 22;9), SIL-TAL1 (del 1), E2A-PBX1 (t 1;19), MLL-AF4 (t 4;11) are higher in Pakistani pediatric ALL patients than western counterparts (74.2% vs 27%, p=0.01) while reverse is the case of TEL-AML (16.5% vs 25%, p=0.01) associated with good prognosis (Table 1). Conclusions and Discussion: Our data shows significant difference between Leukemia patients from Pakistan and western populations in terms of splice variants of different fusion oncogene frequencies, which is in accordance with other reports(5,6) which may be related to ethnicity. These results explain the molecular genetic basis of already-reported poor prognosis and survival of paediatric ALL and adult AML patients in Pakistan(7). Our studies have implications in clinical management of Leukemia at healthcare policy-making bodies and clinical centers.

Figure 1. Frequency of Leukemic oncogene splice variants.

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RARE CYTOGENETIC FEATURES IN ACUTE PROMYELOCYTIC LEUKEMIA

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Acute promyelocytic leukemia (APL) is strongly associated with t(15;17)(q22;q12~q21) giving rise to the formation of two functional fusion genes, PML/RARA on the derivative chromosome 15 and RARA-PML on the derivative chromosome 17. A small group of patients with APL appears to have a submicroscopic insertion of the RARA gene into chromosome 15 or microscopically visible rearranged rearrengent of derivative 17.The prognostic significance of these genetic abnormalities is still under discussion. Among 13 newly diagnosed acute myeloid leukemia patients in 2010, 3 (23%) were classified as an acute promyelocytic leukemia with t(15;17) according to the WHO 2008 criterias. Typical t(15;17) was recognised by classical cytogenetic analysis in one case (case1, F/61y.o). Hematologic workup showed t-AML after previously treated breast cancer with PML-RARa fusion gene transcripts, FLT3 ITD and WT1 mutation. She was in minimal residual disease after 1th induction chemotherapy with cytarabin, idarubicin and ATRA. In the second patient (case2, M/27y.o), chromosome analysis showed a banding pattern compatible with an insertion (17;15) or "two way transloca-

tion" between derivative 17 and derivative 15. Interpretation was clarified by FISH using the PML/RARA Dual Fusion Translocation probe (Vysis) and whole chromosome paint (wcp) 15 and 17 probes, which demonstrated of two fusions and both paints on the abnormale chromosome 17 and no wcp 17 on either of two chromosomes 15. The findings of the microscopically visible der(17)t(15;17) (q24;q21) ins(17;15) (q21;q24) represents a new cytogenetic variants in APL. The patient was included in APL 2006 protocol and achieved a complete molecular remission after induction chemotherapy with cytarabin, idarubicin and ATRA. Cytogenetic analysis of the third patient (case 3, M/23 y.o) studied at diagnosis revealed der(15)(15;17)(q22;q21), ider(17)(q10)t(15;17) (q22;q21). Ider (17) originated from the duplication of the long arm of der(17) with an additional copy of RARA-PML, and resulted in a loss of the whole short arm of chromosome 17 (P53 on 17p13.3). The patient was also included in APL 2006 protocol and achieved a complete molecular remission after induction chemotherapy. Due to the rarity of the abnormalities described above, it is not known if there is any impact on the prognosis after induction chemotherapy based on ATRA, at least we found an excellent response to ATRA-based induction treatment and there is a need for longer follow up and more patients to evaluate better the impact of those additional abnormalities in APL.

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MUTATIONS IN IDH1/2 GENES IN ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE: A SINGLE CENTRE STUDY OF 72 CASES

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Background. Normal karyotype acute myeloid leukemia (NK-AML) comprises a clinically and biologically heterogeneous group of AMLs accounting approximately for 45% of all cases. A number of molecular changes including mutations in FMS-like tyrosine kinase 3 (FLT3), nucleophosmin (NPM1) and CCAAT/enhancer binding protein alpha (CEBPA) genes provide either alone or in combination prognostic information particularly in NK-AML. Recently mutations in genes for the isocitrate dehydrogenase 1 and 2 (IDH1/2) have been reported recurrently in AML. The prognostic impact of these mutations still remains to be determined however their potential as candidate markers for monitoring minimal residual disease is of special interest in NK-AML. Aims: In this study we investigated the prevalence of IDH1/2 mutations in a cohort of NK-AMLs. We aimed to correlate IDH1/2 mutation status with clinical features, prognostic impact and additional recurrent mutations in AML occurring in FLT3, NPM1, CEBPA, Wilms tumor 1 (WT1) and Mixed-Lineage Leukemia (MLL) genes. Methods: A total of 72 patients diagnosed and treated in a period of 1997 - 2009 as de novo NK-AML (age 22 - 72) were assessed in this retrospective study. All patients were treated within a single centre trial. First-line treatment consisted of double induction and intensive consolidation therapy with high dose cytarabine, autologous or allogeneic stem cell transplantation. Diagnostic leukemic blasts were analyzed for mutations in IDH1/2, FLT3, NPM1, CEBPA, WT1 and MLL genes using fragment analysis and/or direct sequencing. An allele specific polymerase chain reaction assay with a sensitivity of ~1% was introduced to detect IDH1 R132H mutation in post-treatment samples. Results: Overall 28% (20/72) of patients were found to carry heterozygous IDH1/2 mutation with IDH1 mutations detected in 7 patients (10%, R132H only) and IDH2 mutations in 13 patients (18%; 12 cases with R140Q and 1 case with R172K). We revealed 9 patients (13%) with a synonomous (GGC/GGT) IDH1 single nucleotide polymorphism rs11554137. Additionally one patient presented with so far not identified T106M (ACG/ATG) IDH1 sequence variant. In our cohort IDH1 and IDH2 mutations were mutually exclusive. Both IDH gene mutations tended to coexist with NPM1 (11/20) and/or FLT3 (8/20) mutations. However in only 2 cases we found IDH2 R140Q mutation accompanied with concurrent mutation in CEBPA or MLL genes respectively. IDH1/2 mutation status did not correlate significantly with clinical characteristics of patients regarding age, sex, leukocyte count, bone marrow or peripheral blood blast counts. The differences were not statistically significant when comparing overall and disease-free survival between the IDH1/2 mutated and wild-type groups. *Summary/Conclusions*. Our data provide evidence about recurring IDH1/2 mutations that are mutually exclusive in our cohort of NK-AML and tend to overlap with prognostic mutations such as FLT3 and/or NPM1. Further evaluation of IDH1/2 mutations in post-treatment samples is in process and will determine the usefulness of these mutations in monitoring minimal residual disease in AML.

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TIROFIBAN VS ABCIXIMAB: IS TIROFIBAN UNDERDOSED?

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Background. Tirofiban is a very promising anti-platelet drug, but has shown to lack the efficacy of abciximab in preventing ischaemic events, possibly due to underdosing. *Aims*. The aim of this study was to determine the efficacy and bleeding effect of escalating doses of tirofiban in comparison with abciximab in baboons. *Methods*. The femoral artery of baboons was shunted to the femoral vein. The artery was mechanically injured and external stenosis was applied. This resulted in thrombus formation measured by decreased blood flow. Thrombi were mechanically dislodged resulting in a pattern of cyclic flow reductions (CFRs). Escalating doses of tirofiban (n=5) and abciximab (n=4) were administered and the effect of this treatment on the CFRs was measured. Bleeding was assessed in an incision and a template bleeding model. Results: Tirofiban completely inhibited arterial thrombus formation after injection of 90 µg/kg (+ 1.35 µg/kg/min infusion). This dose is 9 times higher than the manufacturers recommended therapeutic dose (10 µg/kg bolus + 0.15 µg/kg/min infusion) for an adult human during angioplasty, and more than 3 times higher than the adjusted higher dose recommended (25 µg/kg bolus + 0.15 µg/kg/min infusion). However, in ex vivo platelet function tests, full platelet inhibition was observed at the therapeutic dose. Abciximab completely inhibited arterial thrombus formation after injection of 250 µg/kg (therapeutic dose for adults is a 250 µg/kg bolus + 0.125 µg/kg/min infusion). The maximum mean blood loss in the incision bleeding model was only a 3.4 fold increase from the saline phase in the tirofiban group at 27 times the therapeutic dose, compared with the 18.7 fold increase seen with abciximab at its effective dose (250 µg/kg). Conclusion: Tirofiban is a safe and effective antiplatelet drug in baboons, but is only effective at a dose 9 times higher than the recommended therapeutic dose for adult humans. It compares favourably to abciximab, but with less bleeding. We recommend that further in vivo testing should be done to determine the optimal therapeutic dose of tirofiban.

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EFFECT OF THE ANTIPSYCHOTIC DRUGS ON BLOOD CLOTTING

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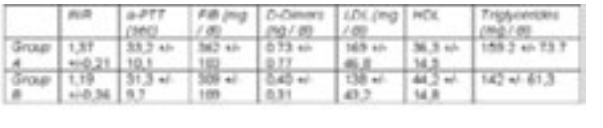
Background-Aim. A comparative study of cardiovascular risk factors such as lipid profile and blood clotting in psychiatric patients, as some antipsychotics (clozapine, olanzapine) are associated with sedation and weight gain, but causes increased growth adhesion and platelet aggregation. Material - Methods: We studied 46 patients on antipsychotic medication (31 men and 15 women) mean age 52.7 years (group A). In all we checked the coagulation system (prothrombin time, partial thromboplastin time, fibrinogen and D-dimer), as well as the lipid profile, and results were compared with those 50 people (32 men and 18 women) a random sample of the general healthy population similar age (mean 54.3 years) and was the control group (group B). *Results*. These are summarized in the table below. *Summary-Conclusions*. 1) It turns out therefore that although the prothrombin time and partial thromboplastin do not appear differentiated between the two groups, however, fibrinogen levels, particularly levels of d-dimer in psychiatric patients is particularly high in relation to general population, showing a statistically significant difference (p <0,05). 2) Also, regarding the lipid profile we recorded significant charges in the group of psychiatric patients compared with controls, the result of suppression of weight gain and worst-often neglected maintenance.



From the above, especially if one account and other predisposing factors (eg smoking in psychiatric patients who touches the vast majority

- 41 of 46 patients in our study) indicate that the cardiovascular risk in this category of patients is much higher than the general population, which should be not noticed our attention.

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LOW RESPONSIVENESS TO ASPIRIN AND CLOPIDOGREL IN PATIENTS FROM GRAN CANARIA (SPAIN) - INCIDENCE AND INFLUENCE FACTORS

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Background. Platelets are the key mediatiors in the pathophysiology of the atherothrombotic disease. Platelet function tests have been used to monitor patients response to antiplatelet therapy. Several studies suggest that low responsiveness to antiplatelet therapy, as measured in various function tests, correlates with high rates of incidences of ischemic events. One widely used method for platelet function analysis is multiple electrode aggregometry. So far most data with MEA have been established in the middle-european population. No data is so far available in west mediterranean population. *Methods*. 226 individuals were tested following informed consent and IRB approval. Hirudin blood was drawn (Dynabyte, Munich, Germany) and analyzed within 3 hours. 51 individuals were treated with aspirin (100 mg/d) alone, 27 with clopidogrel (75 mg/d) alone and 79 with a combination of aspirin and clopidogrel. In addition 69 blood donors were analyzed as a control group. MEA was measured as previously reported using the agonists ADP (ADPtest, 6.4 µM), arachidonic acid (ASPItest, 0.5 mM) and TRAP-6 (thrombin receptor activating peptide, TRAPtest, 32 µM). Results. Based on the results of the healthy volunteers a normal range was defined based on the 90% central interval, i.e. the range of values between the 5° and 95° percentile. These values are close but not identical to the normal ranges provided by the manufacturer of the Multiplate instrument. Differences may be due to differences between the groups used for the determination of the reference ranges (gender, age, genetic background, nutrition). Patients on aspirin or aspirin and clopidogrel showed a significantly reduced aggregation in ASPItest compared to the blood donors (Mann-Whitney U-test, $p < 0.05$). 11.7 % of patients treated by aspirin only had an aggregation of > 30 U, which is used by several authors as the cut-off for a full cyclooxygenase blockade. Only 1 patient (2%) of the aspirin-only group had an aggregation in the reference range for ASPItest. In the aspirin + clopidogrel group 6.4% of patients had an aggregation > 30 U. Patients treated with clopidogrel showed a significantly lower aggregation compared to the blood donors (Mann-Whitney U-test, $p<0.05$). However 37% of all clopidogrel-treated patients had an aggregation > 47U which was defined as a cut-off for increased risk for stent thrombosis and other ischemic events by Sibbing et al. Patients treated by clopidogrel alone had a low response rate of 44%, while patients treated by clopidogrel and aspirin in combination showed a low response to clopidogrel in 35% of the cases. Aspirin alone does not influence the TRAPtest, while clopidogrel has a weaker, but significant effect on the TRAPtest. *Conclusions*. Reference ranges have been established in Canary Island populations. Much less low response to aspirin than clopidogrel has been found. 8,6% of the aspirin-treated patients showed a low response to aspirin treatment. 37% of clopidogrel-treated patients had a low response to clopidogrel. No additive effect of aspirin on top of clopidogrel in respect to ADP induced aggregation. Higher ADP induced aggregation in aspirin-treated patients compared to blood donors.

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ACTIVATION OF CASPASE-3 AND -9 AND DOWN-REGULATION OF SURVIVIN PROTEIN AS A MECHANISM OF APOPTOTIC DEATH IN K562 LEUKEMIA CELLS UPON EXPOSURE TO A DERIVATIVE FROM 4-ARYL-4H-CHROMENES FAMILY

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It has been recently reported the activity of 4-aryl-4H-chromenes family, to induce apoptosis in human cancer cells. Herein we report a derivative of 4-aryl-4H-chromene compound with higher apoptotic activity against Erythroleukemia K562. The cells were seeded in 24-well plates at 1×105 cells/well and treated with 50-80 nM of the 2-amino-4-(3-nitro phenyl)-3-cyano-7-(dimethylamino)-4Hchromene (3-NC). This compound was found to be highly active growth inhibitor with IC50 of 65±3.5 nM as determined by MTT assay. Proliferation of K562 cells was diminished by more than 70% and viability was decreased by about 50% upon 72 h of treatment with 50-80 nM concentration of the compound. Apoptosis as the mechanism of cell death was investigated morphologically by Hoechst 33258 staining, caspase-3 activation assay, as well as the formation of DNA ladder. K562 cells underwent apoptosis upon a single dose (at IC50 value) of the compound and also increased caspase-3 activity by more than 2-fold following 72 h treatment. Caspase-9 was also activated which could be detected 48 hours post-treatment. Furthermore, Western blot analysis revealed that the treatment with the compound down-regulated the expression of certain IAP protein including survivin. . Considering the negative effects of survivin on caspase activity, one can propose that the treatment of cells with 2-amino-4-(3-nitro phenyl)-3-cyano-7-(dimethylamino)-4Hchromene leads to degradation of survivin protein unleashing caspase activity and causing apoptotic cell death. Given the fact that survivin confer resistance to certain cancer cells and considering apoptotic cell death of cancer cells due to activation of caspases upon degradation of survivin, one can propose the down regulation of survivin as an effective approach for cancer therapy under conditions in which these proteins confer resistance to cancer therapy. These data further suggest that 2-amino-4-(3-nitro phenyl)-3-cyano-7-(dimethylamino)-4Hchromene may provide a novel therapeutic approach for the treatment of leukemia.

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THE ROLES OF MACROMOLECULES IN IMATINIB RESISTANCE IN CHRONIC MYELOID LEUKEMIA

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Background. Imatinib is the first anticancer agent targeting BCR/ABL oncogene for the treatment of chronic myeloid leukemia. In the beginning of imatinib treatment very promising outcomes were observed in chronic myeloid leukemia patients. But resistance to imatinib is the main drawback for imatinib treatment. Fourier transform infrared spectroscopy (FTIR) is a sensitive, rapid and nondestructive method that can show changes in macromolecules. FTIR can be widely used in the analysis of various biological systems in any physical state requiring only minute amounts of samples. The method can be used to obtain valuable metabolic and structural information of the cellular components such as lipids, proteins, carbohydrates and nucleic acids in the level of functional groups in the progress of differentiation processes such as disease progression. Aims. In the present work we examined the changes in macromolecules in imatinib resistant K562 cells at the molecular level using Fourier transform infrared (FT-IR) spectroscopy. Methods. Human K562 CML cells were exposed to step-wise increasing concentrations of imatinib and 3 µM imatinib resistant K562 cells were generated and named as K562/IMA-3 cells. Antiproliferative effects of imatinib were determined by XTT cell proliferation assay. Changes in macromolecules in parental and resistant cells were studied by FT-IR spectroscopy. Results. K562/IMA-3 cells were shown to be more than 50-times more resistant to imatinib as compared to K562 cells. Imatinib resistance caused significant changes which mainly indicated that the membrane/lipid order increased, lipid peroxidation end products increased, the lipid-to-protein ratio increased and, the transcriptional status increased, structural and organizational changes in the nuclear morphology and changes in the proteome revealed by the FT-IR spectra. In addition, changes in the proteome and structural changes in both proteins and nucleus were observed in the K562/IMA-3 cells. Summary/Conclusions. The results indicate that raising imatinib resitance caused significant structural and organizational changes in the K562 cells. Similarly drug resistance related macromolecular changes in leukemia and other cancer types can be analyzed using FT-IR technique.

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METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) POLYMORPHISMS IN PATIENTS TREATED WITH INTERMEDIATE-HIGH DOSE OF METHOTREXATE (MTX)

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Background. Association with MTHFR polymorphisms and toxicity or outcome of disease was investigated in haematological patients treated with methotrexate, with no univocal results. Methods and Aims:We retrospectively studied 22 patients (14 M / 8 F), mediane age 40 years , (range 27-63), affected by Non Hodgkin lymphoma (3 Mantle cells, 9 burkitt, 6 DLBC with 5 CNS localization, 1 anaplastic T cells, and 1 Acute Lymphoblastic Leukemia, submitted to different chemotherapeutic regimens, all containing intermediate high dose of MTX for a total of 61 courses. We divided patients in two groups:group A submitted to intermediate dose of MTX (1-2 gr/m2); group B with high dose of MTX (3-5gr/m2). We considered in particulary hepatic toxicity and incidence of mucositis, in association with delayed elimination of MTX. All patients were tested for MTHFR polymorphisms C677T and A1289C. *Results*. Group A: 9 patients, for a total of 25 courses. In 6/25 courses (24%), we have a delay in MTX elimination (>72h); hepatic toxicity of grade II-III were recorded in 7/25 (28%), mucositis in 16/25 (64%), 12 of grade I, 3 of grade II, 1 of grade III. Two patients showed a very high delay in MTX elimination (>120 hours), with mucositis of grade II without hepatic toxicity. Among 9 patients, 7 were mutated for C677T (5 heterozygosis, 2 homozygosis) and 2 were not mutated for both polymorphisms. The two patients with higher toxicity were not mutated. Group B: 14 patients, for a total of 36 courses. A delay of MTX elimination was seen in 20/36 (55%), hepatic toxicity in 18/36 (50%), 2 of grade III, 7 of grade II, 9 of grade I, mucositis in 21/36 (59%), 11 of grade I, 7 of grade II, 3 of grade III. In 9 out of 20 delayed MTX courses we recorded a very high delay (>120h) with hepatic toxicity in 6/9 and mucositis in 9/9 courses. Among these 14 patients, 3 showed C677T heterozygosis, 2 C677T homozygosis, 3 showed A1289C homozygosis and 6 showed both mutations in heterozygosis. In 12 out of 14 patients grade III-IV of haematological toxicity was seen: among these 12 patients, 8 were treated with an association with ARA-C, 3 were treated with polichemotherapy, while one patient received only MTX. Conclusions:We were not able to find any correlation between toxicity and MTHFR polymorphisms in this subset of patients.

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HLA ALLELES AND HAPLOTIPES: IMPLICATIONS FOR HEMATOPOIETIC STEM CELL DONOR SELECTION

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Background. Despite expansion of unrelated donor pools to a current state of more 10 million registered donors worldwide, matching each HLA allele between unrelated donors and recipients remains a problem for many patients because of HLA polymorphism. Consideration of other related donor available is appropriate for any patient who is in need of an allogeneic hematopoietic cell transplant but has no matching sibling or an unrelated donor or cord blood transplant. Analysis of common alleles and haplotipes may help to predict the chance of getting a suitable donor, and it also suggests strategies for improving success in the search. *Aims*. Our objective is to establish what HLA alleles and haplotypes are most common in our area. These results can be used to help to delineate search strategies for potential donors: related or unrelated *Subjects and methods*. The study includes 200 families residing in Andalusia, Spain. Informed consent was obtained in all cases. They were HLA-A*, B*, C* y DRB1* typed by high-resolution (Dynal RELITM SSO typing Kit, Dynal Biotech ASA, Oslo and Inno-Lipa HLA, Innogenetics N.V. Ghent, Belgium). Results. Only 35 HLA-A*, 55 HLA-B*, 23 HLA-C* and 39 HLA-DRB1 alleles were found. These alleles have been previously described in Caucasians as expected from the geographic origin of the studied families. The distribution of predominant alleles studied herein reveals great similarities with those in Spain, Western European and North American populations. In the analysis of haplotypes, their frequencies in the studied population were stimated by counting the number of any given haplotype among the total number of haplotypes. A high number were included within the most frequent haplotypes described in Spain. However, the results reveal that the ten most frequent haplotypes in our population were not the same as in other populations *Summary*. The data we present here on common alleles and haplotypes may help to predict the chance of getting a suitable related donor, and it also suggests strategies for improving success in the search

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PHARMACOGENETICS OF COUMARINS DOSING : PREVALENCE OF CYP2C9 AND VKORC1 POLYMORPHISMS IN THE LEBANESE POPULATION

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Background : Polymorphisms in the genes encoding the cytochrome P450 2C9 enzyme (CYP2C9) and the Vitamin K Epoxide Reductase (VKORC1) are known to contribute to variability in sensitivity to coumarins. Patients with certain common genetic variants of CYP2C9 (*2 & *3) or a VKORC1 polymorphism (- 1639A Allele) require a lower dose of coumarin and are also at higher risk for over-anticoagulation and serious bleeding. In August 2007, the FDA label for warfarin was updated to highlight the benefit of genetic testing to predict warfarin response. Aims : Since the prevalence of these variants in the Lebanese population has not yet been reported, our aim was to determine the genotypes of CYP2C9 and VKORC1 in our population and to compare allele frequencies with previous findings from other ethnic groups. Methods : CYP2C9 (*1/*2/*3) and VKORC1 (*A/*G) allelic variants were assessed by Polymerase Chain Reaction-Restriction Length Polymorphism (PCR-RFLP) assays in a diversified sample of 161 unrelated healthy Lebanese volunteers. Results : The allele frequencies of CYP2C9 *2 and *3 were 0.112 and 0.096 respectively, whereas VKORC1 -1639A was 0.528. Carriers of the CYP2C9 *2 or *3 represented 34.2% of the subjects, while those of the VKORC1 -1639A represented 73.9%. Conclusion : Our data show no significant difference in the frequency of CYP2C9 allelic variants when compared to the Caucasian population, whereas the allelic frequency of VKORC1 - 1639A was very high. Over 50% of the Lebanese population seem to be carrying more than two independent "risk"alleles, and is therefore potentially at high risk of over-anticoagulation.

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LIPOPROTEIN PROFILE IN IRON DEFICIENCY ANEMIA

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Background. The relationship between iron metabolism and atherosclerosis is under investigation. Previous studies in subjects with iron deficiency anemia (IDA) have shown affected activity of antioxidant enzymes and higher concentrations of markers of oxidative stress. Aims: The aim of the study was to investigate the lipoprotein profile of IDA patients. *Methods*. A total of 20 patients (males/females: 7/13, mean age 41±15 years) with IDA were retrospectively examined. They were eligible for participation if they met the following criteria: Hb<12gr/dl, MCV<80fL, RDW-CV>15%, serum ferrum <37 μg/L, serum ferritin <15μg/L. 20 age and sex-matched healthy subjects were also included in the study as the control group. Patients with endocrine, hepatic, renal diseases and under hypolipidemic medication were excluded. Serum iron parameters (ferrum, ferritin) were determined by electrochemiluminescence immunoassay, lipid parameters [total cholesterol (TC), high density lipoprotein (HDL), triglycerides (TG)] with enzymatic colorimetric test (Elecsys Modular E170, ROCHE) and hematological indices [hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)] by cytometry method (XE-5000 SYSMEX, ROCHE). Student's t test and Pearson's correlation analysis were performed. Results: In patients with IDA, TG levels were found to be significantly elevated (101,30±53,07 vs 95,56±42,43 mg/dl, p<0,05) and HDL-C concentrations significantly lower (49,15±12,92 vs 56,62±8,19 mg/dl, p<0,05) when compared to controls. TG/HDL-C ratio, which is considered indicative of the predominance of small, dense LDL particles, was found higher in IDA subjects (2,17 vs 1,70, p<0,05). No significant

differences were observed in TC and LDL-C concentrations as well as in TC/HDL-C ratio between patients and controls. Ht and Hb values presented a significant inverse correlation with those of TG (r= -0,35, p=0,031 and r= -0,34, p=0,039 respectively) and a significant positive one with those of HDL-C (r=0,386, p=0,020 and r=0,420, p=0,011 respectively). *Summary/Conclusions*. The findings suggest that lipid profile disorders are observed when serum iron and ferritin levels are decreased. The effect of IDA and its degree on atherosclerosis should be further investigated.

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IN VITRO DETERMINATION OF APOPTOTIC EFFECT OF HEPARIN ON LYMPHOBLASTS BY USING FLOW CYTOMETRIC DNA ANALYSIS AND MEASUREMENTS OF CASPASE-9 ACTIVATION AND CYTOCHROME C LEVEL

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Background: Heparin induces apoptosis on peripheral neutrophils, mononuclear cells of healthy subjects and on lymphoblasts of patients with acute lymphoblastic leukemia (ALL), in vitro. Aims: Caspase-9 activtity and cytochrome C level were studied to indicate the apoptotic effect of heparin on lymphoblasts by intrinsic pathway of apoptosis. Methods: Twenty bone marrow samples of the patients with ALL were included in the study. Cytochrome C level and caspase-9 activity were concomitantly determined with the percentage of apoptotic lymphoblasts when incubated in 0,10 and 20 U/ml heparin concentrations at 0,1 and 2 hours. Results: The percentages of apoptosis at first hour were higher than those at 0 and 2 hours in 10 and 20 U/ml heparin concentrations, separately ($p< 0.05$). The mean percentage of apoptosis in 20 U/ml heparin levels was significantly higher than those in 0 and 10 U/ml heparin levels at 1 and 2 hours ($p<0.05$). The highest apoptotic effect of heparin on lymphoblasts was determined at first hour in 20 U/ml heparin concentration. The mean caspase-9 activitity at first hour was significantly higher than those at 0 and 2 hours in 10 and 20 U/ml heparin levels, separately ($p<0.05$). The mean caspase-9 activity in 20 U/ml heparin concentration was significantly higher than those in 0 and 10 U/ml heparin concentrations at 1 and 2 hours ($p<0.05$). The highest caspase-9 activity was determined in 20 U/ml heparin levels at first hour. The mean Cytochrome C level at first hour was significantly higher than those at 0 and 2 hours in 10 and 20 U/ml heparin concentrations, separately ($p<0.05$). The highest cytochrome C level was determined in 20 U/ml heparin concentration at first hour. Conclusion: We claimed that heparin induces apoptosis on lymphoblasts by the activation of intrinsic pathway.

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META-ANALYSIS REGARDING SIMVASTATINUM TREATMENT IN MALIGNANT LYMPHOMA

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Background: Simvastatinum (S) is a competitive inhibitor of 3-hydroxide-3-methylglutaryl CoA reductase, a cholesterol and some isopreonid products synthesis regulatory enzyme, like geranyl-geranyl pyrophosphate. Except the hypocholesterolemiant effect it also has anti-neoplastic pleiotropic properties. Aims: Our purpose was to study the existing literature arguments for the use of simvastatinum as an adjuvant therapy in malignant lymphoma. Methods: We analyzed the 10 existing studies in MEDLINE, in January 2011 regarding S therapy in lymphoma. Our study included: the types of lymphoma where it was use, therapeutic action, the results of the experimental studies and its experience in clinical study usage. Results: S was administrated in various lymphoma cells: a mouse model of Hodgkin's lymphoma (HL), Epstein-Barr virus (EBV)-driven lymphoma cells, Waldenstrom macroglobulinemia (WM) cells, adult T-cell leukemia cells (ATL), chronic lymphocytic leukemia (CLL) cells and in lymphoma patients. S induced caspase-related apoptosis via depletion of prenylation-substrates in several HL-cell lines. S induces apoptosis, G2/M cell cycle arrest, accumulation of p21(Waf1/Cip1), and effectively inhibits hyperproliferation of human Namalwa Burkitt lymphoma cells that display general apoptosis resistance and hyperproliferation. S inhibited cell growth, survival and IgM secretion on WM cells by inhibiting synthesis of geranylgeranyl pyrophosphate. S binds to the inserted domain of leukocyte function antigen 1 and inhibits its function, resulting in down-regulation of nuclear factor kappaB activity and induction of apoptosis of transformed B cells and EBV-transformed lymphoblastoid cell lines. S blocks the interaction of adhesion molecules that are important for cell-cell interactions including those between EBV-transformed B cells. S decreased Akt and extracellular signal-regulated kinase mitogen-activated protein kinase pathways. In ATL statins inhibited geranylgeranylation of small GTPases Rab5B and Rac1. In CLL cells S induced apoptosis concurrently with lowering of BCL-2/BAX ratio; its pro-apoptotic effect is tumor-specific, not affecting normal lymphocytes. Combinations of simvastatin + fludarabine and simvastatin + cladribine had a synergic effect in inducing apoptosis. In S lactone inhibited P-glycoprotein mediated rhodamine 123 transport in a murine monocytic leukaemia cell line that over-expresses the multi-drug resistance protein 1a/b. In vitro S induced apoptosis in a dose- and time-dependent way. In a mouse model for HL it effectively impaired tumor growth. It delayed development of EBV lymphomas and prolonged survival of animals. It induced inhibition of proliferation, had cytotoxic effect and produced apoptosis of WM cells. Furthermore, S enhanced the cytotoxicity induced by bortezomib, fludarabine and dexamethasone. Statins hinder the survival of ATL cells and induce apoptotic cell death. In combination with dexamethasone and doxorubicin, statins have a chemo-sensitizing effect and the patients with relapsed lymphoma can be treated with a dose-escalating regimen of simvastatin for 7 days followed by CHOP. High-dose simvastatin given immediately prior to chemotherapy was safe and tolerable up to a dose of 15 mg/kg/day. Summary: S inhibits geranylgeranylation - a critical process for the regulation of lymphoma tumor cell survival and proliferation. The pro-apoptotic effect of S is tumor-specific, not affecting normal lymphocytes. S has a chemo-sensitizing effect, useful in patients with relapsed lymphoma. By the P-glycoprotein inhibition it can reduce the multi-drug resistance.

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USING A NEW INTEGRATIVE APPROACH WITH SIXTEEN REFRACTORY CASES IN PEDIATRICS HEMATOLOGY/ ONCOLOGY AT KING ABDULAZIZ UNIVERSITY HOSPITAL IN SAUDIA

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Background. The goal of health care is to find the most effective and cost-effective approach to prevention, health promotion, and treatment of illness, considering the use of both conventional medicine and complementary and alternative medicine (CAM) therapies. Integrative oncology, the use of CAM in conjunction with standard medical treatment, seeks to improve the supportive care available to patients, while also determining through scientific clinical trials which adjuvant CAM therapies are medically sound, effective, and compatible with standard chemotherapy and radiation. Aims. To report the effectiveness and safety of a natural experimental agent used with conventional therapy in sixteen relapsed cases with different hematological malignancy who were refractory to conventional treatment. Methods. A phase I controlled clinical trial to use the Novel Experimental PM 701 combined with conventional chemotherapy in induction and maintenance phases in the treatment of refractory and relapsed cases in Pediatric Hematology/Oncology, in a period of 2005 to 2009, treated at King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia. This natural agent has been fully studied with animal models and tissue cultures at the King Fahd Medical Research Center's laboratory at King Abdulaziz University and showed potent selective apoptosis of cancer cells, effective and safe in animal models. Results. All the sixteen cases showed complete remission through induction phase, by using the novel experimental PM 701 combined with conventional therapy. Fifteen cases alive ;with eleven patients off treatment. One patient with known case T-ALL died after a year refractory to the treatment; due to the shortage of supply of the experimental PM701 while in maintenance. Summary. We report in this phase I clinical trial a successful, effective, and safe management with the combination of conventional therapy with experimental medicine (PM 701) in the treatment of sixteen refractory and relapsed cases in Pediatric Hematology / Oncology. Complementary and alternative therapies need further control studies in the future to prove the efficacy and safety to be used on oncology patients.

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APOPTOSIS INDUCTION MEDIATED BY HUMAN PARVOVIRUS B19 NON-STRUCTURAL PROTEIN (NS1) IN HUMAN ERYTHROLEUKEMIA CELL LINE THROUGH ACTIVATION OF P53 GENE

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Background. Many human leukemias do not express detectable levels of p53. It has been demonstrated that loss of p53 gene expression is likely to be an important step in leukemogenesis. This could be result from the loss of proper trans-acting factors or mutational/epigenetical silencing of cis-acting elements in regulatory regions of the gene. Apoptosis induction in leukemic cells by agents which trigger cancerous cells specifically is an active area in the field of treatment of leukemia. *Aims*. The objective of this in vitro study was to determine the application of NS1 protein of parvovirus B19 to induce p53 activity and apoptosis in K562 cell line as a p53-null erythroleukemia cell line. Methods. We have developed a lentiviral vector system to deliver the NS1 gene into K562 cells. Using qRT-PCR and protein analysis, we determined levels of p53 gene expression in transduced cells. Morphological studies as well as annexin-V and caspase-3 analyses were performed to assess apoptosis in NS1-transduced cells versus control. *Results*. Transduction of K562 cell line by lentiviral vector was resulted in 84% efficiency of NS1 gene delivery as measured by flowcytometry. Gene expression analysis in both RNA and protein levels showed strong activation of p53 gene in NS1-transduced cells versus mock virus-transduced cells. NS1 gene induced mean of 63% of K562 cells to undergo apoptosis within 4 days, which was five-fold increase over background level. Analysis of caspase involvement showed that NS1 gene could activate caspase-3 and induce apoptosis. *Conclusions*. NS1 protein of parvovirus B19 could be a good candidate in treatment of leukemia, due to high tropism of B19 virus to hematopoietic system and its ability to induce apoptosis through activation of p53 pathway in leukemic cells as demonstrated in the present study.

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INFLUENCE OF NOVEL 5-AMINO-1,2-DIHYDROPYRROLE-3-ONE DERIVATIVE WITH ANTIPROLIFERATIVE ACTIVITY ON THE RAT BLOOD CELLS PARAMETERS AFTER TWO MONTH ADMINISTRATION IN THERAPEUTICAL DOSE

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Background. According to the preliminary studies the 5-amino-1,2-dihydropyrrole-3-one derivative (DD-1, 1-(4-(2-ethyl)morpholine)-4-(benzo[d]thiazol-2-il)-5-amino-1,2-dihydropyrrole-3-one)) suppresses proliferative activity of the tumor cells (HCT-15 Colon Cancer, UACC-62 Melanoma, A549/ATCC Non-Small Cell Lung Cancer, SR and K-562 leukemias et al.). DD-1 was designed as inhibitor of ATP-binding sites of protein kinases and synthesized by scientific production of Chemical-Biological Centre of Taras Shevchenko National University of Kyiv. The aim of the study was determination of DD-1 effects on blood cells parameters in healthy rats and under the action of 1,2-Dimethylhydrazine induction of colon tumors after two month administration. Methods. The outbred male rats was divided into four groups (10 rats in each group): I - control group (treated 0.1 ml of the sunflower oil containing 15% of DMSO per os daily and 0.1 ml of saline subcutaneous every week), II - group treated with 1,2-Dimethylhydrazine (20 mg/kg in saline subcutaneous every week, for induction of colon tumors, and 0.1 ml of the sunflower oil containing 15% of DMSO per os daily), III - group treated with DD-1 (2.3 mg/kg in 0.1 ml of the sunflower oil containing 15% of DMSO per os daily and 0.1 ml of saline subcutaneous every week), IV - group treated with DD-1 (2.3 mg/kg in 0.1 ml of the sunflower oil containing 15% of DMSO per os daily) and 1,2-Dimethylhydrazine (20 mg/kg subcutaneous every week) during two month. A DD-1 dose of 2.3 mg/kg corresponds to the 100% inhibition of tumor cells proliferation in vitro. Rat blood cells parameters were determined by routine method.Results. It is shown that DD-1 in the dose of 2.3 mg/kg does not effect erythroid parameters (erythrocytes count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)) and leukocytes vs. control group after two month administration. 1,2-Dimethylhydrazine does not change none of blood cells parameters after two month administration. In IV-group treated with DD-1 and 1,2-Dimethylhydrazine MCH was decreased (18.05±0.31, $p<0.05$) and MCHC has a tendency to decrease (320.29±5.51, $p=0.07$) vs. control group (19.43±0.16, 335.69±3.66, respectively) after two month administration. The other parameters did not change. Conclusions. DD-1 does not affect the blood cells parameters in healthy rat in indicated dose and does not restrict application of this compound as a potential anti-tumour drug.

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IAP INHIBITORS INDUCE APOPTOSIS IN CHILDHOOD ACUTE LEUKEMIA CELLS VIA NF-KAPPAB-ACTIVATION AND TNFALPHA-SECRETION IN A RIP1-DEPENDENT MANNER

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Children with high risk acute lymphoblastic leukemia (ALL) or relapse of ALL 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 improve the poor prognosis. Since *"Inhibitor of Apoptosis"*(IAP) proteins are expressed at high levels in acute leukemia and block apoptosis at a central point of the apoptotic machinery, they may present a suitable molecular target for therapeutic intervention. We already showed that neutralizing IAP proteins by small molecule IAP inhibitors is an effective approach to sensitize childhood acute leukemia cells for death receptor- or chemotherapy-induced apoptosis. Here, we report that in some acute leukemia cell lines small molecule IAP inhibitors alone, also at nanomolar concentrations, induce apoptosis. Cell lines which are sensitive for apoptosis induction by IAP inhibitors alone show rapid degradation of cIAPs, activation of NF-kappaB and secretion of TNFalpha, leading to an autocrine, apoptosis inducing TNFalpha-loop. Further analysis of signaling pathways reveals that IAP inhibition causes TNFalpha-dependent loss of mitochondrial membrane potential, caspase activation and apoptosis. In addition this signaling pathway is dependent on the availability of RIP1. RIP1-knockdown leukemia cells show significant reduction in IAP inhibitor induced loss of mitochondrial membrane potential, caspase activation and apoptosis. Also primary leukemia cells are in part sensitive for IAP inhibitor induced cell death. Since not all acute leukemia cell lines and primary leukemic cells are sensitive for IAP inhibitor induced apoptosis, it is very important to find markers, which indicate IAP inhibitor-sensitivity and provide a new successful treatment approach for acute leukemia.

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SELECTIVE MICRO ERYTHROCYTES APHERESIS (SEMEA): A NEW THERAPEUTIC PROCEDURE FOR THE TREATMENT OF POLYCYTHEMIA VERA (PV)

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Background. Polycythemia Vera is a myeloproliferative neoplasm characterized by increased red cell mass; in its course, this pathology often shows complications and death's causes associated with thromboembolic phenomena. In some periods of the disease, the typical framework of peripheral blood count is the presence of hypochromic microcytic polyglobulia with a highly mixed red cell population (with elevated RDW, but normal Hb and Hct). However high number of microcytes contributes to issues related to the overload of the microcirculation, while these cells, having low level of Hb, do not contribute to the

oxyphoretic capacity of the blood. Classically, the reduction in RBC mass is obtained with bloodletting by phlebotomy, in order also to induce hyposiderosis and iron-restricted erythropoiesis. Aims. In this study we report the preliminary data obtained with a new procedure of selective erythroapheresis, called SEMEA: SElective Micro Erythrocytes Apheresis, that we have designed in order to remove from the circulation mainly the microcytes. We have evaluated if this innovative and original procedure is able, compared to classis phlebotomy, to take away an increased red cell mass, and if it is also able, respect to non-selective erythroapheresis, to take a selected population of cells: the microcytes. *Methods*. During 2010, 10 male patients affected with PV, aged 64.5±7.94, were treated, after informed consent, by SEMEA, each underwent to a cycle of 6 apheresis in 30 days. Therapeutic procedures were performed with cellular separator Haemonetics MCS+, using the 971E kit for collection of PBSC, but opportunely modifying some parameters: a) AC ratio from 1:9 to 1:12; b) start collection set to 70%; c) detected RBCs set at 12%; d) removed volume of RBCs concentrates per cycle: 40 mL; e) no recycling; f) changing the original bowl with another one of 225-mL. *Results*. Observed data are expressed as mean±SD. Before our treatment: Hct = 48.98±4.4%, Hb = 14.3±1.51 g/dL, RBC = 6.83±0.2 x106/μL, MCV = 71.25±7.94 fL, RDW = 17.33±1.51%. Observed values on all collected units: RBC = 3.12±0.26, MCV = 69.75±6.65, RDW = 17.35±1.24. After SEMEA: Hct = 46.78±3.94, Hb = 13.9±1.71, RBC = 6.05±0.1, MCV = 72.5±6.85, RDW = 16.83±1.34. Conclusions. At the end of this first experimental course of treatment, we observed that: 1) the procedure was effective in reducing polyglobulia; 2) slight increase of MCV in vivo; 3) the MCV of collected RBCs was always 2-3 fL lower than basal MCV of the patient; 4) not significant change in Hb before and after SEMEA. Moreover the treatment has been well tolerated, in fact patients showed no adverse reactions despite the longer duration of the procedure compared to standard therapeutic erythroapheresis (50 vs 15 minutes). This original method, in addition to the reduction in RBCs count, has the advantage of affecting the real pathological cellular fraction of PV, as demonstrated by decreasing of RDW. On the basis of our results, we are encouraged to propose SEMEA to patients affected with PV showing a marked polyglobulia with hyposideremic anisopoikilocytosis after a series of bloodletting by phlebotomy.

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DATA OF ADVIA 120® RBC MATRIX ARE USEFUL TO DISCRIMINATE B12/FOLATE DEFICIENCY, CHEMOINDUCED AND BETA THALASSEMIA MINOR ANAEMIA

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Background. In automated peripheral blood cell analysis, ADVIA® technology (Siemens) provides a lot of information regarding also red blood cells (RBC). Among these information, those regarding red blood cells (RBC) contained in RBC matrix might be useful to define different aetiology of anaemia. Aims. Aim of this study is to define if information contained in RBC matrix is useful to define different aetiology of anaemia, before that data regarding iron balance, B12, folates, DAT and IAT and haemoglobin electrophoresis are available. Methods. We evaluated 148 patients with moderate/severe anaemia. M/F was 57/91. Median age was 65 years (R23-87). 14 patients presented B12 and folates documented anaemia (9%), 68 with chemoinduced anaemia (45%), 9 with beta thalassemia minor (6%) , 57 with sideropaenia (38%). Data were analyzed using RBC matrix of ADVIA 120® automated blood cell analyzer. In this matrix volume of RBC is plotted with haemoglobin concentration of RBC. In this matrix ther'are 9 box (RBC macro - cytic/hypocromic, macro/normo, macro/hyper, normo/hypo, normo/normo, normo/hyper, micro/hypo, micro/normo, micro/hyper). In each box ADVIA 120® provide absolute and percentual number of RBC with above mentioned charachteristics. Results. Among 14 patients with B12 and/or folate deficiency, 11 (78.6%) presented RBC simultaneously macrocytic/normochromic >2% and normocytic/hyperchromic>2%. In remaining 134 patients with anaemia without B12 and/or folate deficiency only 10 (7.5%) showed the same charachteristics, with a Chi Square Yates corrected of 46.962 ($p<0.0001$), with an Exact Fisher text with $p<0.0001$, with a sensibility of 78%, a specificity of 92% and a predictive negative value of 97.6%. Among 68 patients with chemorelated anaemia, 10 (14.7%) presented RBC normocytic/hyperchromic >2% . In remaining 80 patients only 3 showed the same charachteristics, with a Chi Square Yates corrected of 4.2 ($p<0.04$), with an Exact Fisher text with $p<0.038$, with a sensibility of 15%, a specificity of 96% and a predictive positive value of 77%. Among 9 patients with beta thalassemia minor, 8 (89%) presented RBC simultaneously microcytic/hypochromic >2% and microcytic/normochromic>2%. In remaining 139 patients no patient showed the same charachteristics, with a Chi Square Yates corrected of 113.8 ($p<0.0001$), with an Exact Fisher text with $p<0.0001$, with a sensibility of 89%, a specificity of 100% and a predictive positive value of 100% and a predictive negative value of 99%. Summary and Conclusions. ADVIA 120® RBC matrix is useful to define B12 and/or folate deficiency anaemia (RBC simultaneously macrocytic/normochromic >2% and normocytic/hyperchromic>2%), chemorelated anaemia (RBC normocytic/hyperchromic >2%), beta thalassemia minor (RBC simultaneously microcytic/hypochromic >2% and microcytic/normochromic>2%). These data need confirmation on a larger cohort of patients.

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JUVENILE HAEMOCHROMATOSIS

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Juvenile haemochromatosis (JH) is the most severe form of inherited iron overload usually affecting either sex before the age of 30. Sadly the first manifestation of JH can be sudden cardiac failure. In Europeans the disease is associated with heart disease, diabetes, infertility and liver disease. In Asian patients JH associated heart disease is rare. The genetic picture with JH is not simple. JH is usually attributed to mutations in either hemojuvelin (HJV,HFE2) or hepcidin (HAMP). However JH can also be due to combinations of mutations in HFE and hepcidin or transferrin receptor 2 (TFR2) and HFE. JH rarely results from mutations in TFR2 alone. Hence in the majority of cases autosomal recessive inheritance of mutations in HJV, HAMP or TFR2 explain JH. Rarely combinations of mutations (digenic inheritance) are the explanation. Most commonly JH is due to mutations in the HJV gene. Mutations in HAMP gene are rarer and tend to be more severe. Nevertheless both conditions are rare. In Europeans the most common HJV mutation is G320V and in Asians G99R. The range of mutations is such that detailed genetic analysis is required for individual families. The age of onset may be determined by the ethnic background and the location of the mutation in HJV. Mutations in highly conserved residues result in severe disease. As part of the haemochromatosis screening service that has been set up in Oxford we have received requests to investigate the genetic basis of iron loading in a number of C282Y negative patients and their families. We have had 80 requests from referring departments to perform mutation screening in the genes encoding ferroportin, hemojuvelin, hepcidin and transferrin receptor 2. Apart from one case all had unexplained increased serum ferritin, some elevated transferrin saturation. Here we describe those requests relating to JH. In the first case the proband sadly died and it was a consequence of the postmorten that the diagnosis of JH was made. He was found to be negative for C282Y HFE mutations and the family were initially denied further screening. The proband was found to be homozygous for the common G320V HJV mutation. The second case was a family of Asian origin living in the UK where the proband was found to be homozygous for the common Asian mutation G99R. This family is not related to families living in the Midlands with the same mutation which had been described previously. The third case is a British male with congestive heart failure. He was referred to Oxford for further screening. He was found to be homozygous for the rare C80R HJV mutation that had previously only been described in a compound heterozygote living in France. From our findings we strongly recommend that any patient with unexplained raised serum iron parameters, found to be negative for HFE mutations is screened for mutations in HJV, HAMP, TFR2 and ferroportin.

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FERROPORTIN DISEASE, 4 NOVEL MUTATIONS

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The different genetic forms of HH share as their pathogenic mechanisms the deficiency or dysregulation of the hormone hepcidin or defects involving the hepcidin receptor, ferroportin. Ferroportin is the sole known cellular iron exporter in humans. Normally the cell surface concentration of ferroportin is regulated by its interaction with hepcidin. Hepcidin binding triggers the internalisation and degradation of the ferroportin-hepcidin complex, causing a decrease in cellular iron release into plasma. Mutations in the ferroportin SLC40A1 gene cause iron over-

load syndromes with AD inheritance. Ferroportin mutations result in spectrum of phenotypes, depending on the functional alteration of the protein. At one end of the spectrum, loss of function mutations, leads to increased macrophage iron and elevated serum ferritin, but normal transferrin saturation, and is known as "ferroportin disease". At the opposite end of the spectrum a distinct phenotype, similar to classical haemochromatosis, is associated with gain of function mutations. These mutations cause ferroportin resistance to the effects of hepcidin by preventing internalisation of the hepcidin-ferroportin complex From our cohort of patients that had been sent in for ferroportin gene screening 2 known and 4 novel mutations were identified. The novel mutations in exon 5, W158C, D157A and D157Y are all in highly conserved amino acid residues. Four different mutations have been found at codon 157. The mutation D157Y was found in an individual from north India. A molecular mechanism for mutations in this position can be explained by the D157G mutation in ferroportin leading to hepcidin-independent binding of JAK2 and ferroportin downregulation. Two previous ferroportin mutations had been found in India and Thailand and these were V162del and C326Y. They are rare mutations in this ethnic group. The mutation found in exon 8, H507R is a highly conserved amino acid residue which is predicted to be in the transmembrane domain (codons 492-514 in exon 8).

Table 1: Ferroportin mutations and their origin

Family	Protein	Exon	Nucleotide change	Amino Acid change	Origin
A	Ferroportin	3	c.239 G>A	G80E	Caucasian
B	Ferroportin	3	c.430 A>G	N144D	Caucasian
C	Ferroportin	5 (HOMOZ)	c.470 A>C	D157A	Caucasian
D	Ferroportin	5 (HOMOZ)	c.469 G>T	D157Y	Indian
E	Ferroportin	5 (HOMOZ)	c.474 G>T	W158C	Caucasian
F	Ferroportin	8 (HOMOZ)	c.1520 A>G	H507R	Caucasian

Table 2: Genotype and phenotype of families with type 4 haemochromatosis

Family	Sex	Age at diagnosis	TSat %	Ferritin µg/L	Serum iron µmol/L	TIBC mol/L	FPN mutation
A	F	43	H	HYPERFERRITINAEMIA	H	WD	G80E
B	M	30	H?	3782	38	38.3	N144D
C	M	60	H	>3000	H	WD	D157A
D	M	27	H	725	H	WD	D157Y
E	M	54	H	>2000	H	WD	W158C
F	M	66	H?	1350	RAISED	WD	H507R/CT

A published mutation p.Y501C has been found in the same transmembrane domain, this causes non-classical ferroportin disease. All individuals in the family were also heterozygous for C282Y mutation. Ferroportin disease is a clinically and genetically heterogeneous iron overload syndrome. Our results demonstrate the importance of screening patients found to lack HFE mutations for ferroportin mutations.

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TRANSFERRIN POLYMORPHISM AND ANEMIA IN HIV

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Background. Anemia is a common finding in developing countries, especially among women. Moreover, anemia is frequently observed in HIV, with prevalence up to 30% in patients with asymptomatic infection and in as many as 80% in those with AIDS. Importantly, anemia is associated with decreased quality of life and shortened survival. The main cause of deaths in HIV is opportunistic infection. Therefore we explored if anemia, and more especially if iron related parameters correlated with the risk to get opportunistic infections. More specifically we examined the impact of the genetic polymorphism of human transferrin (TF) since. it has been reported that the transferrin CD phenotype was associated with variations in certain measures of iron status. In Caucasians the common (CC) TF phenotype is almost found exclusively. Aims: To determine the prevalence and risk factors of anemia and the influence of HAART on anemia. To explore the relationship between transferrin polymorphism, anemia and the frequency of opportunistic infections. Methods: We conducted a cross-sectional study among 200 HIV positive and 50 HIV negative women in Butare University Teaching Hospital in Rwanda. Informed consent was obtained from all women. Complete physical examination was carried out and WHO HIV disease stage classification, hematological parameters, CD4 count, and iron related parameters were determined. TF phenotypes were determined using starch gel electrophoresis. *Results:* The prevalence of anemia was significantly higher among HIV positive women (29%) than in HIV negative women (8%), p<0.001. Risk factors for anemia (OR and 95% CI shown in brackets) were lower body mass index (OR:3.4[2.4-4.1]), Zidovudine use (OR:1.14[1.01-1.29]), lack of HAART (OR:1.44 [1.21-1.67]), oral candidiasis (OR:1.4[1.2-1.6]), pulmonary TB (OR:1.8[1.7-2.2]), cryptococcal meningitis (OR:1.6[1.21-1.8]), *Pneumocystis jiroveci* pneumonia (OR:1.41[1.20-1.65]) and CD4 lymphocyte count <200 cells/mL (OR:2.41[CI:2,01-3.067]). The mean hemoglobin of 10.9 ± 1.6 g/dL at HAART initiation significantly increased to 12.3±1.5g/dL in 8 months (p<0.001). For the transferring phenotype: The TF phenotype frequencies of TF CD, CB and CC were 14.5 %, 3 % and 82.5 %, respectively. The homozygous TF DD phenotype was not found. Subjects with TF CD phenotype had a significantly lower frequency of opportunistic infections than subjects with TF CC phenotype, 24% and 48% respectively (P=0.026). Subjects with TF CD phenotype had significantly lower values for serum beta2 microglobulin (P=0.0004) and transferrin (P=0.006) compared with TF CC subjects. There was a trend towards lower serum iron concentrations in subjects with TF CD (P=0.08). Overall hematological parameters, ferritin, transferrin saturation, CRP and CD4 count did not differ according to TF phenotype. Conclusions: The prevalence of anemia increases with HIV stage. HAART is associated with a significant improvement in hemoglobin levels. The outcome of HIV infection differs according to the various TF phenotypes. Iron status may play a role in this association. Subjects with TF CD phenotype have a major advantage in HIV infection since they have a lower frequency of opportunistic infections. They also have lower concentrations of serum beta2 microglobulin, associated with good prognosis in HIV infection.

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AGE-RELATED CHANGES OF PERIPHERAL BLOOD COUNTS IN MAN

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Anaemia is becoming a common concern in geriatric health. Even though its prevalence var-ies quite significantly among different groups depending on factors such as ethnicity, lifestyle or fitness, the appropriateness of current WHO definition of anaemia in the elderly may therefore be questioned. We have evaluated peripheral blood parameters from 1,724 individuals (908 women aged 18 to 101 years and 816 men aged 18 to 96 years), who were seen at the University of Heidelberg Medical Center in the absence of a known haematological history. Patients with a known malignant haematological or oncological disease, with chronic infec-tion or inflammation were excluded. Patients with disorders affecting the kidneys, thyroid or stomach as well as patients with a bleeding history, hemolysis or who had been previously diagnosed to have anaemia were excluded from this study. Average haemoglobin levels for men beyond the age of 70 and for women beyond the age of 80 were found to fulfil the WHO criteria for the diagnosis of anaemia. While in our cohort already ~ 20 % of men and women between 60-69 years of age were by definition anaemic, these numbers were steadily in-creasing to up to 63 % in females and to 76 % in males beyond the age of 90. Based on the results of our study and in accordance with the literature to this topic we suggest age-adjusted criteria for the diagnosis of anaemia in the elderly in conjunction with a geriatric assessment.

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HEPCIDIN, A NEW HORMON OF IRON HEMOSTASIS

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Background. Anemia of chronic disease (ACD) results from 3 major processes: slightly shortened red cell survival, impaired reticuloendothelial system iron mobilization, and impaired erythropoiesis. Hepcidin is an acute-phase protein with specific iron regulatory properties, which, along with the anemia seen with increased hepcidin expression, have led many to consider it the major mediator of ACD. Hepcidin is a principal iron regulatory hormone and its expression is stimulated by cytokines. The aim of this study was to determine serum levels pro-hepcidinin in ACD anemia. Methods: The study included 115 patients, 72 males and

46 females. Anemia was defined as hemoglobin below 12 g/dl in females and 13 g/dl in males. We have 68(57.6%) anemic patients, 37(31.4%) have ACD, 17(14.4% IDA), 11(9.3%) ACD= IDA and 50(42.4%) no anemic patients. TNFα, interleukin IL6 and levels were determined by Immulite 1000. DRG ELISA kits were used for prohepcidine determinations. Independent Sample Test, Anova test, Chi-Square Tests was used for statistical analysis. Results: Serum prohepcidin, IL6, TNFα concentrations observed in ACD vs IDA is (329.42+263.22 vs102.63+38.63 p=0.000, 11.8+8.9 vs 4.4+5 p=0.000, 9.8+6.4 vs 8+5.3 p=0.012respectively). Serum prohepcidin concentration have a strong correlation with serum ferritin and IL6 concentration(r =.226, p=0.041 r =.309p=0.006 respectively) and IL6 have a strong correlation with serum ferritin and TNFα (r=.215 p=0.031, r=.507 p=0.000 respectively). Conclusions: We suggestion that hepcidin is a principal iron regulatory hormone in ACD and its expression is stimulated by IL6 cytokine.Serum prohepcidin concentration is the best marker to diagnose ACD.

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HEMOGLOBIN EXTREMADURA [?64(E8)GLY>SER + ?133(H11)VAL>LEU]: A NEW MOLECULAR ANALYSIS AND CORRECTION OF A HAEMOGLOBIN PREVIOUSLY PUBLISHED

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Unstable hemoglobin (Hb) variants account for 9.5% of structural hemoglobinopathies. The majority of these unstable variants are the result of gene point mutations resulting in the substitution of a single amino acid by another. The presence of two mutations in the same allele is infrequent: of the 781 variants of the beta globin cluster described, only 32 are due to two point mutations (4.1% Hemoglobin (Hb) Extremadura is a structural variant that is included within the so-called unstable Hb. It was described in 1989 by Villegas et al., employing the most pioneering techniques available at that time, reverse phase HPLC to separate the abnormal chain (βX) digesting it with trypsin and analysis of the fragments with an automatic analyzer. The carrier was a 27-year-old who had a slight slight splenomegaly and hemolytic anemia (Hb 11.0 g / dL, PCV 31.5%, RBC 3.4 $x10^{12}$ / L; MVC 92fL, MCH 32.1 pg; MCHC 34.9 g/dL; Retis 5%), Heinz bodies (+). The Extremadura is electrophoretically silent Hb on cellulose acetate at alkaline pH, isoelectric focusing (IEF) and ion exchange HPLC, except on citrate agar at acid pH observed a diffuse band between Hb S and Hb C. By reverse phase HPLC eluting behind the chain βA (βA, βX and αA). Over these 20 years have maintained the values at diagnosis (Hb 10.5 g / dL, PCV 34.2%, RBC 3.2x10^{12}/L, MCV 106.4 fL, MCH 32.5 pg, MCHC 30, 6 g/dL; Retis 2.5%). She has had a daughter, who also presents mild splenomegaly and signs of minimal hemolysis (Hb 12.1 g/dL, PCV 38.0%, RBC 3.5 x10^{12}/L , MCV 109.7 fL, MCH 34.9 pg, MCHC 31.8 g / dL; Retis 2.2%), presence of Heinz bodies. At that time it made the study of globin chains by reverse phase HPLC, showing abnormal β chain compatible with the mother. It was decided to complete the study by sequencing the gene β globin, both mother and daughter, being a double mutation in the CD 64 of the 2nd exon GGC-->AGC (Gly-->Ser) and the CD 133 of the 3rd exon GTG-->CTG (Leu-->Val). The correct molecular characterization of these two point mutation Hb variants facilitates the understanding of how they influence changes in Hb stability, solubility and function (oxygen affinity), ultimately responsible for the clinical manifestations of the hemoglobinopathies, and permits the prediction of possible interactions with other Hb mutations, thus allowing more accurate genetic counseling) illustrating how infrequent these double mutations.

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HEMOGLOBIN SETIF IN NORTHERN GREECE. EPIDEMIOLOGICAL SURVEY

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Hemoglobin Setif (α 94 Asp-Tyr) was first described in 1972 in Algeria and since then several cases have been reported in families in Iran, Lebanon, Saudi Arabia and other Middle East states. The substitution in Hb Setif is in the α1β2 contact region of the molecule. This site of amino acid substitution in Hb Setif is an important contact point between α and β chains in the oxyconformation. Oxygen affinity is only slightly reduced nevertheless mild instability is produced. It has been reported that the most remarkable property of Hb Setif is its capacity to induce pseudo sickling of red cells in vitro. This does not have any clinical significance. Identification of Hb Setif is made by hemoglobin electrophoresis and ion exchange high pressure liquid chromatography (HPLC) as well as DNA studies. Hb Setif moves to the position of Hb S on starch gel and cellulose acetate at both alkaline and acidic pH. Hb Setif and Hb A separate on cation and anion exchange HPLC. The percentage of Hb Setif is 12-15% in heterozygotes, and it is reported that it could be as high as 30% in a compound heterozygotes with alpha and beta thalassemia mutations in the same person without any clinical consequences. The aim of this study was to investigate the incidence of Hb Setif in Northern Greece. A total of 78.254 subjects were screened for hemoglobinopathy in our prevention unit that covers the regions of Central and Western Macedonia in Northern Greece with a population of around 2.5 million. The carrier identification was carried out by a standard scheme, which included, CBC and red cell indices using the Coulter ONYX, a Cation Exchange HPLC variant system (Bio-Rad, Variant β-Thalassemia Short Program), to determine HbA, HbA2 and HbF levels.The different abnormal structural hemoglobins were investigated by electrophoretic techniques both at alkaline pH on cellulose acetate and at acid pH on citrate agar. Sickling test and tests for HbH inclusion bodies were also performed. Haemoglobin A2 was also quantified by column micro chromatography and serum ferritin levels were measured by micro Elisa technique. Only 6 individuals were found to be heterozygotes for Hb Setif and two cases were compound heterozygotes of Setif / beta thalassaemia. In heterozygotes the percentage of Hemoglobin Setif was from 16,2-17,4% and Hb A2 was within the normal limits. The combination with beta thalassemia was characterized by low hemoglobin Setif levels in the range as low as 2.2 to 2.7% unlike of the double heterozygosity. Both heterozygotes and compound heterozygotes did not manifest any clinical problem. Our data indicate that Hemoglobin Setif is rare in our country and clinically silent. Identification is important for genetic counseling of families since the coinheritance with beta thalassemia does not affect the hematological and clinical manifestations of the carriers. The fact that levels of hemoglobin Setif in compound heterozygotes with beta thalassemia genes are low as seen in our two cases is an interesting finding.

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STUDY OF THREE FAMILIES WITH HEMOGLOBIN AGRINIO IN SPANISH POPULATION: THREE HOMOZYGOTES CASES

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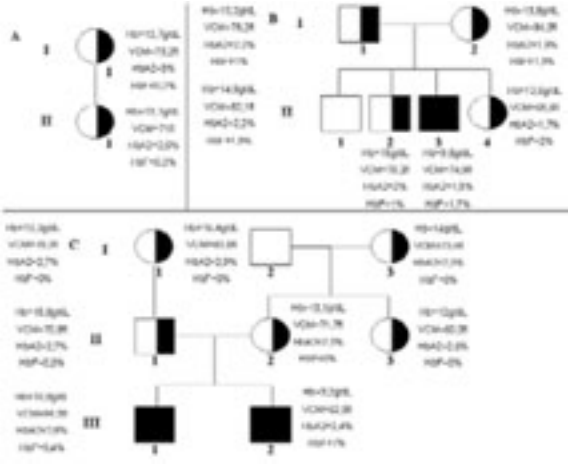
Background. Most α-thalassemia determinants are deletions involving one or both of the duplicated α-globin genes, however, non-deletional α-thalassemia mutations affecting RNA translation, RNA processing, or causing post-translational instability have been described. Hb Agrinio [CD29 (B10) Leu>Pro α2] is an hyperunstable structural variant of chain α wich phenotype is due to a postraduccional precipitation of the structurally anomalous chain in the erythroid precursors. This would explain the absence of abnormal globin chain in the electrophoretic and chromatographic studies. The first case of homozygous Hb Agrinio was described a Greek child at 12 months of age had a marked hypochromic microcytic anemia. *Aims*. We show three families with Hb Agrinio. In three of cases caused a intermedia thalassemia because they were homozygous. It is the first time described in spanish population. Methods. 16 samples of 3 different families were collected and studied to show microcytosis with normal HbA2 and HbF. Genomic DNA was extracted from peripheral blood leukocytes, employing a Bio-Robot EZ1. α gene deletions were ruled out by α-thalassemia StripAssay and the molecular characterization was studied by automated DNA sequencing with BigDye v1.1, specific for α2 gen. Results. We found 11 heterozygous cases, 3 homozygous cases and 2 cases with the triplication of α genes without Hb Agrinio. Moreover, the triplication appears in 2 subjects with Hb Agrinio. The individual genotipe of cases in study are:

Family a) I1: αAgα/αα; II1: αAgα/αα.

Family b) I1: αAgα/αα; I2: αAgα/αααα; II1: αα/αααα; II2: αAgα/αα; II3: αAgα/αAgα and inclusions body; II4: αAgα/αα.

Family c) I1: αAgα/αα; I2: αα/αααα; I3: αAgα/αα and G6PDH deficit; II1: αAgα/αα; II2: αAgα/αα; II3: αAgα/αααα; III1: αAgα/αAgα; III2: αAgα/αAgα and inclusions body.

Conclusions. Automatic sequencing is a great important method for the clinical diagnosis of non-deletional α-thalassemia syndromes. Homozygous cases show a severe hypochromic anemia, with a lower Hb and a decrease of VCM, which is supported by the literature on the subject. The VCM abnormally high for the case of homozygous Hb Agrinio could be explained by the fact that the individual has received a transfusion recently. In patients II.3 of family C and II.4 of family B, the VCM is too low considering that present Agrinio heterozygous Hb. However, this could be explained by iron deficiency anemia associated. Hb Agrinio heterozygous causes a thalassemia trait while in homozygotes behave as an intermedia thalassemia because they are affected both α2 genes, which have a higher rate of gene transcription compared to α1. This Hb is hyperunstable, why suffer instantaneous postradutional precipitation. Here, a residue highly conserved in evolution, essential to create the hydrophobic environment necessary for distal heme binding is affected, disrupting contacts with residues 55, 58, 59 and 101 of the α chain. The diagnosis of less common variants of this disease is essential to be applied to genetic counselling and prenatal diagnosis, reducing health and social costs associated with processing of these pathologies



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FIRST CASE OF HEREDITARY ATRANSFERRINEMIA IN SPAIN

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Background. Hereditary atransferrinemia (OMIM 209300, Orpha number ORPHA1195) was first described in 1961 as a rare autosomal recessive metabolic disorder characterized by microcytic hypochromic anemia due to a functional deficiency of transferrin. Transferrin is the main plasma iron-binding protein encoded by the gene TF. In atransferrinemia, there is a reduction in delivery of iron to erythroid cells in the bone marrow, reduced haemoglobin synthesis, increased iron absorption, and severe iron overload of parenchymal organs. The disease has been reported in only 12 patients from 10 families world-wide. Just four of these families have been studied at the molecular level. *Aims*. Here, we report a new patient with this rare disorder, the first known case in Spain and the 13th patient to be reported in literature. We aim to characterise this family at the genetic and molecular level. *Methods and Patients.* The pre-treated transferrin levels of the proband were extremely low (12.5 mg/dL normal values: 202 - 336 mg/dL). The parents and other relatives had low transferrin levels (value range 124 - 184 mg/dL) as predicted for healthy carriers. Blood samples were taken from the patient and relatives and DNA was extracted. Lymphocytes were isolated using the Ficoll method and grown in cell culture. Cytokine treatment was added to induce transferrin expression and RNA was isolated. Primers were designed for genomic amplifications (PCR) and transcriptional analysis (RT-PCR and qPCR). PCR fragments were sequenced by the conventional Sanger method and mutational analysis was performed using Mutation Surveyor DNA Variant Analysis Software (SoftGenetics). *Results.* Genetic analysis of the transferrin gene in our case revealed common polymorphisms, silent mutations and three novel variations in intronic regions. In addition, the proband and her mother had a new missense mutation in heterozygous state; NM_001063.3:c.[1561C>A]+[=]; NP_001054.1:p.[Ala418Glu]+[=]. This mutation is located in a conserved region of the protein. Paternal genetic analysis did not reveal any mutation in the coding region. The patient is currently being treated satisfactorily with periodic infusions of purified apotransferrin. *Summary/Conclusions*. The detected Ala418Glu amino acid substitution predicts a structural change in the protein that probably affects transferrin function. As Atransferrinemia is an autosomal recessive disorder, we postulate the presence of a second mutated allele inherited from the father (healthy carrier). We are currently studying the TF promoter region and the implication of intronic variations in RNA missplicing.

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EVIDENCE OF PROGNOSTIC VALUE OF RETICULOCYTE COUNT AND RETICULOCYTE HEMOGLOBIN CONTENT IN SICKLE CELL DISEASE

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Background. Reticulocytes are generally accepted to be young red blood cells. Whenever red cell formation in the bone marrow is active, the proportion of reticulocytes in blood increases. To date, reticulocyte hemoglobin content (Ret-he) is the most widely studied of the reticulocyte indices. The hemoglobin content is considered to be constant throughout the lifetime of erythrocytes and circulating reticulocytes unless structural changes take place compromising the amount of cytoplasm or causing cellular fragmentation. During intramedullary development, reticulocytes actively synthesize hemoglobin. Furthermore, fetal haemoglobin (HbF) seems to be protective to sickle cell anemia patients and is also used for monitoring these patients. *Aims.* Evaluation of Reticulocyte count (RET) and Ret-he and their relation to HbF and HbS proteins in patients with homozygous sickle cell anemia Methods. Ninety four patients with homozygous sickle cell disease (40 males and 54 females) were retrospectively studied and the levels of RET, Ret-he, HbF and HbS were evaluated. The measurement of reticulocyte parameters were generated by the Sysmex XE 2100 whiles the levels of HbS and HbF determined by cation-exchange HPLC (High Performance Liquid Chromatography). Results. Mean levels of RET, Ret-he, HbF, HbS were 200100±112700 /UL, 24.87±5.8 pg, 14.52±9.65%, 71.07±11.61%, respectively. Reticulocyte count was negatively correlated with HbF ($r = -0.216$, $p < 0.05$) and positively correlated with HbS ($r = 0.217$, $p < 0.05$). Additionally, a positive correlation was found between HbF and Ret-He ($r = 0.342$, $p < 0.01$ and a negative correlation between HbF and HbS ($r = -0.440$, $p < 0.001$). *Conclusions.* The protective role of HbF was confirmed and the same conclusion was reached regarding Ret-he and Reticulocyte count. When the absolute amount of reticulocytes decreases, the improvement in bone marrow stress, due to tissue anoxia, is reflected. The fact that the reticulocytes decreased after the HbS increase would support the improved erythropoiesis hypothesis. The decrease of the Reticulocyte count and the increase of Ret-He could be used as indicative indices for the severity of sickle cell disease.

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MTHFR GENE POLYMORPHISMS ARE NOT ASSOCIATED WITH HEMATOLOGIC ALTERATIONS IN WOMEN WITH RECURRENT PREGNANCY LOSSES

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Background. Recurrent pregnancy loss (RPL) is defined as three or more consecutive spontaneous abortions prior to the 20th week from the last menstrual period. It is a multifactorial condition. Low concentration of serum folate was observed in carriers of MTHFR 677TT genotype and it could impair the hemoglobin concentration. The effects of haplotypes for MTHFR 677C>T and 1298A>C polymorphisms in the hematologic parameters are unknown. *Aims*. To investigate the relationship between MTHFR (677C>T and 1298A>C) polymorphisms and hematologic alterations in women with recurrent pregnancy losses (RPL). *Methods:* Two

hundred and forty two non-pregnant women with three consecutive losses prior to 20 weeks of pregnancy and 237 healthy fertile non-pregnant women (group 3) who had at least two children and no known pregnancy losses were studied. The primary RPL group was made up of women who had three or more consecutive losses without carrying a fetus to viability (group 1, N=109) and in the secondary RPL group, women were included who had three or more consecutive losses and with at least one viable pregnancy (group 2, N=133). The hemogram was obtained by using a hematologic cell counter Pentra 120 ABX. Cobalamin was determined by using a Immulite (DPC Medlab) kit. Serum folate was determined by a microbiological assay. The MTHFR (677C>T (rs1801133) and 1298A>C (rs1801131)) polymorphisms were detected respectively by RFLP PCR and Real Time PCR. *Results*. Variant allele frequencies for MTHFR 677C>T and 1298A>C polymorphisms were similar in three groups (P>0.05). The frequencies of haplotypes for MTHFR 677C>T and 1298A>C polymorphisms in three groups were also similar (P=0.555). The number of blood cells (erythrocytes, leucocytes and platelets) and hemoglobin concentrations were similar in three groups according to genotypes or haplotypes for MTHFR 677C>T and 1298A>C polymorphisms (P>0.05). No difference was observed in Cbl concentrations according to three groups of genotype women for two polymorphisms (P>0.05). Women from RPL groups took supplementation with folic acid when the blood was collected, thus serum folate concentrations were similar in group 1 and 2 according to genotypes for MTHFR 677C>T polymorphism and higher than serum levels found in the control group, however in the control group, carriers of 677TT genotype had lower serum folate when compared with CT and CC genotypes. No difference was found in the serum folate concentrations in three groups according to MTHFR 1298A>C polymorphisms. Conclusions: The MTHFR (677C>T and 1298A>C) polymorphisms are not associated with hematologic alterations in women with RPL.

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STRATEGY FOR MOLECULAR CHARACTERIZATION OF ALPHA THALASSAEMIA IN OMANI POPULATION

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Background. Alpha-thalassaemia is known to be prevalent in the Sultanate of Oman. However, there are no studies that have reported molecular characterization of alpha thalassaemia from the Sultanate of Oman. Presence of Hb Barts in a neonate is pathognomonic of an underlying alpha thalassaemia. Materials and Methods. In a prospective study, cord blood samples from 7837 neonates, which showed abnormally high Hb Barts by a qualitative and quantitative high performance chromatography[HPLC](Biorad Laboratories, Hercules, CA, USA)were subjected to Genescan studies. [Figure] Samples with a single peak, suggestive of a deletion lesion were subjected to GAP PCR for alpha thalassaemia. 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 using ABI 3100 Genetic Analyzer®. (Applied Biosystems, Foster City, CA, USA). Results: Overall there were 4042 samples (51.58%) with normal HPLC (HbA + HbF). The remaining 3795 cases (48.42%) were associated the presence of Hb Barts, indicative of the presence of alpha-thalassaemia. Amongst these subjects, 229 cases additionally also had HbS, 28 cases had HbD, 30 cases had HbE, and 3 cases had HbC. Genescan studies revealed that 38.77% cases had non-deletional type[two peaks] but 61.23% subjects showed deletional (-alpha3.7) type of alpha-thalassaemia.[figure] . This was confirmed by GAP PCR for deletional cases whereas in non-deletional cases selective amplification and direct sequencing of both alpha2 and alpha1 genes was necessary. Conclusions?? : The incidence alpha-thalassaemia in this cohort of neonates was 48.42%. CBC and HPLC on a newborn sample can lead to the suspicion of an underlying alpha thalassaemia. A strategy to screen these samples with Genescan studies can be help to classify the underlying defect as either deletional or non-deletional type, so that further appropriate molecular characterization can be facilitated. Capillary electrophoresis of alpha-globin genes analyzed by the Genescan software is thus the ideal intermediary step to choose an appropriate molecular method to characterize the alpha-globin genotype.

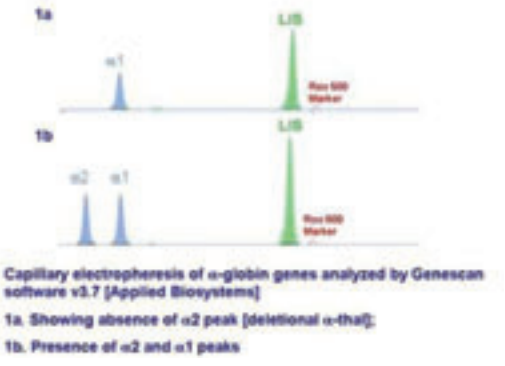

Figure 1. Alpha globin genes analysis by Genescan.

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DEFERASIROX FOR THE TREATMENT OF TRANSFUSIONAL IRON OVERLOAD IN SICKLE CELL ANEMIA: A 2-YR PROSPECTIVE STUDY

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Background: Majority of patients with sickle cell disease (SCD) receive repeated blood transfusions by adulthood. Because the body has no physiological mechanism to actively excrete excess iron, chelation therapy is important for the management of iron overload (IOL) and its complications, including iron deposition into the liver, heart and endocrine organs, eventual death. Deferasirox (DFX) is a once-daily, oral iron chelator that is approved as first-line treatment of chronic transfusional IOL including SCD. Aims and Methods: Objectives of this prospective trial were to evaluate the IOL status, before and after two year-treatment with DFX, using liver iron concentration [LIC, mg/d dry weight] by magnetic resonance imaging (MRI), MRI cardiac (Cardiac T2*, ms), serum ferritin (SF, µg/L), and to evaluate the safety and tolerability of DFX. Results: A total of 31 patients with SCD and IOL received starting dose of 20mg/kg/day of DFX. Two patients discontinued treatment at 8 and 9 months, due to pregnancy and moving to other city, respectively. One patient died at 18 months due to pulmonary infection and hemorrhagic stroke. DFX was interrupted in 3 patients due to confirmed SF levels <500 µg/L at 18-month period of treatment and DFX was not reinstated in none of them during the final 6 months of study. Twenty-five patients completed 2-year treatment. Mean ± SD age 26.9 ± 12.5y; 84% female, 90% afrodescendent, 61.3% on regular blood transfusion; median (range) DFX dose over 24 months and DFX exposure were 20 mg/kg/day (15-25) and 90.5 weeks (35.6-98.0), respectively. Mean SF level (µg/L) did not significantly reduced at 12 months (p=0.052) but significantly dropped at 24 months compared to baseline [from 2344.6 to 1986.3 (p=0.040)]. Mean ± SD LIC significantly dropped at 12 months and at 24 months compared to baseline [from 13.0 ± 5.4 to 10.4 ± 6.3 (p=0.001) and to 9.3 ± 5.7 (p<0.001), respectively]. The proportion of patients with LIC levels (mg/g dw) ≤7.0, >7.0- ≤14.0 and >14.0 from baseline to 24 months by percentage of patients changed from 13.6% to 44.0%, 40.9% to 44.0% and 45.5% to 12.0%, respectively. In all patients, Cardiac T2* was normal (> 20 ms) at baseline, 12 and 24 months of treatment. There was no significant difference between left ventricular ejection fraction values at baseline and after 12 months but this parameter significantly increased at 24 months of treatment compared to baseline [from 62.2 ± 6.0 to 64.6 ± 6.2 (p=0.02)]. The most common drug-related AEs were mild, transient diarrhea (7 pts), headache (7), nausea (5), vomiting (3), skin rash (2), increases in ALT (2), serum creatinine increases that exceeded the ULN (2). No patient experienced progressive increases in serum creatinine or renal failure. Conclusions: Our data confirms that deferasirox is effective in reducing body iron burden in transfused patients with SCD, well tolerated in pediatric and adult patients and with a clinically manageable safety profile. The availability of deferasirox as a once-daily, oral iron chelator would potentially facilitate improved compliance, and thereby reduce morbidity and mortality from iron overload.

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COMPARISON OF CAPILLARY ELECTROPHORESIS TO HIGH -PRESSURE LIQUID CHROMATOGRAPHY IN THE EVALUATION OF HAEMOGLOBINOPATHIES

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The precise detection of structural haemoglobins as well as the accurate precision of Hb A2 and Hb F are very important issues in clinical laboratories. The detection and diagnosis of several haemoglobinopathies is important for the genetic counseling of couples at risk. In this prospective study we compared the high-pressure liquid chromatography method (HPLC), (Variant, Biorad) that we routinely use for diagnosis, with the automated capillary zone electrophoresis (CE), (Sebia Minicap). 126 samples were examined by HPLC and CE. 62 samples were found to be normal, 25 were heterozygous for β-Thalassaemia, 26 samples of people with sickle cell trait or patients with sickle cell syndromes, 2 were carriers of δβ-Thalassaemia, and 11 were found to be carriers of abnormal haemoglobins as E, Lepore, H, E-Saskatoon, D, Hb Questembert. For the normal samples the mean value of Hb A2 with the HPLC method was statistically signigificantly higher in comparison to CE method, (2.934 ± O.44, and 2.7 ± 0.46 respectively) (p=0.00). For the samples from β-Thalassaemia carriers, the mean value of Hb A2 with the HPLC method was statistically signigificantly lower in comparison to CE method, (5.2 ± O.69, and 5.47 ± 0.74 respectively) (p=0.014). For the samples from people with sickle cell trait or patients with sickle cell syndromes, the mean value of Hb A2 with the HPLC method was statistically signigificantly higher in comparison to CE method, (4.4 ± O.97, and 3.63 ± 0.88, respectively) (p=0.00). Interestingly, CE method can measure the percentage of Hb H, fact that fails with HPLC method. Hb E co elutes with Hb A2 in HPLC method and Hb E-Saskatoon co elutes with Hb S. With CE method, Hb E is "identified"as Hb C and Hb E-Saskatoon as Hb O-Arab. As for Hb Lepore it is known that co elutes with Hb A2 in HPLC but in CE it is separated. The carrier of Hb Questembert was not identified neither in HPLC nor in CE. Both methods provide automated detection of variant haemoglobins. The use of HPLC has the advantage of a broad literature with the use also of classical electrophoresis at alkaline and acid gels. The use of CE in combination with the HPLC could become a useful tool in clinical laboratories.

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NEW PARAMETERS FOR THE DETECTION OF MEGALOBLASTIC ANAEMIA

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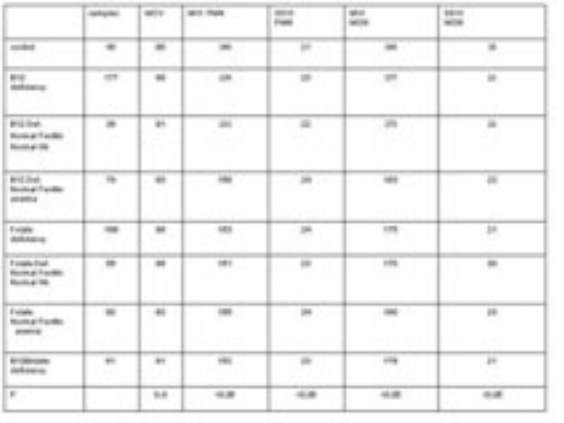
The classical flow charts used widely for the diagnostic approach of anemia due to Vitamin B12 (B12) deficiency or anemia due folate deficiency includes the Mean cell Volume of the red blood cells (MCV) as one of the key tests for the suspicion of these diseases and the differential diagnosis of anemia. Only around half of the patients with B12 deficiency (B12) or folate deficiency have high MCV, in many of the situations because the coexistence of other causes of anemia. The Coulter LH 780 hematology analyzer (Beckman Coulter) has the ability to measure specific parameters of neutrophil and monocyte populations like mean and standard deviation (SD) of cell volume (MVI,SDVI), conductivity (MCI,SDCI), and light scatter (MSI, SDSI). These so-called positional parameters (PP) can detect morphologic changes in neutrophil and monocyte population. Using VCS parameters we investigated the correlation between megaloblastic neutrophils and monocytes in B12 and/or folate deficiencies. AIM The correlation of megaloblastic anaemia due to B12 and/or folate deficiencies with neutrophil and monocyte positional parameters. METHODS The study population included 427 patients. 177 had B12<145pg/mL and 189 patients had folate <2.33ng/mL There were 61 cases with both serum folate and B12 deficiency (46 of them had normal ferritin, 31 had anaemia). Anemia was defined according the WHO anemia criteria (Hb<12 g/dL in women and Hb<13 g/dL in men). We collected blood samples from 43 healthy control subjects. The VCS parameters and full blood count was obtained by the Coulter LH 780 hematology analyzer. B12, folate and ferritin values were obtained using paramagnetic particle chemiluminescent immunoassay (Access2). P value less than 0, 05 were considered significant.



The MVI and SDVI of neutrophils and monocytes may be used for the detection of megaloblastic neutrophils and monocytes. Megaloblastic neutrophils and megaloblastic monocytes may be seen in B12 and/or folate deficiency. Positional parameters have significant statistical role in the detection of those deficiencies in contradiction with the widely used MCV, because they are not affected by the presence at the same time of iron deficiency or other reasons of anemia. Although plausible, this hypothesis needs to be sustained clinically by a prospective study.

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EVALUATION OF AN IMPROVED MICROCUVETTE FOR FAST AND EASY POINT OF CARE HEMOGLOBIN DETERMINATION

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Background. Point of care testing for hemoglobin is often carried out using disposable microcuvettes based on the photometric determination of azidemethemoglobin [1]. The use of such microcuvettes enables a fast and accurate determination of hemoglobin outside the laboratory. One major problem arising from the use of microcuvettes is the formation of air bubbles during sample filling as it can cause incorrect hemoglobin readings. An improved microcuvette geometry comprising the integration of a slot which avoids this problem was developed by EKF-diagnostic GmbH (Hemo_Control microcuvette) [2]. Aims The aim of the study is the validation of the improved microcuvette on the Hemo_Control photometer in comparison to the classical reference method in hemoglobinometry and also to compare it to another widely-used microcuvette system (HemoCue B-Hemoglobin system). Methods. To evaluate the accuracy of the system, 112 venous blood samples (concentration range 30-256 g/L) were measured over 10 days with the Hemo_Control system, the Hemocue system and the reference method NCCLS [3]. 8 Hemo_Control devices, 10 lots of Hemo_Control microcuvettes, 4 HemoCue B devices and two lots of Hemocue cuvettes were used in the study to confirm calibration stability of the POCT system. Venous blood is the sample material of choice for comparison in order to avoid within sample variation. To evaluate the precision of the Hemo_Control system over 20 days, a control material was used because blood samples are not stable for that period of time. On each of 20 testing days, two separate runs with duplicate samples of three levels of hemoglobin concentration were measured. In the precision study 13 Hemo_Control devices, 19 Hemo_Control microcuvette lots and 4 operators were involved. The precision was calculated according to NCCLS EP5A [4]. Results. The results show an excellent correlation between the haemoglobin measurements from the Hemo_Control system and the NCCLS reference method for venous samples irrespective of the lot of microcuvettes. The correlation coefficient was 0.998 with an interception of 0.003 g/dL; the standard error was 0.26 g/dL. This lot to lot precision means that recalibration of the system is unnecessary. Furthermore the correlation coefficient to the results of Hemocue B devices was 0.997 with an interception of -0.46 g/dL. The standard error was 0.37 g/dL. The measured total precision of the Hemo_Control system was to 1.1 %. Assessing 100 venous blood samples, the within-run-imprecision gave a Cv of 0.63%. Summary/Conclusions The new microcuvettes show excellent accuracy and precision. The filling behaviour of the new microcuvette is considerably improved and the risk of air bubbles is substantially reduced. The measuring time is also shorter.

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URINARY HEPCIDIN LEVEL AS AN EARLY PREDICTOR OF IRON DEFICIENCY IN CHILDREN

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Background: The ideal screening test would be capable of identifying iron deficiency in the absence of anemia. This would allow for the treatment of iron deficiency in the pre-anemic stage, preventing iron deficiency anemia and it's associated mental, motor, and behavior effects. Such test is not widely used at this time. Aim of the work: To detect role of urinary hepcidin level in early prediction of iron deficiency in children. Methods: This case control study was performed on100 children in Hematology Unit of Pediatric Department, Zagazig University Hospital, Egypt. Parental informed consent was obtained from all participant cases be eligible for enrollment into the study. The medical ethics committee of the hospital approved the protocol. Children classified to 25cases of iron deficiency stage one (iron depletion), 25 cases stage two (iron-deficient erythropoiesis), 25 cases stage three (iron deficiency anemia) and 25 healthy children as a control group. Estimations of iron parameters were done (iron, ferritin, transferrin, free protoporphyrin, total iron binding capacity and transferrin saturation). Urinary hepcidin 25 level was detected by competitive ELISA. 96-well plates were coated with anti-human hepcidin antibody and biotinylated hepcidin-25 as tracer. Analysis of collected data was done by statistical software (SPSS for Windows, version 11; SPSS;Chicago, IL). All values were given as mean ± SD. One-way analysis of variance (ANOVA)was used to assess differences among means of the study groups. Multiple regression analysis was used to assess the influence of multiple variables on a single variable. Receiver operating characteristic (ROC) curve analysis was used to determine the discriminative properties of various cutoff levels of urinary hepcidin Levels. A value of < 0.05 was considered significant. Results: Our results revealed that urinary hepcidin-25 levels were significantly lower in all stages of iron deficiency than in control group, more significant reduction in its level was observed with the progress in degree of iron deficiency($p<0.01$). Urinary levels of hepcidin showed significant positive correlation with hemoglobin(Hb),mean corpuscular volume(MCV), hematocrit value, serum iron level, ferritin level and transferrin saturation(P value0.01).On the other hand urinary hepcidin levels showed significant negative correlation with serum transferrin and total iron binding capacity. Urinary hepcidin at cutoff point ≤0.94nmol/mmol Cr could Predict iron deficiency stage 1 with sensitivity 88% and specificity 88%. Cutoff point ≤0.42nmol/mmol Cr could predict iron deficiency stage 2 with sensitivity 96% ,specificity 92%, positive predictive value92.3% and negative predictive value 95.8%. Cutoff point ≤0.08nmol/ mmol Cr could Predict iron deficiency stage 3 with sensitivity 96%, specificity 100%, positive predictive value 100% and negative predictive value 96.2%. Conclusions: We can conclude that urinary hepcidin-25 level was a simple and non invasive test and could predict iron deficiency very early, before appearance of hematological affection.

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STUDY ON THE RESULTS OF ERYTHROPOIETIN TREATMENT IN CANCER PATIENTS FROM SOUTHERN TRANSYLVANIA

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Background. Erythropoietin reduces transfusion needs, improves quality of life and increases the response to treatment of patients with cancer. Aim: We aimed to study the efficacy and safety of ß-epoetinum in cancer patients with different types of cancers. Methods: Of the 624 patients with oncological and onco-hematological diseases, hospitalized in Emergency Clinical Hospital Sibiu, treated with ß-epoetinum Sibiu in June 2009 - December 2010, all the 86 patients, who started therapy during this period were selected, in which we determined the serum hemoglobin initially and than at intervals of one month. ß-epoetinum dose was 30,000 IU/week, and if the serum hemoglobin did not increase after one month at least with 1 g/dl, the dose was doubled. In patients with an average hemoglobin decrease after one month of treatment, therapy with ß-epoetinum was stopped. The results were compared also depending the type of cancer and they were statistically analysed. Results: The average age of the 86 patients was 65.36+/-13.33 years. Distribution by gender: women - 53, men - 33. At the start of treatment, mean serum hemoglobin was 9.32+/-1.20 g/dl. It grew up to 10.92+/-1.26 g/dl ($p<0.00001$) after one month, 11.47+/-0.97 g/dl ($p<0.00001$) after 2 months, 12.22+/-1.20 g/dl ($p=0.011$) after 3 months. After the first month of treatment, mean hemoglobinemia increased 1.6 g/dl, after 2 months - with 2.15 g/dl, and after 3 months - by 2.9 g/dl. The average monthly increases were obtained in patients with chronic nonlymphoide hematological malignancies (1.93 g/dl, 2.66 g/dl, 3.97 g/dl), followed by those with chronic lymphoid hematological malignancies (1.86 g/dl, 1.66 g/dl, 2.79 g/dl). The smallest serum hemoglobin levels increases were observed in patients with solid neoplasms: after a month average increase of 1.17 g/dl, and 2 months - 2.09 g/dl. Most patients tolerated the treatment well. From the 624 patients treated with ß-epoetinum made in the 18 months, one patient developed splenic infarction and thrombosis of the inferior vena cava (with a favorable evolution on anticoagulant therapy), 1 - thrombophlebitis of a leg, 2 - alergodermia, 2 - high blood presure and 5 - dyspeptic symptoms possibly related to the treatment. There were no recorded deaths related to the ß-epoetinum therapy. Summary: ß-epoetinum therapy had an eficient control of the anemia in the majority of the oncological and hemato-oncological patients, reducing transfusion needs. The largest increases in serum hemoglobin were observed in patients with chronic nonlymphoide hematological malignancies, followed by chronic lymphoid hematological malignancies and those with solid malignancies. Side effects were rare and no deaths resulted.

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EVALUATION OF ANEMIA IN PATIENTS WITH CHRONIC KIDNEY DISEASE NOT SUBMITTED TO DIALYSIS TREATMENT

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Background: Anemia is a common condition in patients with chronic kidney disease (CKD) due to insufficient erythropoietin (EPO) production. Iron deficiency and inflammation are often associated with and contribute to the impaired erythropoiesis in these patients. Aims: Evaluate the possible role of inflammation in the anemia and iron deficiency in various stages of renal failure. Methods: Adult patients with CKD and anemia neither undergoing dialysis nor using erythropoietin and iron supplementation. Haematological parameters (Sysmex XE2100), iron status and inflammatory activity were carried out in 77 patients with CKD (26 Stage II, 36 Stage III and 15 Stage IV of impaired glomerular filtration), 37 normal individuals (CG), and 22 normal subjects with iron deficiency anemia without renal disorder (IDA). Results: 33 patients had renal anemia (RA) without iron deficiency, and 44 had functional iron deficiency (FID) (serum ferritin <100 µg/dL and/or transferrin saturation (TS) <20%). Comparing patients with RA with and without iron deficiency, it was observed levels of protein C-reactive protein (CRP) higher in group with FID ($p <0.0001$, Mann-Whitney test) and higher levels of soluble transferrin receptor (sTfR) in group with FID ($p = 0.0144$). The degree of haemoglobinization of reticulocytes (Ret-He) was preserved in patients with CKD, similar to normal CG, both higher than IDA. A significant correlation (Spearman coefficient) between Ret-He and Mean Cellular volume (MCV) was observed in RA group ($p= 0.6132$, $p= 0.0001$), RA with FID ($r= 0.6167$, $p= < 0.0001$), CG ($r= 0.3892$, $p= 0.0172$) and IDA group ($r= 0.4337$, $p= 0.04356$). It was showed a progressive decreasing in hemoglobin level as renal dysfunction worsened. Although ferritin levels did not showed difference among the stages of DRC, levels of transferrin iron binding capacity (TIBC) and sTfR were increased and TS declined with increasing renal impairment. EPO levels were lower in the CKD than normal group and IDA. Inflammatory activity (CRP levels) were higher in the group with AR and FID ($p <0.0001$) than in RA and CG ($p <0.0001$). There was no correlation between haematological and iron parameters with CRP. Conclusions: According to the results we could conclude that the frequency of iron deficiency in those patients with CKD is quite high (57%), and that both anemia and iron deprivation are more evident in patients with more severe renal dysfunction. The role of inflammatory activity evaluated by CRP levels

in the physiopathology and progression of anemia was not observed, but other inflammatory markers such as interleukins and hepcidin may provide evidence about that association. Diagnosis of iron deficiency in patients with CKD is not always sufficiently investigated. Deprivation of iron in patients not undergoing dialysis should be diagnosed and, if possible, corrected. An increase in hemoglobin levels may improve the quality of life of these patients.

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EFFICACY OF COMBINED ORAL DEFERIPRONE AND SUBCUTANEOUS DESFERRIOXAMINE IN IRON-OVERLOADED CHILDREN WITH BETA-THALASSEMIA DISEASE: A TWO-YEAR EVALUATION

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Background. Iron overload is a serious complication found in transfusion-dependent patients with beta-thalassemia disease. Subcutaneous infusion of desferrioxamine (DFO) for five days per week creates the compliance and tolerability problem especially in pediatric patients. *Methods*. The daily dose of 50 mg/kg increasing up to 75 mg/kg of Thailand locally-manufactured deferiprone (DFP) plus twice weekly subcutaneous infusion of DFO 40 mg/kg to 58 transfusion-dependent children with beta-thalassemia disease. Their mean age was 11.0 years (SD, 3.7). Regular leukocyte-depleted packed red cell was given at a dose of 15 to 20 ml/kg every three to four weeks to maintain pre-transfusion hematocrit at 27%. *Results*. The patients had previously received regular transfusion for a mean duration of 9.1 years (SD, 3.4). All of them had been chelated by subcutaneous DFO for a median duration of 4.5 years (interquartile range 2.4-6.7) with an average dose of DFO at 27.7 mg/kg/d (interquartile range 22.3-31.8) for five days a week. The median serum ferritin level at the beginning of the study was 2909.4 ng/ml (interquartile range 2384.0-3708.8) while the mean transfusional iron load during the studied period was 0.31 mg/kg/d (SD, 0.07). None of the patients were hepatitis B carriers or had seropositive hepatitis C infection. All patients survived. The efficacy of combined therapy was evaluated in the 53 patients completed 24 month follow-up period. The median declination of serum ferritin was gradually increased: 169.6 ng/ml at 4 months, 404.4 ng/ml at 8 months, 663.7 ng/ml at 12 months, 746.7 ng/ml at 16 months, 772.3 ng/ml at 20 months and 1225.7 ng/ml at 24 months. Finally, the median serum ferritin level at 24 month was 1,438.7 ng/ml (interquartile range 1,004.6-3258.1).Eight out of 10 patients who had serum ferritin less than 1,000 ng/ml underwent cardiac MRI and LIC study. All patient had normal myocardial T2* with a median of 39.7 ms (interquartile range 33.3-43.4). However, the median hepatic T2* was 4.5 ms (interquartile range 3.0-6.8). Three patients were defined as no hepatic iron loading (<2mg/g) while five patients were defined as mild hepatic iron loading of 2-5 mg/g. Five patients dropped out from the study due to parental concern after adverse events including mild neutropenia (n=3), mild thrombocytopenia (n=1) and proteinuria (n=1). Another 11 episodes of adverse effects and abnormal laboratory results were found in eight patients during the studied period: GI discomfort (n=3), neutropenia (n=1), mild thrombocytopenia (n=2), elevated alanine aminotransferase (ALT) >250 u/L (n=3), serum creatinine >1 mg/dl (n=1) and arthropathy (n=1). Neutropenia and elevated ALT became normalized within one month of DFP interruption and DFP could be safely rechallenged in the following month. DFP was discontinued in patient with arthropathy although he was completely recovered after DFP interruption for one week. The rest of the events were mild, transient and spontaneous recovery without treatment discontinuation. No patient experienced agranulocytosis. *Conclusions*. The preliminary study of using locally-manufactured DFP combined with a two-day infusion of DFO showed an effective and tolerable means in chelating overloaded iron in transfusion-dependent children with beta-thalassemia disease.

1573

ACCURACY OF NONINVASIVE HEMOGLOBIN MEASUREMENT IN ANEMIC PATIENTS WITH KNOWN LOW HEMOGLOBIN LEVELS

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Background. Hematology/Oncology patients with known anemia (low hemoglobin) are subject to routine invasive blood sampling in order to the monitoring of hemoglobin levels and the determination of red blood cell transfusion. Aims. The intention of this study is to determine whether noninvasive hemoglobin readings (SpHb) from patients with low hemoglobin are accurate compared to invasive hemoglobin measurements (tHb). Methods. Eligible in/outpatients at the Japan Red Cross Medical Center diagnosed with hematological or oncological diseases were enrolled following informed consent. Male and female patients, age ranging 41 to 85 years, had hemoglobin measurements performed by venous blood draws analyzed on a Sysmex XE-2100 hematology analyzer. Concurrently, noninvasive SpHb spot check measurements were obtained with a ReSposable finger sensor (R2-20 and R2-25, revision E) connected to a Radical-7 Pulse CO-Oximeter (SET software version 7.6.0.1.) (Masimo Corp., Irvine, CA USA). Both adult and pediatric size sensors were used. SpHb values were compared to tHb by calculating bias and precision and with a Bland-Altman plot. If the internally calculated signal confidence indicator on the Radical-7 was less than 50%, then a SpHb value was not displayed on the device. Low signal confidence occurs due to low perfusion in the finger, excessive motion, or other confounding factors. Data pairs that included low signal confidence readings were excluded from analysis. *Results*. Fifty patients (36 male/15 female) with an average age of 66 years were enrolled. A total of 50 analyzed data pairs included tHb ranging from 6.1-13.1 g/dL(from Sysmex device) and noninvasive hemoglobin SpHb ranging from 6.4-14.6 g/dL. Data were separated based on sensor type/finger size (R2-20 vs. R2-25). For patients with smaller fingers using sensor R2-20, the bias and standard deviation were -0.31 ± 0.66 g/dL. For patients using the adult size sensor (R2-25) the bias and standard deviation were 0.62 ± 1.02 g/dL. A subset of subjects (n=8) with very low hemoglobin (<8 g/dL) showed a bias of 0.34 ± 0.23 g/dL. Conclusions. Based on the results of these 50 patients, noninvasive SpHb with the Radical-7 showed good agreement with invasive tHb measurements from the laboratory reference device. Among patients with very low hemoglobin (<8 g/dL), SpHb showed even better agreement with the laboratory reference device with a bias and standard deviation of 0.34 ± 0.23 g/dL.The bias and standard deviation were consistent with a previous report of SpHb accuracy in volunteers undergoing hemodilution. Use of the Radical-7 with SpHb in this clinic allowed for instant, accurate and noninvasive hemoglobin monitoring in hematology/oncology patients, avoiding further blood loss in this already anemic population. This device is considered to be useful to determine the trigger point of red blood cell transfusion without venesection.

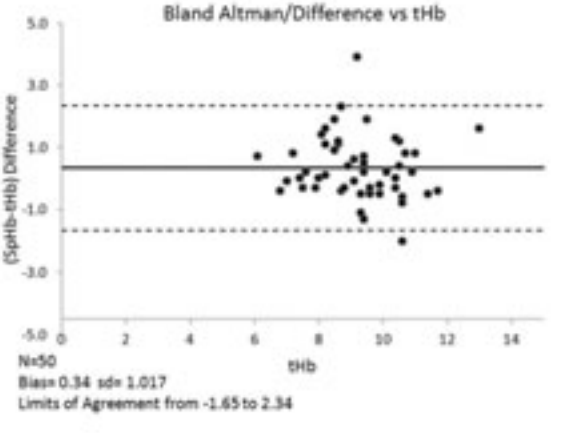

Figure 1. Bland Altman/Difference vs tHb.

1574

IDIOPATHIC PULMONARY HEMOSIDEROSIS A RETROSPECTIVE STUDY FROM A PEDIATRIC HEMATOLOGY-ONCOLOGY CENTER

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Background. Idiopathic pulmonary hemosiderosis (IPH) is a rare disease of unknown etiology, characterized by recurrent episodes of diffuse alveolar haemorrhage and sideropenic anemia which occurs most frequently in children. Sudden decrease in hemoglobin and hematocrit associated with the onset of active respiratory disease is strongly suggestive of IPH. Diagnosis of IPH should be considered when children have iron deficiency anemia and pulmonary signs and symptoms such as cough, hemoptysis and dyspnea. *Materials and methods*. We carried out a retrospective study using the medical charts of patients diagnosed with IPH, admitted at the Hematology-Oncology department- University Pediatric Clinic in Skopje which is the only institution that provides tertiary care and copes with this problem in our country. Results: Throughout a 45 years period (1965-2010) 43 patients (21 males and 22 females) were diagnosed with IPH, with an incidence of 0,38 per million yearly. Mean age at diagnosis was 6±3,64 years (range 0,25 to 15) with mean duration of follow up 77,86±100,65. The diagnosis was established through the evidence of recurrent pulmonary hemorrhages, associated with severe repetitive mycrocytic hypochromic anemia, and was confirmed by the detection of hemosiderin-laden macrophages in Perl reaction from gastric washings or bronchoalveolar lavage. Open lung biopsy was made just in one patient. Initial treatment consisted of prednisone in 40 patients (93%). Ten patients (23,2%) required long-term corticosteroid because of recurrent attacks; 19 patients (44,18%) required other immunosuppressants (Immuran or Leuceran) in addition to prednisone to control their hemoptysis. Ten patients died (9 of massive pulmonary hemorrhage and 1 from pneumonitis caused of Varicella) 1 to 12 years post diagnosis. Unfortunately, 23 patients were lost of evidence after mean follow up period of 50,04±49,08 months. Ten patients are alive: 2 with long term follow up of 35 and 27 years and being without treatment of 27 and 23 years respectively. Five year survival for IPH patients in our study was 78% (by Kaplan-Meier method). *Conclusions*. Pediatricians should raise suspicion for intrapulmonary bleeding in a patient who has recurrent dyspnea, hemoptysis and iron deficiency anemia. Our results show that long-term survival in IPH is possible with introducing early and long-term immunosuppressive therapy.

1575

THE USE OF RECOMBINANT HUMAN ERYTHROPOIETIN IN ANEMIC PATIENTS WITH LUNG TUBERCULOSIS (PILOT STUDY)

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Tuberculosis (TB) is chronic infection which widespread in Russia and other countries. Hematological disorders are relation to chronic infections well known. But data about pathogenesis and treatment of anemia in TB patients are inadequate. To investigate a prevalence of anemia and erythropoiesis in TB infection patients we have observed 70 adults with lung TB. We determined Hb concentration, reticulocyte count, erythrocyte indexes (MCV, RDW), serum iron, total iron binding capacity and serum ferritin concentrations. Erythropoietin (EPO) values were measured immunoenzymometrically by using ELISA-EPO (IBL, Germany) kits. The adequacy of EPO level in relation to the degree of anemia was evaluated individually by computing the ratio between the logarithm of the observed value and the logarithm of the expected result O/E log (EPO) ratio according to the regression line constructed with the control group. Control group consisted of 27 iron deficiency anemia (IDA) patients. Total 8 patients of all detected anemic TB patients were treated with recombinant human erythropoietin (rHuEPO)(epoietin-alfa). Of 8 treated patients 6 were males and 2 patients were females. All patients received 300 IU/kg of rHu-EPO three times per week subcutaneously during 5-6 weeks. Anemia was detected in 40 (57,1%) of 70 adult patients. IDA was registered in 27,5% of anemic adults only. All other low Hb patients had anemia of inflammation. All anemic patients (both IDA and anemia of inflammation) showed inadequately low EPO production to degree of the anemia. As compared with controls, the mean O/E log (EPO) ratio was significantly lower in all anemic TB patients (0.63 vs. 1.0 in IDA controls). Six (75%) out of 8 patients showed a complete response to rHuEPO therapy, with Hb reaching normal levels within the first 4 weeks of the treatment. All 8 anemic TB patients, treated with rHu-EPO, showed complete reticulocyte reactions. We did not observe any adverse or side effects during the therapy with rHu-EPO. Thus anemia is widespread in adult lung TB patients. Main cause of low Hb in adult TB patients is anemia of inflammation. Inadequately low EPO production to degree of the anemia in TB patients is pathogenetic substantiation of erythropoiesis-stimulating agent's (ESA) therapy. Results of the pilot study demonstrated high effectiveness of rHuEPO therapy of anemic TB patients. We speculate that increased Hb level isn't the one benefit of rHuEPO therapy in these patients. Other advantage of the rHuEPO therapy seem is an improvement of quality of life them, so far as this effect is typical for all ESA. We hope that ESA therapy will be to improve outcomes and prognosis in anemic TB patients too.

1576

THROMBOPHILIC STATUS IN ASYMPTOMATIC GREEK CHILDREN AND YOUNG ADULTS PATIENTS WITH BETA THALASSEMIA INTERMEDIA

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Backround: Beta thalassemia is known to be characterized by a hypercoagulable state, with prothrombotic factors present and thrombotic events development in a number of patients. Aim: The aim of the present study was to evaluate the frequency of coagulation abnormalities in young asymptomatic patients with thalassemia intermedia. Methods: In this prospective study, 25 non transfused young patients were evaluated. The study group consisted of 12 (48%) boys and 13 (52%) girls, with an average age of 11.8±4.6 years (range 4.5 - 20 years), a mean hemoglobin value of 9.34±1.4g/dl, a mean hematocrit value of 29.01±4.4%, a median PLT value of 378000/ml (range 253000- 1179000) and a median serum ferritin value of 66 ng/ml (range 20-967). Of those examined, 6/24 (25%) had undergone splenectomy. Laboratory tests included prothrombin time (PT), activated partial thromboplastin time (aPTT), antithrombin III (ATIII), protein S (PS), protein C (PC), activated protein C resistance (APCR), fibrinogen, prothrombin fragment (F 1 + 2), thrombin-antithrombin complex (TAT), fibrinolysis products (D-dimmers), lupus anticoagulant (LA), anti-cardiolipin antibodies (ACA), beta 2 glucoprotein I antibodies (anti- β2 GPI- I) and genomic testing of MTHFR C677T, Factor V Leiden G1691A and Factor II G20210 mutations. Results: Normal findings resulted from prothrombin and partial thromboplastin time, fibrinogen and activated protein C resistance value testing. Anti-phospholipid antibodies were negative in all patients. With regards to natural coagulation inhibitors, low activity was found for ATIII, PC and PS in 4%, 56% and 48% of patients, with mean values of 92.3±11.2%, 69.6±14.8% and 66.3±14.1%, respectively. Increased d-dimers, as well as TAT and F1+2 values were found in 12%, 60% and 12% of patients, with median values of 182 (93-1462)ng/ml, 5.1(1-96)μgr/l and 135(71-1155)pmol/l, respectively. Heterozygosity and homozygosity for the MTHFR C677T mutation was found in 48% and 12% of patients, whereas heterozygosity for FV Leiden G1691A and G20210FII was found in 8% and 12.% of patients, respectively. Double heterozygosity for MTHFR/ FV Leiden and MTHFR/ G20210II mutations was found in 16% of patients. Conclusions: The original feature in the present study lies in the population characteristics of young asymptomatic patients with beta thalassemia intermedia of greek origin. The study results confirm the presence of haemostatic changes in patients with beta thalassemia intermedia, starting at an early age. Additionally, increased prevelence of thrombophilic mutations in the study group compared to Greek population is observed, adding to the risk of thrombotic event development.

1577

LEFT VENTRICULAR VOLUMES, MASS AND FUNCTION NORMALIZED TO THE BODY SURFACE AREA, AGE AND GENDER FROM CMR IN A LARGE COHORT OF WELL-TREATED THALASSEMIA MAJOR PATIENTS WITHOUT MYOCARDIAL IRON OVERLOAD

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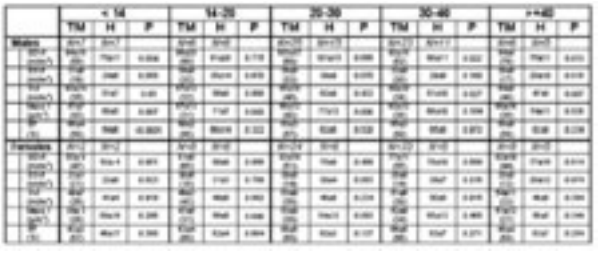
Background. Cardiovascular Magnetic Resonance (CMR) allows an accurate and reproducible quantification of left ventricular (LV) parameters. In Thalassemia major (TM) patients different *normal* LV values have been reported due to chronic anemia and eventually pre-existing iron burdens. Moreover, in this population it is unknown the influence of sex and age on LV parameters and no ranges of normal have been reported using MASS® software. *Aims*. The aim of this study was to establish the ranges for normal LV volumes, mass and ejection fraction normalized to the influence of body surface area (BSA), age and sex from CMR in a large cohort of well-treated TM patients without myocardial iron overload. Materials. Among the 923 TM patients who underwent CMR within the MIOT network for the assessment of cardiac iron overload, function and fibrosis, we selected 142 patients with no known risk factors or history of cardiac disease, normal electrocardiogram, no myocardial fibrosis and no myocardial iron overload (all the cardiac segments with a normal T2* value).



Moreover, we studied 71 healthy subjects matched for age and sex. LV function parameters were quantitatively evaluated in a standard way by SSFP cine images using MASS® software. LV end-diastolic volume, end-systolic volume, stroke volume, and mass were normalized to BSA (EDVI, ESVI, SVI, massI). Results. Table 1 shows the comparison of the CMR parameters with differentiation for sex and age in TM patients and healthy subjects and the cut-off of normality defined as mean - 2 standard deviation (SD). TM patients showed significantly lower BSA than the controls (P<0.0001). Significantly higher EDVI and SVI were found only for males < 14 years and > 30 years. Significantly higher LVEF were found only for males < 14 years. In TM patients all LV volumes indexes were significantly larger in males than in females (P<0.0001 in all cases). The EF was not different between the sexes. In males the ESVI and the EF were significant different among the age groups (P=0.006 and P=0.001, respectively). In females no significant differences were detected among the age groups. Conclusion. In a large cohort of well-treated TM patients significant differences in LV parameters compared to controls were limited to males < 14 years and > 30 years. Appropriate "normal"reference ranges normalized to BSA, sex and age should be used to avoid misdiagnosis of cardiomiopathy in TM patients.

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RIGHT VENTRICULAR VOLUMES AND FUNCTION NORMALIZED TO BODY SURFACE AREA, AGE AND SEX IN A LARGE COHORT OF WELL-TREATED THALASSEMIA MAJOR WITHOUT MYOCARDIAL IRON OVERLOAD

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Background. Cardiovascular Magnetic Resonance (CMR) has provided the opportunity to quantify right ventricular (RV) parameters with excellent reproducibility and accuracy. The role of the RV is gaining ground in thalassemia major (TM) patients and this population could experience different *normal* RV values due to chronic anemia and eventually pre-existing iron burdens. Aims. The aim of this study was to establish the ranges for normal RV volumes, mass and ejection fraction (EF) normalized to the influence of body surface area (BSA), age and sex from CMR in a large cohort of well-treated TM patients without myocardial iron overload. Materials. Among the 923 TM patients enrolled in the Myocardial Iron Overload (MIOT) network who underwent CMR for the assessment of cardiac iron overload, function and fibrosis, we selected 142 patients with no known risk factors or history of cardiac disease, normal electrocardiogram, no myocardial iron overload (all the cardiac segments with a normal T2* value) and no myocardial fibrosis.

All patients had been regularly transfused and chelated since early childhood. Moreover, we studied 71 healthy subjects matched for age and sex. RV function parameters were quantitatively evaluated in a standard way by SSFP cine images using MASS® software. RV end-diastolic volume (EDV), end-systolic volume (ESV) and stroke volume (SV) were normalized by body surface area (EDVI, ESVI, SVI). Results. Table 1 shows the comparison of the CMR parameters with differentiation for sex and age in TM patients and healthy subjects and the cut-off of normality defined as mean - 2 standard deviation (SD). TM patients showed significantly lower BSA than the controls (P<0.0001). TM males (except age group 14-20 yrs) showed significantly higher RV EF compared to controls. In TM patients all LV volumes indexes were significantly larger in males than in females (P<0.0001 in all age groups). The EF was not different between the sexes. In males as well as in females the RV volumes were no significant different among the age groups, while in males the EF was significant different (P=0.004). Conclusion. In a large cohort of well-treated TM patients males showed significantly higher RV EF compared to controls. Appropriate "normal"reference ranges normalized to BSA, sex and age should be used to avoid misdiagnosis of cardiomiopathy in the clinical arena in TM patients.

1579

QUANTITATIVE T2* MRI FOR RENAL IRON OVERLOAD ASSESSMENT IN A COHORT OF HEALTHY SUBJECTS: NORMAL VALUES AND CORRELATION WITH AGE AND GENDER

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Background. Renal dysfunction, mainly caused by the is the iron overload consequent to regular transfusions, is frequently detected in adult

subjects with thalassemia .Multiecho T2* MRI is a well-established technique for iron overload assessment in heart and liver but there are very few reports concerning the kidneys in both healthy subjects and transfused patients. *Aims*. Our aims were to assess the feasibility and reproducibility of the MRI technique for measuring kidneys T2* values, to establish the lower limit of normal in a cohort of healthy subjects and to correlate the obtained values with age and gender. Methods. Twenty healthy subjects (13 men and 7 women, mean age 29.1 ± 7.2 years) underwent MRI exam. One transverse slice through the kidneys was obtained by a T2* gradient-echo multiecho sequence. T2* measurement was performed with a previously validated software program (HIPPOMIOT IFC-CNR®). For each kidney, T2* values were calculated in three different regions of interest. The ROI T2* values were averaged to obtain a representative value for both kidneys. The mean kidney T2* value was also calculated. The lower limit of normal for the T2* value was calculated on log-transformed data as mean minus 2 standard deviations. Results. Measurement of renal T2* values was feasible in all subjects. Average processing time was about 2 minutes. For the mean T2* value the coefficient of variability (CoV) and the intraclass coefficient correlation (ICC) were respectively 6% and 0.9 for the intra-operator reproducibility and respectively 12% and 0.8 for the inter-operator reproducibility. There was not a significant difference between left and right kidney T2* values (53.1 ± 8.1 ms vs 51.5 ± 9.0 ms; P = 0.183). The lower limit of normal for the mean kidney T2* value was 36 ms. The mean kidney T2* value did not show a significant difference between men and women (men 50.6 ± 8.9 ms vs women 55.6 ± 5.7 ms, P=0.153). There was no correlation between mean kidney T2* value and age (r=-0.049; P=0.838). Conclusions. In conclusion, renal T2* measurements appear to be feasible, reproducible, non-time-consuming and reliable. The renal T2* values in healthy subjects were independent of age and gender. This approach can be extended to thalassemia patients.

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PROSPECTIVE SURVEY ON HEART AND LIVER IRON AND HEART FUNCTION IN THALASSEMIA MAJOR PATIENTS TREATED WITH SEQUENTIAL DEFERIPRONE-DESFERRIOXAMINE VERSUS DEFERIPRONE AND DESFERRIOXAMINE IN MONOTHERAPY

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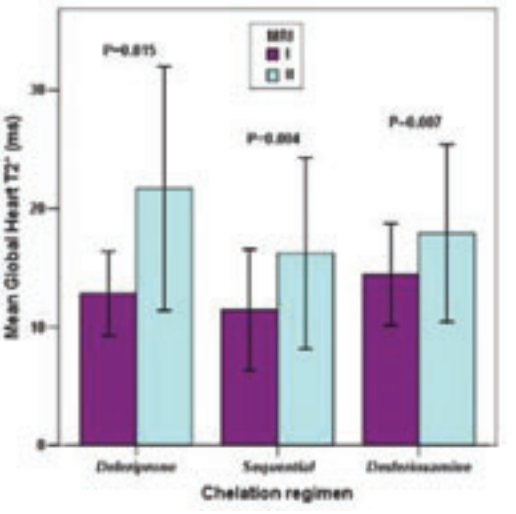
Background: Magnetic Resonance (MR) is the unique non invasive suitable technique to evaluate quantitatively the changes in cardiac and hepatic iron and in cardiac function in thalassemia major (TM) patients under different chelation regimens. Aims: Our aim was to prospectively assess the efficacy of the sequential deferiprone-deferrioxamine (DFP-DFO) versus deferiprone (DFP) and deferrioxamine (DFO) in monotherapy in a large cohort of TM patients by quantitative MR. Methods: Among the first 1135 TM patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network, 392 patients performed a MR follow up study at 18±3 months. We evaluated prospectively the 35 patients treated with DFP-DFO versus the 39 patients treated with DFP and the 74 patients treated with DFO between the 2 MR scans. Iron concentrations were measured by T2* multiecho technique. Biventricular function parameters were quantitatively evaluated by cine images. Results: Excellent/good levels of compliance were similar in the DFP-DFO (97.1%) versus DFP (94.9%) and DFO (95.9%) groups. No significant differences were found in the frequency of side effects in DFP-DFO (15.6%) versus DFP group (9.4%) .



The percentage of patients who maintained a normal global heart T2* value (≥20 ms) was comparable between DFP-DFO (96%) versus DFP (100%) and DFO (98.1%) groups. Among the patients with myocardial iron overload (MIO) at baseline (global heart T2*<20 ms), in all three groups there was a significant improvement in the global heart T2* value (DFO-DFP: 4.8±3.9ms P=0.004, DFP: +8.8±8.6 ms P=0.015 and DFO: 3.7±5.5 ms P=0.007; Figure 1) and a reduction in the number of pathological segments (DFO-DFP: -3.2±3.8 P=0.026, DFP: -6.0±5.6 ms P=0.031 and DFO: -2.9±3.7 ms P=0.001). In DFO-DFP and DFP groups there was a significant increment in the left ventricular ejection fraction (EF) (4.3±5.1% P=0.035 and 5.0±6.4% P=0.045, respectively) as well as in the right ventricular EF (6.7±6.6% P=0.017 and 6.8±3.7% P=0.001, respectively). The improvement in the global heart T2* and in biventricular function were not significantly different in DFO-DFP compared to the other groups. Among the patients with hepatic iron at baseline (T2*<9.2 ms), only in DFO group there was a significant improvement in the liver T2* value (2.0±3.5 ms P=0.010). Liver T2*changes were not significantly different in DFO-DFP versus the other groups. Conclusions: Prospectively we did not find significant differences on cardiac and hepatic iron or in cardiac function in TM patients treated with sequential DFP-DFO versus the TM patients treated with DFO or DFP in monotherapy.

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PROSPECTIVE COMPARISON ON HEART AND LIVER IRON AND HEART FUNCTION BY MR IN TM PATIENTS TREATED WITH COMBINATION DEFERIPRONE-DESFERRIOXAMINE VERSUS DEFERIPRONE AND DESFERRIOXAMINE IN MONOTERAPY

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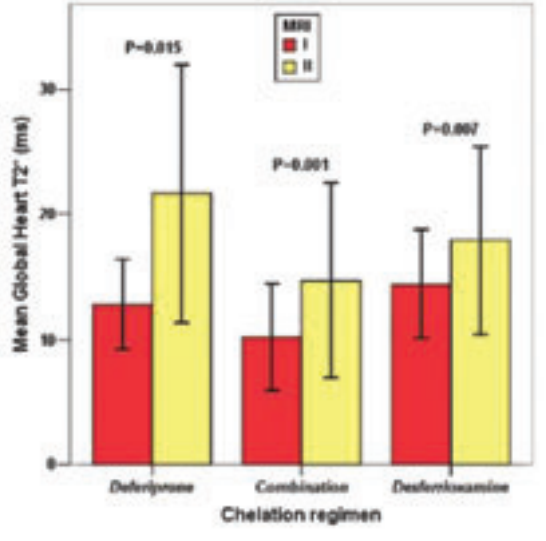
Background. Using T2* Magnetic Resonance (MR) a randomized placebo controlled study from Sardinia demonstrated combination therapy with deferiprone and desferrioxamine (DFP+DFO) significantly more effective than DFO in improving myocardial iron. One non-randomized study from Sardinia and one observational study from Greece seem to confirm for DFP+DFO therapy the most rapid clearance of cardiac iron. No data are available in literature about prospective comparisons on cardiac iron and function and liver iron in TM patients treated with DFP+DFO versus DFP and DFO in monotherapy. *Aims*. The aim of our multi-centre study was to assess prospectively in a large clinical setting the efficacy of the DFP+DFO versus DFP and DFO in TM patients by quantitative MR. Methods: Among the 1135 TM patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network we selected those with an MR follow up study at 18? 3 months who had been received one chelator alone between the 2 MR scans We evaluated prospectively the 51 patients treated with DFP+DFO versus the 39 patients treated with DFP and the 74 patients treated. Iron overload was measured by T2* multiecho technique. Biventricular function parameters were quantitatively evaluated by cine images. *Results*. The dosages were: combined therapy DFP 61.9±24.3 mg/kg per 6.1±1.4 days/week and DFO 40.7±6.0 per 3.5±1.1 days/week; DFP 73±13 mg/kg per 6.1±1.4 days/week; DFO 40.7±6.5 per 5.4±0.93 days/week. Excellent/good levels of compliance were comparable in the DFP+DFO (90.2%) versus DFP (94.9%) and DFO (95.9%) groups. The percentage of patients who maintained a normal global heart T2* value (≥20 ms) was comparable between DFP+DFO (96%) versus (100%) and DFO (98.1%) groups. Among the patients with myocardial iron overload (MIO) at baseline (global heart T2*<20 ms), in all three groups there was a significant improvement in the global heart T2* value (DFO+DFP:+4.5±6.1 ms P=0.001, DFP:+8.8±8.6 ms P=0.015 and DFO:3.7±5.5 ms P=0.007; Figure 1) and a reduction in the number of pathological segments (DFO+DFP:-2.4±3.8 P=0.004, DFP:-6.0±5.6 ms P=0.031 and DFO:-2.9±3.7 ms P=0.001). A significant improvement in the left systolic function was found only in DFP (+5.0±6.4% P=0.045) group. A significant improvement in the right systolic function was found in DFP+DFO (3.2±6.7% P=0.024) and DFP (6.8±3.7% P=0.001) groups. The changes in the global heart T2* as well as in biventricular function were not significantly different in DFO+DFP versus DFO or DFP groups. Among the patients with hepatic iron at baseline (T2*<9.2 ms), changes in liver T2* in DFO+DFP group (4.9±6.0 ms) were significantly higher versus the DFO group (2.0±3.5 ms) (P=0.012) and at the limit of the significance versus DFP group (1.3±3.3 ms). *Conclusions*. Prospectively in TM patients at the dosages used in the clinical practice combined DFP+DFO did not show superior reduction in myocardial iron or better improvement in biventricular function in comparison to DFO or DFP monotherapy, but it showed a greater reduction in liver iron versus DFO group.



1582

INTRA- AND INTER- OPERATOR REPRODUCIBILITY IN THE ASSESSMENT OF CARDIAC AND HEPATIC T2* VALUES USING 3T MRI SCANNERS

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Background. Detection and monitoring of tissue iron overload represent the key clinical factor in the management of patients with iron loading anemias. The Magnetic Resonance Imaging (MRI) multiecho T2* performed at 1.5T scanners is the most available noninvasive technique to assess hepatic and cardiac iron content. However, recently high field scanners, primarily 3T scanners, have been installed widely and are used in the clinical setting, bringing the need to assess the feasibility and reproducibility of T2* measurements at 3T. Feasibility of T2* measurements at 3T was documented by several studies, but the reproducibility of T2* measurements at 3T has never been investigated. A good reproducibility is vitally important for longitudinal follow-up of patients and also greatly reduces the sample size required for research. Aim: This study aimed to evaluate the reproducibility of T2* measurements at 3T. *Methods*. 38 transfusion-dependent patients (22 males, 36 ± 7 years) underwent MRI for T2* assessment at 3T. A single transverse slice through the liver was acquired using a T2* GRE multiecho sequence. The T2* value was determined over a large ROI of standard dimension, chosen in a homogeneous area. Three parallel short-axis views of the left ventricle were obtained using T2* GRE multislice multiecho sequence. The left ventricle was segmented into a 16-segments standardized model and the T2* value on each segment was calculated as well as the global value and the mid-ventricular septum T2* value. MRI image analysis was performed using a custom-written, previously validated software (HIPPO MIOT®, IFC-CNR). To assess the reproducibility of T2* values, data related to 20 patients were randomly selected from the entire data set. To evaluate the intra-observer variability, the 20 images were blindly reanalysed by the same observer who analysed the entire data set after two weeks. To evaluate the inter-observer variability, the selected images were presented in random order to an other operator, who didn't know the results obtained by the other one. The difference between two different analyses was evaluated by calculating the coefficient of variation (CoV) and the interclass correlation coefficient (ICC). The CoV was calculated as the ratio of the SD of the half mean square of the differences between the repeated values, to the general mean. The ICC was obtained from a two-way random effects model with measures of absolute agreement. An ICC ≥ 0.75 was considered excellent, between 0.40 and 0.75 good, and < 0.40 unsatisfactory. Results: The results of the intra- and inter-observer variability analysis performed in the selected images acquired at 3T are summarized in Table 1. An excellent ICC was always obtained (>0.929).

Table 1. Reproducibility data for T2* values at 3T.

	Intra-observer reproducibility		Inter-observer reproducibility	
	ICC	CoV (%)	ICC	CoV (%)
Liver	0.999	4.69	0.998	6.17
Global heart	0.998	2.53	0.995	3.82
Mid-ventricular septum	0.994	4.86	0.988	6.62
Segment basal anterior	0.967	12.51	0.931	15.69
Segment basal anteroseptal	0.994	7.04	0.986	9.27
Segment basal inferoseptal	0.989	6.47	0.977	8.52
Segment basal inferior	0.971	10.73	0.931	15.57
Segment basal inferolateral	0.958	11.79	0.899	16.92
Segment basal anterolateral	0.963	11.84	0.935	14.69
Segment medium anterior	0.977	9.70	0.973	10.07
Segment medium anteroseptal	0.988	6.96	0.980	8.27
Segment medium inferoseptal	0.986	7.10	0.991	5.33
Segment medium inferior	0.950	13.43	0.929	16.52
Segment medium inferolateral	0.964	10.84	0.961	11.07
Segment medium anterolateral	0.991	5.59	0.970	9.99
Segment apical anterior	0.976	10.25	0.965	12.33
Segment apical septal	0.996	4.05	0.970	11.11
Segment apical inferior	0.978	9.43	0.969	6.72
Segment apical lateral	0.994	4.77	0.983	8.14
All segments	0.979	8.94	0.965	11.39

Conclusions. The intra- and inter-operator reproducibility for T2* measurements at 3T were very good and comparable with these ones previously found for T2* measurements at 1.5T. As expected, the coefficient of variation for the global heart T2* was the smallest, due to a "compensation"of outliers that may lead to high variability in a single segment measurement.

1583

PREVALENCE SURVEY OF HAEMOGLOBINOPATHIES AND IRON DEFICIENCY AMONG NATIVES AND IMMIGRANTS PREGNANT WOMEN. ONE YEAR REFERRALS IN HAEMOGLOBINOPATHY PREVENTION IN NORTHERN GREECE

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Haemoglobinopathies constitute the most frequent monogenic disorders worldwide and Thalassaemias are the most frequent genetic disorders in Greece. The risk of giving birth to a thalassaemic child depends on the incidence of the thalassaemic gene in the population under study. The aim of the study was to determine the prevalence of haemoglobinopathies and iron deficiency in Greek pregnant women and in immigrant pregnant women and their haematological characteristics and epidemiological issues. The carrier identification was carried out by a standard scheme, which included, CBC and red cell indices using the Coulter ONYX, a Cation Exchange HPLC variant system (Bio-Rad, Variant β-Thalassemia Short Program), to determine HbA, HbA2 and HbF levels. The different abnormal structural haemoglobins were investigated by electrophoretic techniques both at alkaline pH on cellulose acetate and at acid pH on citrate agar. Sickling test and tests for HbH inclusion bodies were also performed. Haemoglobin A2 was also quantified by column micro chromatography and serum ferritin levels were measured by micro Elisa technique. 664 pregnant women were recruited in the study. 376 (56.6%) of them were native Greek women and 288 (43.4) were immigrants. 396 (59.6%) were at the first trimester of pregnancy, 226 (34.4%) were examined at the second trimester and 38 (6%)at the third trimester. 353 of the pregnant women that were tested in the first trimester had hemoglobin levels more than 11gr/d, 37 had hemoglobin levels from 9 to 10.9 gr/dl and 6 had hemoglobin levels less than 9 gr/dl. The hemoglobin levels of pregnant women during the second trimester was more than l0.5 gr/dl in 199 women, from 9 to 10.4 gr/dl in 22, and less than 9 gr/dl in 5 of them. The hemoglobin levels of pregnant women in the third trimester was more than 11gr/dl in 20 of them, from 9 to 10.9 gr/dl in 14 and less than 9 gr/dl in 4 of them. Iron deficiency with ferritin levels less than 12 ng/ml had 213 (32.1%) women, while 131 (19.7%) had 12-20 ng/ml, 272 (41%) of them had ferritin from 20 to 50 ng/ml and 48 (7.2%) had more than 80 ng/ml ferritin levels. Native pregnant women were found to have higher levels of ferritin in all trimesters and this was statistically significant p=0.000. We found that the number of the native pregnant women that are examined at the first trimester is statistically significant in comparison to immigrant pregnant women (263 to 133, p=0.000).The mean age of the native women was 28.7± 5.6 years in comparison to 26.68 ±5.3 years p=0.000. 575 (86,6%) of the pregnant women did not carry any haemoglobinopathies, 75 (11,4%) carried a beta thalassemic gene , 13 (1.85%) were found carriers of sickle cell gene, one carrier (0.15%) of hemoglobin D. Native pregnant women seem to ask for consultation earlier in pregnancy, are older in age and have higher incidence of thalassemic genes. The effective prevention and management of haemoglobinopathies in Greece will depend on the integrated community-based programs for education, carrier screening and genetic counseling.

1584

IS ANEMIA CORRECTION AFTER SPLENECTOMY DEPENDENT ON THE CLINICAL SEVERITY OF HEREDITARY SPHEROCYTOSIS PRIOR TO SPLEEN REMOVAL?

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Hereditary Spherocytosis (HS) ranges from asymptomatic to a life-threatening transfusion-dependent anemia. Splenectomy corrects the anemia, though the intrinsic erythrocyte membrane defect persists. Our aim was to study how the anemia, erythropoiesis and iron status were affected in HS patients after splenectomy, in accordance with the HS severity presented prior to splenectomy, to ascertain if patient's improvement could be related to the clinical severity experienced before spleen removal. In 60 HS patients (43 splenectomized and 22 unsplenectomized) and 35 controls, we performed a basic hematological study, osmotic fragility (OF) test and reticulocyte count; determined the plasma levels of bilirubin, erythropoietin (EPO), soluble transferrin receptor (sTfR), folic acid, vitamin B12, iron, ferritin, and transferrin; and calculated reticulocyte production index (RPI). Our data showed that splenectomy lead to correction of the anemia (normal values of erythrocytes, hemoglobin and hematocrit), however mean cell hemoglobin concentration (MCHC) sustained its high value and OF was higher in splenectomized than in unsplenectomized patients; red cell distribution width (RDW), bilirubin, EPO, sTfR, reticulocytes, RPI, folic acid, vitamin B12, iron, ferritin, transferrin, were reduced in relation to unsplenectomized, though significantly higher than controls. In splenectomized patients erythrocytes, hemoglobin concentration and hematocrit presented a trend to decrease with worsening of HS (as classified prior to splenectomy) and bilirubin and OF a trend to increase. In summary, splenectomy lead to a correction in the anemia and this improvement seems related to the severity of HS prior to splenectomy.

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INTRAVENOUS IRON MONOTHERAPY FOR THE TREATMENT OF NON-IRON DEFICIENCY ANEMIA IN CANCER PATIENTS UNDERGOING CHEMOTHERAPY

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Background. Anemia is a common complication of malignancy, occurring in over 50% of patients and may increase to more than 90% in patients with certain types of cancer and in those undergoing chemotherapy or radiation therapy. Anemia often enhances symptoms of fatigue, weakness, and dyspnea and thus, it may worsen Quality of life (QOL) and performance status in cancer patients. Blood transfusion is an effective way to treat anemia, but the effect is often temporary, and is linked to many serious adverse effects. Erythropoiesis-stimulating agents (ESA) produce significant increase in Hb level, decrease transfusion requirements, and improve QOL. However, 30-50% of patients do not respond. Additionally, many concerns were recently raised over the routine use of ESA to treat anemia in cancer patients; in several studies, ESAs were found to shorten overall survival or time to tumor progression. Aims: This trial will assess the efficacy and feasibility of IV iron therapy in cancer patients with non-iron deficiency anemia who are undergoing treatment with chemotherapy or radiation therapy without the use of ESAs. The study was approved by the Institutional Review Board (IRB) and informed consents were obtained. Methods: Adult patients with solid cancers and non-iron deficiency anemia (hemoglobin (Hb) level < 11 g/dL, ferritin level > 100 ng/ml, transferrin saturation > 20%, with normal folate and vitamin B12 levels) who are receiving anti-cancer treatment with chemotherapy and or radiation therapy were included in the study. Anemia related to hemolysis, bleeding, or bone marrow infiltrations were excluded. All patients received 200 mg of ferrous sucrose given in short intravenous (I.V) infusion weekly for a total of 12 weeks without the use of ESAs, blood transfusion, or other supplements. Hb level was measured at baseline, every 3 weeks, and at week 14. Adverse events related to IV iron were prospectively reported. Results: During the study period, 19 patients (14 females, 5 males) were included. The mean age (+ SD) was 58 (+ 10.2) years. All patients had cancer and were on active chemotherapy; 3 patients withdrew from the study at week 1, 4 and 6; convenience was the only reason in all. Three other patients were transfused while on iron therapy and considered as treatment failure. Thirteen patients had at least 8 weeks of IV iron therapy (10 had full 12

weeks and 3 had 8 weeks). The mean Hb level at baseline was 9.6 g/dL. Two weeks following the last dose of I.0V iron therapy, 9 (56.3%) patients had more than1.0 gm increase in their Hb while 3 (18.6%) others had <1.0 gm increment. The mean Hb level following completion of treatment was 11.8 g/dL; an overall mean increase by 1.9 (0.8, 5.3). No therapy-related toxicities were reported among all patients. *Conclusions.* IV iron treatment alone is safe and can reduce blood transfusion requirements and improve Hb levels in cancer patients undergoing anti-cancer therapy. This approach requires further randomized studies.

1586

THE RELATIONSHIP OF MEGALOBLASTIC ANEMIA AND THYROID FUNCTION DISORDERS BETWEEN GENDER AND MENAPAUSE

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Background. Megaloblastic anaemia (MA) preferentially affects cells with high metabolic turn-over such as hematopoietic precursors and gastrointestinal epithelium. Ageing is associated with thyroid function abnormalities and cobalamine deficiency that are mostly caused by autoimmune disorders leading to production abnormalities and gastric mucosa destruction respectively. *Aims*. In our study, we want to evaluate the relationship of MA and thyroid function disorders to sex and menopause. *Methods*. 157 patients (64 male, 93 female) who were deficient of either cobalamine and /or folic acid and had thyroid function abnormality were included in the study. Age, diet habits, smoking, medical history of all and menopause status of women patients were recorded. Hemogram, reticulocyte count, peripheral smear, cobalamine, folic acid, ferritin, serum iron, total iron binding capacity, homocystein, glucose, urea, creatinin, lipid profile, LDH, total biluribin, AST, ALT, ALKP, GGT, total protein, albumin, FT3, FT4, TSH, anti-thyroglobulin antibody, anti-microsomal antibody, PT and APTT of all patients were measured. FSH was sampled from women who had menopause. ECG and PA Chest X-Ray were evaluated in each patient. Patients who had been diagnosed as megaloblastic anaemia, thyroid function abnormalities, chronic renal failure, pregnant or lactation, coronary artery disease, diabetes mellitus, chronic liver disease were excluded from the study. Data were analysed by SPSS 13 for Windows. Pearson ki-square and Student's t test were used for statistical study. *Results*. 93 (59.2 %) cases were women and 64 (40.8 %) cases were men. 124 (79 %) cases had only MA and 33 (40, 3 %) cases had MA with thyroid dysfunction. MA only group, had more women than men and more premenopausal women then post-menopausal one but these were not statistically significant (p>0.05). 19 (20.5 %) of women had MA and thyroid dysfunction and 52.6 % of cases were in menopause and 47.4 % were not. MA were more common in women having menstrual cycle, but this has not reached statistical significance (p>0.05). The peak incidence of all cases was between 40-49 years old and mean age was 44.6 ± 15.4. In only MA group, 50 (40.3 %) of cases were under 40 years whereas 74 (59.7 %) of cases were over 40. MA was significantly higher in patients over 40 (p= 0.042). 33 cases were found in MA and thyroid dysfunction group; 7 (21.2 %) of them were under 40 years old and 26 (78.8 %) were over 40 and this was statistically significant (p=0.042). *Conclusions*. As a result; it was thought that regardless of sex and menopause status, all people after 45 years old should be examined for MA and thyroid dysfunction. It will be beneficial to make larger population studies in this field.

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FLOW CYTOMETRIC DETECTION OF PNH-LIKE CLONES ON PERIPHERAL BLOOD GRANULOCYTES IN PATIENTS WITH APLASTIC ANAEMIA IN PAEDIATRIC AGE GROUP

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Background: Aplastic anaemia (AA) and paroxysmal nocturnal hemoglobinuria (PNH) are both clonal bone marrow stem cell disorders with closely interlinked pathophysiology. Bone marrow failure has been regarded as one of the triad of clinical manifestations of PNH, and PNH in turn has been described to evolve in patients recovering from aplastic anemia. Several studies investigated the link between PNH and AA, but very few tested this link in pediatric age group. Subjects and Methods: In this study, the presence of CD55 and/or CD59 defective (PNH-like clone) peripheral blood granulocytes was evaluated in 30 newly diagnosed children with AA and in 20 healthy matched controls using flow cytometric immunophenotyping. Results: Minor population with deficiency of both CD55 and CD59 was detected in 8 cases (26.7%), while CD55 deficiency was detected in 4% of cases (13.3%) and CD59 deficiency in 5% of cases (16.7%). Conclusion: The results of this study highlight the strong association between AA and PNH. Expansion or regression of these PNH-like clones in response to therapy needs more evaluation in relation to disease outcome and response to immunosuppressive therapy.

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CHANGES IN PLASMA LEVELS OF ADAMTS13 IN PAINFULL CRISIS AND ASYMPTOMATIC SICKLE CELL ANEMIA

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Background. Sickle cell disease is a congenital disease with severe haemolytic anemia. This clinical statement is characterized with ischemic changes as a result of obstruction of vascular system by sickling erythrocytes. ADAMTS 13 (A Disintegrin and Metalloprotease with Thrombospondin type 1 repeats) is a metalloprotease that cleaves von Willebrand factor (vWF) multimers in plasma. Deficiency of ADAMTS 13 is the major pathogenic factor in thrombotic thrombocytopenic purpura (TTP). In some diseases in which thrombosis incidence is increased (ischemic stroke, ischemic heart disease, malignancies, collagen vascular disease etc.), ADAMTS 13 deficiency is reported. Aims:The aim of this study is to demonstrate if there is a relationship between ADAMTS 13 and painful crisis of SCA or not. Methods:Thirty sickle cell patients who admitted to emergency department for painful crisis and their controls after at least 1 month from the time of their painful crisis improved, and 30 healthy individuals were included to the study. The patients with acute coronary syndrome, renal disease, hepatic disease, malignancies, collagen vascular disease, active infections, acute inflamation (regardless of etiology), the patients using clopidogrel, ticlopidine, glycoprotein IIb/IIIa antagonist and pregnant women in which the levels of vWF and ADAMTS-13 can be affected were excluded from the study. The ADAMTS-13 and vWF antigen plasma levels were determined by ELISA method quantitatively. Results:The levels of vWF of the patient groups were found significantly higher than levels of the control groups (p=0.0001). There was no statistically difference when the ADAMTS-13 levels of both 2 groups were compared with the levels of the control group (p=0.295 and p=0.082). The ADAMTS-13 / vWF ratio was statistically significantly lower in both painful crisis and asymptomatic period when compared with the control group (p=0.0001). *Summary*. While there is no relationship detected between ADAMTS 13 and SCA, the high levels of vWF might be an indicator of vascular disease in patients with SCA.

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ORAL IRON CHELATION CHALLENGES IN CHILDREN

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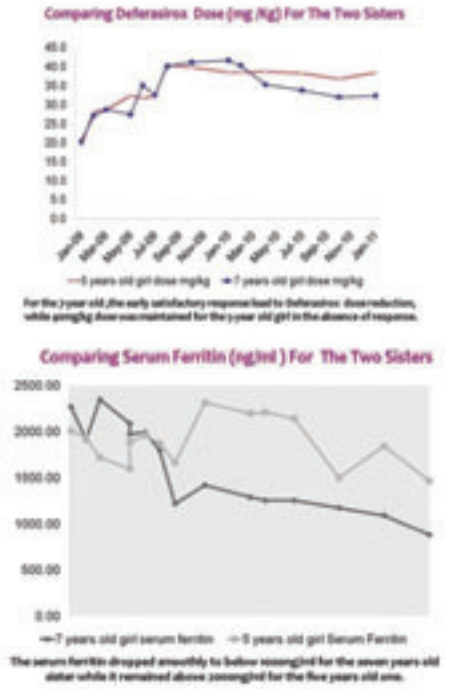
Background. The availability of oral iron chelation therapy signifies a notable change in clinical practice that has relieved patients undergoing chronic blood transfusion from the burden of desferrioxamine infusions. Despite this, getting children on chronic blood transfusion to take iron chelation therapy regularly is still challenging for clinicians and parents. *Aims*. The aim of this case review is to present some of the challenges faced by doctors who deal with iron overload and oral chelation therapy in children. Making few suggestions and recommendation was another goal. Method. This was a case review analysis in which two sisters, five and seven years old , suffering from b-Thalassemia major and undergoing regular blood transfusion were monitored closely to follow their response to deferasirox, an oral iron chelator. This was in a form of file review as well as regular meeting with patients and parents. *Results*. These two sisters shared the same genetic background, diet, motivational tool (star chart), dose of deferasirox, and followed up at the same center to receive the same volume of blood per kg every three weeks. In spite of having all these similarities, they responded differently and the serum ferritin dropped smoothly to below 1000ng/ml for the seven years old sister while it remained above 2000ng/ml for the five

years old sister. For the younger girl (5-year old) it was reported by parents that it was difficult to get her to drink the full amount of the medicine.



We observed that low body weight for the girl made it hard to maintain the ideal deferasirox dose as any increment using the available tablet strength causes significant change in dose per kilogram. Producing lower tablet strength may help make dose adjustment easier in children. High deferasirox clearance in children between two to six years was reported in early clinical studies and could be contributing to the younger girl slow response. This has raised the question of the benefit of dividing deferasirox dose in children below five years to get a better control. *Summary*. Oral iron chelation therapy carries its own challenges in children on chronic blood transfusion. Children are resistant to drink the full amount of deferasirox and adjusting the dose utilizing the available tablets' strength has been challenging. Increased deferasirox clearance in children below five years may raise the question of the benefit of dividing deferasirox dose to get to a better control. The use of star chart as a motivational tool is old but still found to be effective in improving medication compliance among our chronically transfused young patients. Observing the individuality of each patient is warranted as each patient is unique and therapy need to be tailored to suit him.

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CLINICAL OUTCOME OF PATIENTS WITH MYELOPHTHISIC ANEMIA ARISING FROM ADVANCED GASTRIC CANCER

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Background. Although it is not common to encounter patients with myelophthisic anemia arising from advanced gastric cancer, clinical features and optimal treatment are not yet to be elucidated. Prognosis for gastric cancer patients with bone marrow metastases is extremely poor. The current study was performed to evaluate clinical outcome of patients with myelophthisic anemia arising from advanced gastric cancer. Methods We retrospectively reviewed the medical records of 26 advanced gastric cancer patients with bone marrow metastases between September 1986 and February 2009 at Soonchunhyang University Hospital. Results The median age was 46 years (range 24-61 yeras). All patients had poorly differentiated adenocarcinoma, including 17 signet ring cell carcinomas. The majority of the patients showed thrombocytopenia, anemia, and elevation of lactate dehydrogenase. Sixteen patients (61.5%) were received palliative chemotherapy with a median of 4 cycles. (range, 1-13 cycles). Median survival durations after bone marrow metastases for entire patients were 37 days (95% CI, 12.5-61.5 days). The median survival times from bone marrow involvement were 11 days in the best supportive care group (range 9.5-12.5 days) and 121 days (range 94.7-147.3 days) in the palliative chemotherapy group (p <0.001). Patients died of tumor progression (11 patients, 45%), brain hemorrhage (6 patients, 25%), infection (5 patients, 21%), and DIC (1 patient, 4%). There were no chemotherapy related deaths. *Conclusions.* It is difficult to decide whether to proceed with aggressive treatment for gastric cancer patients with bone marrow metastases because of the hematologic findings, e.g. anemia, thrombocytopenia, and DIC. However, this study suggests that palliative chemotherapy should be actively considered in patients with myelophthisic anemia arising from advanced gastric cancer.

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PULMONARY HYPERTENSION IN SICKLE CELL DISEASE: STUDY OF 137 PATIENTS RANDOMLY SELECTED FROM A PUBLIC HEMATOLOGY HOSPITAL IN RIO DE JANEIRO, BRAZIL

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Background. Sickle cell disease (SCD) is the most common monogenetic inherited hemoglobin disorders worldwide. Pulmonary Hypertension (PH) is one of the leading causes of morbidity and mortality and, in the other hand, hemolytic disorders are potentially among the most common causes of pulmonary hypertension. The pathogenesis of pulmonary hypertension in hemolytic disorders is multifactorial, including hemolysis, impaired nitric oxide bioavailability, chronic hypoxemia, chronic vosoclusives episodes, chronic liver disease, and asplenia. *Methods*. We randomly select 150 patients who have been assisted in the emergency room of Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti (HEMORIO), a public hematology hospital of State of Rio de Janeiro, Brazil, from 2009 June to 2010 August. Thirteen was excluded from the analysis, ten who did not show up to echocardiography, two who suffer at least one episode of acute chest syndrome (one of the exclude criterion) and one who die. Patients signed the informed consent to participate. *Results.* The majority was SS genotype (90%). The mean age was 30.9 ranging from 13 to 60 years old. The male/female ratio was 1.6, 62% of male and 38% of female. PH was diagnosed by echocardiogram. We consider PH when tricuspid regurgitant jet velocity (TRV) ≥ 2.5m/s which correspond to a pulmonary artery systolic pressure of approximately 30-39 mm Hg. 27% was classified as having mild PH (TRV between 2.5 and 2.9m/s), 5.1% as having moderate to severe PH (TRV > 3.0m/s). We could not classify 3 patients because they have not TRV despite they have others signs of PH. There was no significant difference between genders. A logistic regression was performed to estimate the effect of lactate dehydrogenase (LDH), the use of Hydroxyurea (HU) and the age on the prognosis of PH. Aging is related to increase in the incidence of PH (OR=1.05 CI95%=1.01 to1.09). Elevation of the plasma LDH is followed by a little increase of the incidence in PH and should not be a predictor in this sample. HU confers protection (OR = 0.33 IC95% = 0.12 to 0.81). In contrast to patients with traditional forms of pulmonary arterial hypertension, patients with hemolytic disorders have a mild-to-moderate degree of elevation in mean pulmonary pressures, with mild elevations in pulmonary vascular resistance. *Conclusions.* The hemodynamic etiology of pulmonary hypertension in these patients is multifactorial and includes pulmonary arterial hypertension, pulmonary venous hypertension, and pulmonary hypertension secondary to a hyper dynamic state. Currently, there are limited data on the effects of any specific treatment modality for pulmonary hypertension in patients with hemolytic disorders. This data suggest that HU could improve the outcome of SCD patients with PH.

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COMPARISON OF HBA2 QUANTITATION BY HPLC AND CE TO DETERMINE THE SEVERITY OF BETA GLOBIN GENE MUATATION IN THALASSAEMIA TRAIT

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Background. Thalassaemia is a group of inherited disorders of haemoglobins characterised by a significant decrease in the rate of synthesis of one or more globin chains, which in beta thalassaemia will be the beta chain. In adults the haemoglobin A2 (HbA2) comprises 2-3.5% of total haemoglobin. In some patients the proportion of HbA2 is raised. This is diagnostic for beta thalassaemia trait, but can also be seen in some unstable haemoglobins. Mutations which cause decreased β-globin gene expression are classified as either β+ (reduced level of β-globin synthesis) or β0 (no β-globin synthesis). Aim In this study the HbA2 was quantified using two different methods, capillary zone electrophoresis (CE) and high-pressure liquid chromatography (HPLC). The results was correlated with the mutations found via β globin gene sequencing in order to establish whether or not quantitation of HbA2 by either method can be used to predict the β-globin mutation type (β+ or β0). Method The CE, HPLC and β globin gene sequencing were used to evaluate a total of 31 subjects with HbA2 > 3.5% (indicative of beta thalassemia trait). Results The HbA2 results produced by CE were statistically significantly lower than those obtained by HPLC when compared using a paired t-test (p= <0.001). Three of the thirty one patients were found not to have any common beta globin gene mutations, but were included as the HPLC showed an HbA2 percentage of 3.6, 3.7 and 3.9 respectively. Out of the 31 patients, 8 cases were diagnosed as having heterozygous β0 mutations including 4 patients with codons 41/42 (-TTCT) mutations, 2 patients with codons 8/9 (+G) mutations, 1 patient with a codon 15 (-T) mutation and 1 patient with a codon 39 (CAG-TAG) mutation. Twenty patients were found to be heterozygous for β+ mutations including 8 patients with IVS-1-5 (G-C) mutations, 6 patients with IVS-1-110 (G-A) mutations, 3 patients with -29 (A-G) mutations, 2 patients with -88 (C-T) mutations and 1 patient with an IVS-I-6 (T-C) mutation. When compared using the students t-test there was no difference between the HbA2 results obtained using HPLC for the β+ and β0 mutations cohorts (p=0.06). The same comparison performed using the HbA2 results obtained using CE showed a statistically significant difference between the β+ and β0 mutation cohorts (p=0.02). The predictive values of the measurement of HbA2 in establishing whether a patient has a β+ or β0 mutation calculated using Receiver operating characteristic (ROC) curves are 0.701 and 0.763 for HPLC and CE respectively. *Summary/Conclusion.* From the results of this study the CE is more sensitive in distinguishing between β+ and β0 mutations. Although this difference is relatively modest it is suggestive of a trend which may be more profound if the cohort was larger. The HbA2 level was significantly lower with the CE analyser. No attempt was made to compare the different β+ and β0 mutations as the sample size was insufficient.

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MOLECULAR CHARACTERIZATION OF BETA-THALASSEMIA AND HEMOGLOBIN VARIANTS IN THE CZECH AND SLOVAK POPULATIONS: AN UPDATE

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Background. β-thalassemia is considered to be a rare disorder in Middle Europe. Similarly to other non-malaria regions, the presence of β-thalassemia in Middle Europe reflects the historical as well as recent immigration and demographic changes that have influenced the genetic variability of the current populations living in this area. *Aims*. To assess the frequency and spectrum of mutations on the β-globin gene in Czech and Slovak patients with clinical symptoms of β-thalassemia or δβ-thalassemia. The results of the initial part of this research were published almost two decades ago (Indrak et al., Hum Genet 1992; 88: 399-404) and the aim of this work was to update this original report. Patients and Methods: Nearly 380 cases from seven hematological centers of Czech and Slovak Republics were analyzed. Blood samples were available for blood cell count measurements, hemoglobin (Hb) electrophoresis, chromatography and for genetic analyses. *Results*. Twenty-two β-thalassemia mutations were identified in more than 260 heterozygotes from 152 unrelated families of Czech or Slovak descent. Most of the mutations were of Mediterranean origin and accounted for 70% of patients. Newly discovered insertion of transposable element L1 into the β-globin gene represents a novel etiology of β-thalassemia due to a silencing effect of repressive chromatin associated with retrotransposon insertion (Piterkova et al, Heamatologica 2011; 96(s2): 417-8, (EHA meeting abstract)). The list of abnormal hemoglobins now contains 14 β-globin variants, involving the rare high oxygen affinity Hb Olomouc associated with familiar polycythemia and two unique Heinz body hemolytic anemia variants (unstable Hb Hradec Kralove and Hb Hana). *Conclusions.* In the Czech and Slovak populations, β-thalassemia appears to be an uncommon disorder, which, however, must be considered as the prevailing cause of congenital hypochromic microcytic anemia. All but four studied patients were heterozygous carriers, manifesting thalassemia minor, with rare exceptions of dominantly inherited β-thalassemia with phenotype that ranged from severe thalassemia minor to thalassemia intermedia. Three of the four homozygous or double-heterozygous β-thalassemia patients were recent immigrants from malaria countries. Genetic drift and migration in the past as well as recent immigration are responsible for introduction of the Mediterranean alleles, while several mutations, described in single families, originated locally.

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY A SINGLE INSTITUTION EXPERIENCE

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Background. Glucose-6-phosphate dehydrogenase deficiency(G-6PD) is an X-linked disorder affecting red cell metabolism. Its distribution varies significantly among different geographic regions .In Greece where this disease is endemic, an estimated 225.000 males and 92.000 females are affected. The main clinical manifestations are acute hemolytic anemia and jaundice triggered by infection or ingestion of fava beans or oxidative drugs. *Aims*. To present data indicating the frequency of G6PD deficiency in the population admitted to the hospital of Chania city (Crete island) where there is extensive consumption of fresh or dried fava beans and previously have been reported several cases of favism Methods: During 13 -year period (1997-2010), tests for the quantitative measurement of G-6PD activity by enzymatic colorimetric assay by a commercial kit (Trinity biotech, Menarini) were conducted on 1397 samples.Any individual with an activity below 4,6(U/g Hb) and 146 (U/1012 RBC) was considered G-6PD deficient. Results: Of the 1397 (1030 males, 367 females) screened , 267 (147 males, 120 females) were children. Among children 14/267 (5, 24%) were immigrants and 32/267 (11,98%)were found to have G-6PD deficiency. Complete enzyme deficiency was shown in 19/267(7,11%) males whereas 2/267 (0.74%) were immigrants. Moderate enzyme deficiency was identified in 13/267 (4, 86%)females. 4/267 (1, 49%) children were admitted to hospital with G6PD deficiency related acute hemolysis .The children with hemolysis were males, (2/5 were immigrants), younger than 5 years old and have consumed fava beans. Of the adults (220males, 910 females) 65/1130 (5,75%)were deficient in G6PD. Complete enzyme deficiency was shown in 40/1130 (3,53%) males and 6/1130(0.53%) were females . Moderate enzyme deficiency was identified in 19/1130 (1, 68%) females. The overall incidence of the deficiency in screened population (97/1397)was 6,94%. The rate among men was 59/1030 (5,73%) and among females, 38/367 (10,35%), with the male to female ratio 1:2. Conclusion: G6PD deficiency seems to affect more females than males. As neonatal screening for G6PD is long established in Greece ,clinical cases of favism are observed rarely. Acute hemolysis was found only in young children. We believe that the screening with a comprehensive education program should be performed for young children in order to prevent the occurrence of hemolysis.

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THE ROLE OF REGULATORY T CELLS AND FOXP3 EXPRESSION IN GREEK B-THALASSEMIA MAJOR PATIENTS

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Background: The suppressive/immunomodulatory function of CD4+CD25+FoxP3+ regulatory T (Treg) cells is crucial for the maintenance of immune homeostasis. Although a large number of studies have been performed on immune status of patients with β-thalassemia major, including T and B-lymphocyte subpopulations, little information is available regarding the role of Treg cells in this patient group. Aim: The aim of the present study was to determine B cells and T cells subpopulations,

including CD4+CD25bright+Foxp3+ Treg cells, and to correlate possible findings to iron chelation therapy. Methods: Fifty-two patients with beta-thalassemia major (30 males, 22 females) aged 6.5-46 years (mean age: 24.14 ± 10.439 years) participated in the study. Patients were regularly transfused and were on a regular chelation program. Data recorded included age, sex, chelation regimen, iron overload as measured by serum ferritin and heart and liver T2*MRI, cardiac function as measured by left ventricle ejection fraction (LVEF) and history of splenectomy. Iron chelation therapy consisted of deferasirox in 22 patients, deferiprone in 12, deferioxamine in 9 and combination of deferioxamine and deferiprone in 9 patients. Patients with HIV, HBV, HCV and CMV infections were excluded from the study. Twenty-seven healthy children and adults of the same age served as controls. All peripheral blood samples were analyzed on FC 500 Flow Cytometer (Beckman Counter). Four-color cytometric analysis was performed for the detection of CD19+ B cells, CD3+ T cells and T cells subpopulations CD3+CD4+, CD3+ CD8+, CD4+CD45RA+, CD4+CD45RO+, CD4+CD25+, CD4+ CD25bright+ FoxP3+ Treg using specific fluorochrome-conjugated monoclonal antibodies. The percentages of Treg were determined using the anti-CD4, anti-CD25, anti-CD127 (Beckman Coulter) surface and anti-FoxP3 (PCH101, e-Bioscience) intracellular monoclonal antibodies. Results: Percentages of CD19+ and CD4+CD45RO+ memory T cells were found to be significantly higher in patients compared to the control group (CD19+: 14.07±4.14 vs 11.62±2.26 and CD4+CD45RO+: 24.53±8.69 vs 16.38±4.01, p=0.0012 and p <0.0001, respectively). On the contrary, percentages of CD3+ and CD4+CD45RA naïve T cells were significantly lower in patient cohort compared to controls (CD3: 70.42±6.51 vs 74.57±6.28 and CD4+CD45RA: 17.64±6.24 vs 25.26±5.51, p =0.0099 and <0.0001, respectively). Finally, Treg cells were found to be significantly higher in patients compared to the control group (2.77 ±1.05 vs 1.75±0,57, p <0.0001). However, there were no correlation between Treg cells and any of the parameters studied (age, sex, iron chelation regimen, LVEF, serum ferritin, liver or heart MRI, history of splenectomy) (p>0.05). Conclusions: Elevated CD19 B cells as well as CD4+CD45RO memory T cells in thalassemia patients compared to controls might be indicative of chronic antigenic challenge, as a result of repeated blood transfusions. The increase of Treg percentage in patients could be attributed to their immune compensatory function. Further studies are needed in order to investigate the exact role of Treg in the immune system of thalassemia patients, as well as their potential in inducing transfusion tolerance.

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RETICULOCYTE HEMOGLOBIN AS AN INDICATOR OF IRON DEFICIENCY IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Background. The evaluation of iron status in patients with chronic kidney disease is crucial as it provides prerequisite information for deciding recombinant human erythropoietin treatment. As there are cases (e.g. inflammation) when the traditional biochemical markers for the estimation of iron deficiency seem to be inadequate, the introduction of more reliable tools remains a laboratory challenge. Reticulocyte hemoglobulin equivalent (RET-He) is a new parameter provided by modern automated hematologic analysers as a component of complete blood count. It provides an indirect measure of iron availablility for new red blood cell production and it is considered an indicator of iron-deficient erythropoiesis. *Aims*. To investigate the levels of RET-He in patients with chronic kidney disease, its correlation with other parameters of iron status and red blood cell indices and to examine whether it can be used as a useful marker in the assessment of iron status. Methods: A total of 31 patients (males/females:16/15) under hemodialysis referred to the outpatient dialysis unit of our hospital were studied. Iron deficiency was defined as having serum ferritin levels less than 100 ng/ml. For the determination of RET-He and red blood cell indices flow cell hematology XE-5000 Sysmex analyser (ROCHE) was used. Serum ferrum and ferritin concentrations were measured with Modular P800 analyser(ROCHE). Results: The mean RET-He value was 28,33±4,04 pg. Levels below the reference range were determined in 18/31 (58%) patients. RET-He was significantly and positively related to serum ferrum (r= 0,49, p=0,005), but not to serum ferritin. RET-He levels were positively correlated with mean corpuscular volume-MCV (r=0,55, p=0,001), mean corpuscular hemoglobin-MCH (r=0,68, p=0,000) and mean corpuscular hemoglobin concentration-MCHC (r= 0,49, p=0,005). The examined index was strongly and inversely related to red blood cell distribution width-RDW (r= -0,45, p= 0,01). No significant correlation with serum creatinine levels was observed (p>0,05). Summary/conclusions: RET-He levels in patients undergoing hemodialysis treatment were found to be lower than that of the normal population. RET-He was well correlated with conventional indices of iron deficiency. The potential utility of this simple, easily measurable and low-cost laboratory test as a reliable marker of iron status in chronic kidney disease patients should not be underestimated.

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IMMUNOLOGICAL EVALUATION OF ?-THALASSEMIA MAJOR PATIENTS RECEIVING ORAL IRON CHELATOR DEFERASIROX

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Background. Several immunological abnormalities have been described in patients with beta thalassemia major. Deferasirox (DFX), an oral chelating agent used in the treatment of beta thalassemia major, has interesting properties including antiproliferative, apoptotic and antitumour effects. Aim: This study was performed to investigate whether DFX further contributes to the altered immune function in beta thalassemia major patients. *Patients and methods*. We prospectively studied the immune function in 17 consecutive beta thalassemia major patients between July and December 2009 at King Khalid University Hospital, Riyadh. All the patients were receiving regular blood transfusions from an early age and had previously received desferoxamine for iron chelation. The dose of DFX ranged from 20-30 mg/kg and the mean duration of DFX treatment was 24 months at the time of sample collection. Apart from the demographic and clinical data collection, serum immunoglobulins, IgG subclasses, serum levels of complement factors C3 and C4, and lymphocyte subsets were studied. Results: There were 5 males and 12 females with a median age of 26 years (range 15-32 years). Median serum ferritin level was 2528 μmol/l (range 974-13166) before starting DFX treatment and was 1875 μmol/l (range 563-6643) at the time of study. Serum ferritin decreased in 12 patients, increased in 3 and was stable in 2 patients. Fourteen patients were splenectomised. Serum IgG levels were increased in 7 patients, while IgA and IgM levels were increased in 4 and 2 patients respectively. Low C4 levels were found in 9 patients and low C3 in 2 patients. IgG subclasses were normal in most of the patients except IgG-1 levels, which were increased in 4 patients. Absolute total B and T lymphocytes were increased in 14 patients each as compared to the normal range. CD4+ and CD8+ cells were increased in 13 and 12 patients respectively. NK cells were also increased in 11 patients. CD4/CD8 ratio was increased in 8 patients, decreased in 2 patients and normal in 7 patients. Conclusions: The immunological changes observed appear to be non-specific and previously described in thalassemia major patients, and unlikely to be contributed by DFX. Larger studies including other aspects of immune system are needed to understand the effect of DFX on immune function of thalassemia major patients including comparison of immune status before and after starting DFX.

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HYDROPS FETALIS IN A PK-DEFICIENT PATIENT HOMOZYGOUS FOR A PKLR MISSENSE MUTATION IN CIS WITH A NOVEL PROMOTER NUCLEOTIDE SUBSTITUTION

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Background. Pyruvate kinase (PK) deficiency is a congenital disease with heterogeneous severity, ranging from a mild asymptomatic to a severe transfusion-dependent haemolytic anaemia. Rare cases of hydrops fetalis and death in the neonatal period have been reported. Aims: To report the molecular background of a PK-deficient patient with hydrops fetalis associated with a severe haemolytic anaemia. Clinical history: A 6 year old boy, of Indian origin, with a transfusion dependent chronic haemolytic anaemia. He was born with a non-immune hydrops fetalis and severe

neonatal haemolytic anaemia requiring exchange transfusion: Hb=5 g/dL, reticulocytes=311 x 10^9/L and erytrobasts=250/100 leucocytes. His parents are non-consanguineous and have no history of anaemia. Father's red cells PK activity was 6.4 IU/gHb and mother's was 5.5 IU/gHb (n.r. 8.4-15.2). Methods: Haematological parameters and PK enzyme activity were measured by standard procedures. After informed consent, genomic DNA was extracted from EDTA peripheral blood samples and PKLR gene was studied by PCR-sequencing. Results: We identified a previously described PKLR gene missense mutation, c.1220A>G (p.Glu407Gly), in exon 9 at the homozygous state. Additionally, a second nucleotide substitution in the promoter region, -119G>A, not previously described, was also found at the homozygous state. Father and mother are heterozygous for both mutations. Conclusions: We describe a patient of Indian origin with a PK-deficient severe anaemia, homozygous for the missense mutation c.1220A>G (p.Glu407Gly) in cis with the nucleotide substitution -119G>A, a new promoter mutation. Mutation p.Glu407Gly was previously described in an Indian patient with severe haemolytic anaemia at birth, requiring exchange transfusion. The non-conservative substitution of the highly conserved Glu407 acidic residue for the tiny and small residue Gly occurs in the PK-R subunit A domain (Aα8a), near the active site of the enzyme, which is located between the B and A domains. Nevertheless, the second mutation identified in PKLR promoter region, -119G>A, modifies the CAC/Sp1 motif (-119gggtgg-113), and is expected to decrease the gene transcriptional activity. The additive effect of these two mutations may explain the severity of the phenotype.

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INTRAVENOUS FERRIC CARBOXYMALTOSE IN THE TREATMENT OF IRON DEFICIENCY

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Background. Some patients with iron deficiency anaemia cannot tolerate any formulation of oral iron and require intravenous (IV) preparations. We have used iron sucrose since its introduction and we reported our experience previously. The need for repeated infusions placed major strain on nursing time, Day Unit capacity and caused significant patient inconvenience. Ferric carboxymaltose offers increased dosing intervals, though at a higher drug cost. Aims To establish the efficacy of the use of intravenous ferric carboxymaltose in the treatment of iron Deficiency anaemia. *Methods* Over the last two years; we have treated 51 patients with a fixed dose one gram IV ferric carboxymaltose (FCM) over 15 minutes infusion. Six patients (12%) with minimal response were excluded and including patients with beta thalassaemia, pernicious anaemia and anaemia of chronic disease. *Results.* Table.

IDA

No. patients	45 (36F;9M)
No. Treatment episodes	55
Mean pre treatment Hb (g/l)	9.9
Mean post treatment Hb (g/l)	11.2
Mean pre treatment serum ferritin ng/ml	29
Mean post treatment serum ferritin ng/ml	168.8
Mean pre treatment Tsf %	8.4
Mean post treatment Tsf %	16.9

Conclusions. Whilst ferric carboxymaltose carries higher prescribing costs compared with iron sucrose; it significantly reduces hospital visits, is very well tolerated and patients prefer the convenience that this drug offers. Advantages for the hospital include reduced nursing time, fewer hospital appointments and admissions, lower patient transport costs and quicker pharmacy processing, these benefits significantly offset higher drug costs. Treatments with ferric carboxymaltose were well tolerated and no adverse effects were reported and no treatment was discontinued. Conclusion: The safety and the short duration of administration of FCM may favour its delivery in the community, further improving ease of access and convenience for patients. Our study demonstrates that the use of ferric carboxymaltose should be targeted to the treatment of appropriate clinical conditions in order to achieve maximum patient benefit and acceptable costs.

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RELATIONSHIP BETWEEN HAPTOGLOBIN GENOTYPE, INTERLEUKINS AND CLINICAL FINDINGS IN BRAZILIAN SICKLE CELL ANEMIA PATIENTS

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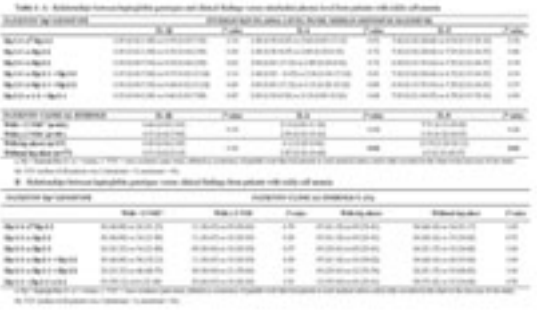
Background: Oxidative stress, particularly in the endothelium, exerts a strong influence on the genesis of sickle cell anemia (SCA) vaso-occlusion and, consequently, on patients' clinical evolution and survival. Recent investigations suggests that the profile of produced cytokines by immune response may influence the morbidity in patients with SCA. Haptoglobin (Hp) is a plasma glycoprotein whose primary function is to bind to free hemoglobin, preventing excretion of iron by the kidneys and protecting blood vessels from its oxidative effects. Three main genotypes (Hp1-1, Hp2-1, Hp2-2) correspond to distinct proteins with different properties. The protein Hp2-2 has the highest molecular weight and the lowest antioxidant capacity. Furthermore, Hp has immunomodulatory properties that seem to influence the pattern of inflammatory response and cytokine secretion. The Hp 1-1 genotype was associated with higher production of IL-6 and IL-10 than the Hp 2-2 genotype. Aims: Determine whether genotypes of Hp and plasma levels of IL-1β, IL-6 and IL-8 correlate with the clinical and laboratory aspects of adult patients with SCA, followed up at HEMOPE, in the state of Pernambuco, northeastern Brazil. Methods: Peripheral blood samples of 94 stable patients (median age: 27 years; 42 female; 52 male; all Afro- Descendants), without transfusion and without use of hydroxyurea, known to be carriers of SCA (HbSS) were analyzed. The genotype of Hp was determined, previously, by allele specific PCR and the plasma or serum levels of interleukins by ELISA. The patients' clinical characteristics were obtained from the records in medical chart. Adequate statistical analysis was performed using the GraphPad Prism Software-V.-5.00. Results: The Table 1 summarizes the partials results.



The patients' Hp genotype distribution was Hp 1-1 (28.7%), Hp 2-1 (41.5%) and Hp 2-2 (29.8%). Statistically, there is no significative differences in interleukins IL-1β, IL-6 and IL-8 plasma levels when compared with the distinct Hp genotypes patients groups. When we compared the frequencies of vase occlusive pain crisis (VOC) with the distinct Hp genotypes we did not find a statistically significant differences. Similarly, when we compared the Hp genotype and leg ulcers (LU) occurrences no statistically a difference was observed. However, when the dosages of interleukins were compared to patients with episode of LU, in past or in the moment of collected, we have found the IL-6 and IL-8 levels were significantly higher than in SCA patients who never has not been any event of LU ($P = 0.03$ and $P = 0.01$, respectively). Conclusions: In this preliminary analysis no significant differences between groups were observed in relationship to Hp genotypes, interleukins and VOC or LU. On the other hand, we have demonstrated that circulating levels of IL -6 and IL-8 were elevated in patients with SCA who developed LU. On the basis of our results, these findings suggest that the increased IL-6 and IL-8 levels may play a role in the development of the LU in our SCA patients. These findings in association with the genetic background of each patient can be associated with the clinical course of the disease.

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1601

COMPARISON OF THE CORRECTION EFFICACY OF IRON DEFICIENCY ANEMIA WITH FERRIC CARBOXYMALTOSE AND IRON [III] HYDROXIDE-SUCROSE COMPLEX IN A PORTUGUESE HOSPITAL

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Background: Ferric carboxymaltose is a new formulation of intravenous iron that allows the administration of higher doses than traditional iron complexes, like iron [III] hydroxide-sucrose complex, which requires the administration of multiple doses in several treatment sessions. *Aims:* The aim of this work is to compare the efficacy of therapeutic with ferric carboxymaltose or iron [III] hydroxide-sucrose complex in the impact of iron and hemoglobin levels reposition in patients with iron deficiency anemia. *Methods:* The population studied consisted in 150 patients, 83 with digestive related anemia (gastric neoplasias, chronic inflammatory diseases and upper gastrointestinal bleeding) and 67 with gynecologic related anemia (obstetric complications, metroragies and hysterectomies). 72 patients were treated with iron [III] hydroxide-sucrose complex, with a weekly dose of 200 mg administered during 60 minutes and 78 patients were treated with a single dose of ferric carboxymaltose administered for 30 minutes. *Results*: The average age of patients was 52.24±17.74 years, 73.83% were females. The average dose of iron [III] hydroxide-sucrose complex was 1157.75±495.52 mg and of ferric carboxymaltose was 811.86±400.47 mg. The average values of laboratory results precedent and subsequent to iron reposition demonstrated that the administration of ferric carboxymaltose in a sigle dose has similar effects in the increase of serum hemoglobin, iron and ferritin to those achieved with several treatment sessions with iron [III] hydroxide-sucrose complex. However, the difference between the number of treatment sessions with one or other therapeutic formulation is significant (1.35±0.61 with ferric carboxymaltose and 3.87±1.68 with iron [III] hydroxide-sucrose complex, p<0.001). *Summary/conclusions:* The administration of ferric carboximaltose has demonstrated to produce similar effects to those obtained with iron [III] hydroxide-sucrose complex in increasing the levels of serum hemoglobin, iron and ferritin but the reduced administration time of the ferric carboxymaltose, associated to the high iron uptake accomplished with a single dose and absence of adverse reactions provides a major convenience both to patients and health practitioners.

1602

OBSTRUCTIVE SLEEP APNEA /HYPOPNEA SYNDROME IN CHILDREN AND ADOLESCENTS WITH SICKLE/BETA-THALASSEMIA

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Background: The prevalence of obstructive sleep apnea/hypopnea syndrome (OSAHS) is reported to be high in patients with sickle cell disease (SCD) and has been associated to other manifestations of the disease, such as the occurrence of vaso-occlusive crises (VOC). It is also known that VOC are the result of complex mechanisms, involving increased cellular adhesion and chronic inflammation. *Aims:* The aim of the present study was to investigate the prevalence of OSAHS in children and adolescents with sickle/beta-thalassemia and to investigate possible correlations to clinical and laboratory parameters. *Methods:* For the purposes of the study 17 young patients with sickle/beta-thalassemia, aged 3.5 to 18 years (mean age 10.97±4.87 years), were evaluated. Mean haemoglobin (Hb) level was 9±0.7 g/dl. Age, sex and annual number of VOC were recorded. Overnight polysomnography was performed and severity of OSAHS was evaluated based on the Apnea Hypopnea Index (AHI). Tonsil's size was measured according to the Mallampati scale. With regards to laboratory parameters, von Willebrand Factor (vWF) antigen and ristocetin co-factor activity (vWF:RCo) were measured and surface expression of CD11a, CD11b, CD11c and CD18 were determined on patients' neutrophils by flow cytometry. *Results:* Of the 17 patients evaluated 16 (94.1%) presented with OSAHS. Mild OSAHS was recorded in 11/17 patients (64.7%), moderate in 2/17 (11.8%) and severe in 3/17 patients (17.6%). There was a negative correlation between the annual number of painful crises and the degree of OSAHS expressed as AHI, as well as with the basal oxygen during sleep and mean oxygen during desaturation, although these correlations did not reach statistical significance. A statistical significance was demonstrated between AHI and tonsils' size (r=0.585, p=0.028). vWF:RCo was significantly lower in patients with mild OSAHS (AHI<5) compared to patients with moderate or severe OSAHS (AHI>5) (98.68 ± 17.42 vs 128.92 ± 15.01, p= 0.006). VWF:RCo activity was inversely correlated to mean oxygen during desaturation (r=-0.485, p= -0.049) and to minimum value of oxygen during sleep (r= 0.499, p=0.069) and significantly correlated to AHI (r= 0.499, p=0.041). With regards to immunity parameters studied, a negative statistical trend between the expression of CD11a adhesion molecule and basal oxygen during sleep was found (r= -0.457, p=0.065). *Conclusions:* The study revealed a high rate of OSAHS in children and adolescents with sickle/beta-thalassemia compared to other reports on sickle cell disease patients, with adenotonsillar hypertrophy being one of the principal causes. Moreover, study findings suggest an association between hypoxemia and vWF levels as well as expression of CD11a adhesion molecule, OSAHS thus contributing to a given prothrombotic state.

1603

THE EVALUATION OF PULMONARY FUNCTIONS IN CHILDREN WITH SICKLE CELL DISEASE

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Background: The lung damage seen in sickle cell disease appears to be a complication of chronic haemolysis and repeated episodes of pulmonary vaso-occlusion. It is a source of acute morbidity, in the long term, a major determinant of survival. Approximately 4 % of sickle cell patients develop sickle chronic lung disease leading to end stage respiratory failure, characyterised by hypoxemia, restrictive lung disease and cor pulmonale. Such episodes start in childhood. *Aims*: In this study we evaluated pulmonary functions together with clinical parameters in children with sickle cell disease. *Materials and Methods:* 24 children with sickle cell anemia and 9 children as control group where include to the study. Complete blood count, hemoglobin electrophoresis and biochemical values were eveluated for both groups. The carbonmonoxide diffusion test performing for both groups. At the same day spirometric respiratory function evaluation and exercise test performed to all groups at department of sports physiology. Results:HbF, SGPT, ferritin, total bilirubine, direkt bilirubine and iron values were high at patient group (p<0.05). Hemoglobin and hematocrit values were low at patient group according to control group (p<0.05). Number of patient's who had one-three vasooclusive crisis were 14 (58.3%), who had 3 or more vasooclusive crisis were 7 (29.2%) and who had no vasooclusive crisis were 3 (12.5%). Acute chest syndrome was seen in 5 patients (20.9%). Impaired isole carbonmonoxide diffusion test was established at the 62.5% of the patient's. At patient group, spirometric FEV1 and MEF25 measurement were lower (p<0.05). At exercise test, oxyjen uptake/heart rate were lower for patient group (p<0.05). *Conclusions*: Our results confirm that lung disease in sickle cell disease begins in childhood. Pulmonary function tests differs significantly in children with sickle cell disease compared with healthy matched controls of similar age. Common abnormalities include restructive physiology and decreased diffusion capasity for carbon monoxide.

1604

MOLECULAR CHARACTERISTICS OF PATIENTS WITH SICKLE CELL ANEMIA AND STROKE IN A BRAZILIAN POPULATION

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Background: Predicting the severity of sickle cell anemia (SCA) is important to provide better informed genetic counseling and better targeting of intensive therapies. Stroke is a catastrophic complication of sickle cell disease (SCD) and a leading cause of death in both children and adults. The risk for stroke can be inferred from abnormally high cerebral velocities assessed by transcranial Doppler (TCD) and can be reduced by chronic transfusion programs. Genetic factors that predispose to this complication are not well established. Classic modulators of SCD severity, such as βS cluster haplotype and α-thalassemia, have had controversial reports. A polymorphism in the promoter region of the TNFα gene

has previously been associated with stroke in children with SCD. *Aims*: We aimed to clarify which genetic risk factors are associated with stroke in SCA in a Northeastern Brazilian population followed at Hematology and Hemotherapy Center of Pernambuco, Recife, Brazil. *Methods:* We have determined the α-globin genotype, the βS haplotype, and the presence of eNOS promoter -786 T[ARROWRIGHT]C single nucleotide polymorphism (SNP), TNF promoter -308 G[ARROWRIGHT]A SNP, factor V (FV) Leiden G1691A, prothrombin (PRT) G20210A, methylenetetrahydrofolate reductase (MTHFR) C677T point mutations and G6PD202A- mutation of patients with proven SCA as confirmed by hemoglobin HPLC pattern. All molecular analyses were determined by PCR-RFLP, except for the α-globin genotypes, which were determined by gap-PCR. Statistical analyses used Fisher's exact test. *Results:* A total of 168 patients with SCA were included in our case-control study. Of these, 53 patients presented clinical signs and symptoms of stroke, defining the case group of this study. The control group consisted of 115 patients presenting normal TCD with absence of clinical signs and symptoms of stroke. The α-gene deletion (-α3.7Kb) showed significant difference (p=0.0008) between case and control groups [3 patients (5.7%) vs. 32 (27.8%), respectively], corroborating the previously reported protective effect of α-gene deletion in stroke. The CAR/CAR βS haplotype group had statistically higher frequence in the case group compared with the control group (p=0.0174). CAR/CAR individuals appear to be at higher risk for stroke than other patients. We found no difference in the other genetic markers frequencies comparing patients with and without stroke in our SCA population. *Conclusions:* From all genetic markers in our study, only α-globin genotype and βS haplotype showed reproducible influence as genetic modulators for stroke prevalence in our SCA population. This demonstrates that genetic heterogeneity among different populations may account for failure to prove reproducibility of previous gene association studies, and warrants further studies in worldwide collaboration to determine the actual relevance of findings involving genetic polymorphisms and their influence on the prevalence and predictability of clinical complications in SCA.

Funding: Financial Support by FACEPE and CNPq.

1605

THE NEW ORAL COMBINED CHELATION REGIMEN MAY IMPROVE SHORT STATURE AND PUBERTAL MATURATION IN JUVENILE β-THALASSEMIA MAJOR PATIENTS

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Iron overload has a critical impact on multitransfused thalassemia major patients (TMp) inducing significant morbidities. Chelation with desferrioxamine (DFO), in addition to the burden of frequent infusions and local reactions, exhibits a toxic effect on bone formation, causing metaphysal dysplasia and platyspondylosis leading to a short stature. Orally active chelators deferiprone (DFP) and deferasirox (DFX) offer the promise of easier administration, better compliance without any toxic adverse effect in lower ferritin levels. Aims. The substitution of a chelation regimen in juveniles TMP with short stature, in order to attain a normal pubertal growth spurt in time. Additional objectives were to evaluate the efficacy on total body iron load and the safety of the oral combined chelation. *Patients and Methods.* 3 juveniles TMp, 1 male 2 females, mean age 11.3±2,3, were included in the study for a two years period. Hospital's Ethics Committee approval and appropriate written informed consent from guardians were obtained prior to participation. The protocol involved the combination of two oral chelators DFP: 100 mg/Kg/day and DFX: 20 mg/Kg/day. Primary endpoint measures were investigated by growth-charts for age percentiles and clinical staging of puberty according Tanner criteria. Regarding efficacy, yearly mean serum ferritin levels (CMIA), quantification of heart and liver iron by Signa-MRI-1.5-Tesla, multi-echo-T2*, liver iron concentration (LIC) mg/gr/dw by Ferriscan and LVEF, FS by cardiac-echo-Doppler, were analyzed. Safety was evaluated by close clinical and laboratory monitoring of adverse reactions according to updated SPC for each drug. Statistical analyses were performed using SPSS. *Results.* Although at baseline TMp had a decline of the height age percentile varying from <2% to <8%, after oral combined chelation they presented an overall increase in standing and sitting height and reached a normal height age percentile. In all 3/3 TMp mean body mass index increased from 15,8 to 19. Sexual development also progressed in the 2 elder from Tanner stage II to IV and in the younger one from stage I to III. LH and FSH, as peripheral hormone secretion increased. In both females menarche occurred at the age of 11 and 12 years old. Although at baseline they had mean ferritin <500 ng/mL, normal LIC and MRI T2*L, T2*H, after two years of oral combined chelation mean ferritin decreased to 173±52 ng/mL but LIC remained unchanged to 0,9 mgFe/g/dw probably because of a tended higher transfusional iron intake in adolescents. MRI T2*L increased from 32,6 to 34 msec, T2*H from 28,8 to 31,2 msec. Mean LVEF improved significantly from 61% to 66% and FS from 34 to 38%. Between MRI T2*H and FS a significant positive correlation (pearson) was found (p<0.05). No drug-related neutropenia or agranulocytosis or other toxicities known to be associated with the two chelators were observed. Pre and post-treatment creatinine remained unchanged in all TMp as well as eGFR calculated by Schwartz formula. *Conclusions.* These results indicate that oral combined chelation (DFP and DFX), seemed to be beneficial in juveniles TMp for attaining normal stature and sexual maturity. Additionally it improved cardiac function. It is also well tolerated, more acceptable for life-long chelation and influences significantly patients' quality of life.

1606

INVASIVE MOULD INFECTIONS (IMI) IN PATIENTS WITH ACUTE LEUKEMIA RECEIVING CHEMOTHERAPY

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Background. Acute leukemia patients on chemotherapy receive itraconazole prophylaxis and are treated according to a febrile neutropenia guideline developed based on the hospital's flora and antibiotic resistance. *Aims.* To determine risk factors for and clinical features of invasive mould infections(IMI) in patients with acute leukemia receiving chemotherapy. *Methods.* Patients diagnosed with acute leukemia between 1st January 2004 and 31st March 2007 and treated with chemotherapy were included in the study, approved by the Institution Review Board. A retrospective review of their casenotes was made to establish patients' clinical profile, disease characteristics and IMI management. These cases were then matched to controls obtained from the Leukemia Registry. *Results.* Nineteen cases of invasive mould infections (IMI) were found in the period, giving an incidence of 11.2%, using only patients undergoing curative chemotherapy as denominator. The incidence in patients receiving the first cycle of chemotherapy was 8.9%; in patients receiving subsequent cycles of chemotherapy was 4%. Thirteen cases were proven by EORTC/MSG criteria, 8 of them by histology without positive cultures. There were 3 proven cases of fusariosis, and 1 each of aspergillosis and nodulisporiosis. Cases were more likely than controls to have had an absolute neutropenia for at least 14 days in the chemotherapy episode in which the IMI was diagnosed (p=0.045), to have been bacteremic prior to the diagnosis of IMI (p=0.045), and to have had fever not responding to a carbapenem (p=0,016). They also had a longer length of stay (51.8 vs 27.2 days, p=0.01). The use of anti-fungal prophylaxis was not less common in cases than controls. Common clinical features noted in cases included fever not responding to a carbapenem (84.7%), cough (42.1%), a rise in alkaline phosphatase(ALP) (36.9%), breathlessness (26.3%), and hemoptysis (21.1%). A pleural effusion was noted on CXR or CT chest in 52.8% of cases, and a characteristic CT thorax in 47.4% of cases. Sixty-eight percent of cases received amphotericin (conventional or lipid preparation) as part of a febrile neutropenia protocol, though only 15.8% received this agent when an IMI was considered. Clinical improvement was attributed to an increase in ANC in 26.3% of cases, and to the introduction of voriconazole in 10.5%. The next cycle of chemotherapy was delayed for 2-4 weeks in 15.8% of cases, and for more than 4 weeks in 31/6% of cases. Three patients (15.8%) died within three months of IMI diagnosis, 47.4% survived more than 1 year. *Conclusions.* Absolute neutropenia more than 14 days is a risk factor for IMI in patients with acute leukemias. Fever not responding to carbapenems, and a rise in ALP may be considered red flags for clinicians to consider the possibility of an IMI. The anti-fungal prophylaxis used during the period under study (itraconazole) did not reduce the likelihood of an IMI and a change should be considered.

1607

POLYMORPHISM IN TLR2 ARG753GLN AND SUSCEPTIBILITY TO FUNGAL INFECTIONS IN CHILDREN WITH LEUKEMIA

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Background: Toll-like receptors (TLRs) are essential pattern-recognition receptors and have a role in activation of both innate and adaptive immune response. TLR2 recognizes various pathogen-associated molecular patterns of bacteria, virus and fungi. Intensive treatment protocols and stem cell transplantation improved survival rates in children with leukemia however invasive fungal infections (IFIs) represent a substantial cause of morbidity and mortality. Single nucleotide polymorphism Arg753Gln affect TLR2 responsiveness and may contribute to the course of infections. Aims: We aimed to investigate the association between TLR2 Arg753Gln polymorphism in children with leukemia and susceptibility to invasive fungal infections. Methods: TLR2 Arg753Gln was assessed in 96 children (1-18 years old) treated for acute lymphoblastic leukemia (ALL) and 103 age and sex matched controls. Toll-like receptor 2 genotyping was performed by hybridization probe assay specific for the TLR2 variant. Informed consent was obtained. Results: The mean age of the patients was 5,9 years old (±3,7 years), 52% were male. According to BFM-95 treatment protocol risk groups; 33,3% of the patients were in standart, 58,3% in medium and 8,3% in high risk group. During initial treatment (BFM-95) 8 (8,3%) IFIs were observed. According to the revised definitions of IFIs; 4 were proven, 2 probable and 2 possible. Leukemia relapsed in 15 patients, other 4 patients (26,6%) had IFI during relapsed ALL treatment protocol (BFM Rez-ALL). TLR2 753Gln mutant allele was found as heterozygous in 1 patient with ALL, this patient had no febrile neutropenia attack or IFI during leukemia treatment. TLR2 Arg753Gln polymorphism was not observed in controls. Conclusions: Limited data is available on the incidence of TLR2 mutation. This study is limited by the small number of positive patients with TLR2 mutation so no epidemiological conclusions can be drawn. However, interestingly the only patients who was heterozygous had no febrile neutropenia attack, IFI or any serious infection during the treatment. The known TLR2 polymorphism identified so far may not cause a crutial role in the pathogenesis of IFIs in children treated for ALL.

1608

FUNGAL COLONIZATION IN CHILDREN WITH CANCER: HOSPITAL ADMISSION AND SENSITIVITY PATTERNS; A SINGLE CENTER EXPERIENCE

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Background: Fungal infections represent a growing problem in children with cancer and fungal colonization is recognized as an important risk factor for these infections. Aim:The aim of the study was to detect the frequency of fungal colonization; fungal species characterization and antifungal sensitivity in pediatric oncology inpatients admitted at the Ain Shams University Pediatric Oncology and compare them to outpatients attending the clinic. A case-control study of fungal colonization in 50 children with cancer and receiving chemotherapy was performed; 22 patients admitted for febrile neutropenia compared to 28 outpatients as a control group. Each patient was evaluated for the occurrence of fungal colonization (defined as at least one positive surveillance culture). Samples were collected by swabbing both buccal mucosa, axillae in all patients, in addition to blood and stool studies during peak of fever, and perianal sample in the presence of inflammation or rash. All samples were cultured on Sabouraud's dextrose agar, thereafter, susceptibility of the identified isolates to antifungal drugs was determined. All isolates were of candida (C.) (100%) and aspergillus (A.) (90%) species. The most common colonizing species were *Aspergillus fumigatus* (90%), C. albicans (84%), Aspergillus flavus and C. glabrata (both 50%). A. flavus and C. tropicalis were significantly higher in inpatients than outpatients (68.2% vs 35.7% and 40.9% vs 7.1%, respectively P<0.05). The isolates were highly sensitive to amphotericin B (AMB) ranging from 50% to 100%. The sensitivity pattern to fluconazole (FCZ) was varying from 0% to 83.3% .C. albicans had higher sensitivity in outpatients to itraconazole (ITZ) (96.2%), and nystatin (88.5%) compared to inpatients (56.3%) and (37.5%) respectively (P<0.05). C. glabrata had higher sensitivity to AMB in inpatients (92.9%) compared to outpatients (27.3%) (P<0.05). Neutropenic patients had higher rate of colonization with C. glabrata (74.2%) and C. tropicalis (32.3%) versus non neutropenic patients (10.5%; P<0.0001) and (5.3%; P<0.05) respectively. Cancer patients are at increased risk for fungal colonization, therefore, they are more vulnerable to fungal infections. Neutropenia was the most important risk factor. Culture and sensitivity is important before starting of antifungal therapy due to the wide variability in drug sensitivity of each strain.

1609

INVASIVE FUNGAL INFECTIONS IN LYMPHOPROLIFERATIVE DISORDERS

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Background. Few epidemiological data regarding invasive fungal infections (IFI) in chronic lymphoid malignancies are available in literature. Aims The aim of our study is to describe epidemiology, clinical manifestations and outcome of IFI in these patients. Methods We reviewed the records of patients (pts) affected by lymphoproliferative disorders, admitted to our department between 2003 and 2010 and treated for probable or proven IFI. Results and conclusions We registered 34 probable and proven IFI. In our population, the incidence of IFI was 2,7%; (moulds 2%, yeasts 0,4%, mixed infections 0,3%). Twenty-two patients were affected by lymphoma, 8 by chronic lymphocytic leukemia, 3 by Waldenström macroglobulinemia and 1 by hairy cell leukemia. The median age was 57 years (r: 17-71). Twenty-six patients (76%) had progressive or relapsed hematological disease, and 76% was treated with multiple lines of chemotherapy. According to the criteria of EORTC/MSG, risk factors were: deep and prolonged neutropenia (10 pts), immunosuppressive therapy after solid organ transplant (2), previous allogeneic HSCT (2), high dose steroid therapy (3), monoclonal antibodies such as rituximab (7) and alemtuzumab (2), nucleosidic analogue administration (2) or multiple of these risk factors (8) during the previous 90 days. Six patients developed a yeast infection; in 4 cases infection was documented by blood cultures (2 *C. albicans*, 1 *C. glabrata* and 1 *Blastoschyzomices capitatus*), in 1 by culture of a freshly placed biliary drainage (*C. glabrata*), and in the other by the microscopic observation of *Candida spp.* in the vitreum after vitrectomy. Twenty-five patients developed an invasive mould infection (IMI). Among 22 probable IMI, of which 19 had lung involvement, 2 a sinusal localization, and 1 a pulmonary infection disseminated to brain. Microbiological evidence of infection was more often provided by the positivity of the galactomannan antigen in the serum (8 pts), in the BAL fluid (1) or in both (3). Cultures resulted positive in 6 cases, 3 from the BAL fluid (2 *A. fumigatus*, 1 *Aspergillus spp*) , 1 from nasal swab (*A. fumigatus*) and 2 from the sputum (1 *A. fumigatus*, 1 *Fusarium*). In the remaining 4 patients both culture positivity from the BAL fluid (1 *A. flavus*, 1 *A. fumigatus*, 1 *Aspergillus spp*, and 1 positive for both *Scedosporium* and *Aspergillus*) and serological demonstration of the galactomannan antigen were present. We diagnosed also 3 proven IMI by the histologic evidence of Aspergillus (2) and Mucor (1) in the lung. Three mixed infections by both moulds and yeasts were observed. Twenty-one patients recovered. Thirteen died within 90 days from the beginning of the infection, all with uncontrolled hematological disease. Among these, 6 patients died due to a progressive hematological disease. In 6 patients, infection (2 by yeasts, 4 by moulds) was a concurrent cause of death. In 1 case IFI with mixed etiology (*Candida spp./Aspergillus spp.*) was the only cause of death as documented at autopsy. Fungal attributable mortality was 20% (7/34). The only significant risk factor for mortality at univariate and multivariate analysis was the persistence of neutropenia.

1610

HAS NOVEL INFLUENZA A/H1N1 2009 ASSOCIATED PNEUMONIA POTENTIAL TO BE MORE PATHOGENIC IN ADULT PATIENTS WITH HEMATOLOGICAL MALIGNANCIES? CASE REPORTS AND REVIEW OF THE LITERATURE

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Background and aims: During the recent winter, the northern hemisphere, especially Europe, experienced a second wave of novel influenza A/H1N1 2009 virus. A devastating complication of this viral infection is pneumonia. In hematological malignancies (HM), however, no studies have evaluated the novel A/H1N1 associated pneumonia outcome during this second wave. In addition, no comprehensive data have described whether outcome of novel A/H1N1 pneumonia during the initial wave is different from that observed after this second wave. To address these issues we analyzed all cases described in the English literature. HM from our department with novel A/H1N1 2009 virus pneumonia were also included in the analysis. Methods: MEDLINE was searched using the following key words: 2009 A/H1N1 influenza, HM

and HSCT. *Results*: The search identified 25 cases of A/H1N1 associated pneumonia (Table 1). All these reports were dated during the initial wave (April 2009-March 2010). Mean age was 43 years (range 15-72). The most common underlying HM was AML (32%) followed by NHL (20%). Sixteen (64 %) had previously undergone allo-HSCT. The median absolute lymphocyte and neutrophil counts were 625 cells/µl (20-3800) and 300 cells/µl (10-6020), respectively. All patients were treated with oseltamivir 150 mg daily for 5-10 days. The median time from illness onset until the start of oseltamivir was 3,5 days (1-22). Critically ill patients were also receiving combinations of antibiotics and anti-fungal treatment. Eight (32%) patients required mechanical ventilation. Mortality rate attributable to novel A/H1N1 pneumonia was 19%. On the other hand, during the second wave (December 2010- March 2011), no cases of novel A/H1N1 associated pneumonia have been reported in HM. Herein, we present the first two known cases (both died due to influenza). Patient 1, a 55-year-old woman presented with typical flu symptoms and bilateral pulmonary infiltrates. On admission showed leukocytes 5.6 x 10^9/l with 80% blasts. A diagnosis of AML FAB M2 was made. Novel A/H1N1 virus was detected by RT-PCR in both nasopharyngeal swab (NPS) and BAL; Oseltamivir 75mg/12h was started on day 3; however she died in ICU 10 days later due to respiratory failure despite broad spectrum antibiotics. Patient 2, a 52-year-old woman diagnosed with Multiple Myeloma in June-2010, achieved complete remission after a bortezomib-based combination. However, on February 2011, she experienced a meningeal relapse. While undergoing chemo- and radiotherapy she developed flu symptoms and alveolar infiltrate in right upper lobe. NPS specimen showed novel A/H1N1 virus by RT-PCR. Two days after the onset of symptoms oseltamivir 75mg/12h and antibiotics were started. For the next 6 days she developed worsening respiratory distress and died 5 days later. There was no correlation between severe lymphocytopenia and mortality risk.

Conclusions: During the second season of the novel A/H1N1 pandemic, it appears that HM with novel A/H1N1 associated pneumonia might present with a high mortality (100% vs 19% during the initial wave) despite early antiviral treatment. Additional studies are needed to confirm these data. Thus, prevention strategies (infection control and vaccination) for patients, family members and caregivers are clearly indicated.

1611

A FULMINANT COURSE OF INFLUENZA A (H1N1) VIRAL PNEUMONIA IN A PATIENT WITH MULTIPLE MYELOMA AFTER TREATMENT WITH BORTEZOMIB

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Background . In 2010, 63 deaths and 2193 hospital admissions were reported in the Netherlands due to the current pandemic of Influenza A (H1N1). Main victims were children aged 0-5 years old, pregnant women and immunocompromised patients. Interestingly, in a recent study of 92 hospitalized patients with flu-like symptoms, 6/13 H1N1 PCR-positive patients received treatment for multiple myeloma [Garland, BBMT 2010]. One of the main treatment strategies for multiple myeloma is proteasome inhibition by bortezomib. As a side effect, bortezomib induces apoptosis in human NK-cells, suppresses NKp46-mediated cytotoxicity and has significant cytotoxic effects on CD4+-T cells and dendritic cells. In children with influenza, NK-cells are known to transduce an important signal to activated CD8+-T cells through the NK-cell inhibitory receptor CD94/NKG2A, which limits lung injury. In a large clinical study, an increased risk of viral infections occurred in myeloma patients treated with bortezomib [Chanan-Kahn, JCO 2008]. In addition, unexplained severe lung injury was reported in 4/13 patients after bortezomib treatment [Miyakoshi, Blood 2006]. Aims. We report the case of a patient with multiple myeloma who died of severe lung injury following treatment with bortezomib and infection with H1N1. Results A 52-year-old man with multiple myeloma completed 3 monthly induction courses consisting of bortezomib (d1,4,8,11), doxorubicin (d1-4) and dexamethason (d1-2). His renal function had improved and serum IgG lambda had decreased from 25 to 11 g/L. Three weeks later he was admitted with fever, coarseness and dyspnea. He was treated with broadspectrum antibiotics. All cultures remained negative. Over the next week he developed diarrhea and respiratory insufficiency. The diagnosis of H1N1 was made and antiviral medication was started. Before bortezomib, peripheral blood levels of T-cells and NK-cells were normal (CD8+ 0.9x10^9/L, CD4+ 0.9x10^9/L and NK 0.09x10^9/L). Immunophenotyping at diagnosis of H1N1 showed slightly elevated CD8+-T-cells (1.0x10^9/L), but diminished CD4+-T-cells (0.37x10^9/L) and NK-cells (0.03x10^9/L). Broncho-alveolar lavage showed a particularly high copy number of viral RNA (CT value 21.9).

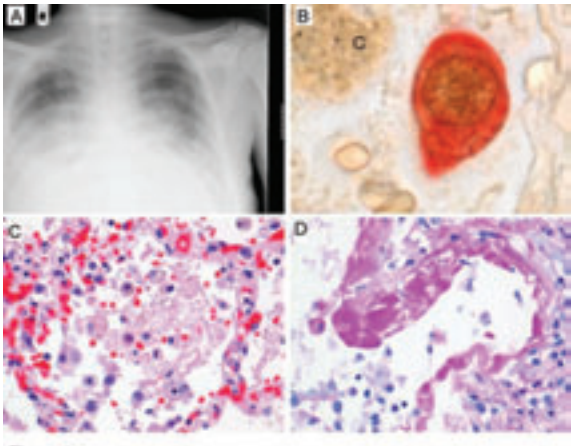

Figure 1.
Imaging, immunohistochemistry and histopathology of the patient infectd with H1N1 virus demonstrating infection of alveoli and related viral pneumonia. A. Thorax radiograph showing bilateral perihilar alveolar consolidation. B. Immunohistochemistry staining of intralesional alveolar epithelial cell with expression of viral antigen (brown nucleus) and keratin (red cytoplasm). C. Diffuse alveolar damage, H&E staining. D. Intraluminal hyaline membranes, PAS staining.

He was supported with mechanical ventilation, inotropic drugs and haemodialysis, but he finally died of lung injury, multi-organ failure and secondary sepsis. On autopsy, the lung parenchyma showed diffuse alveolar damage with hyaline membranes and edema. Immunostaining demonstrated influenza viral antigen in alveolar epithelial cells and alveolar macrophages in lesional lung tissue. H1N1v virus was detected throughout the respiratory tract as well as in brain and jejunum. Virus histochemistry demonstrated that H1N1v virus was also able to replicate in human alveolar epithelial cells. *Conclusions* . This fatal case of H1N1 demonstrates a rare finding: viral infection and replication of H1N1 in the alveolar epithelium. Furthermore, bortezomib treatment may lead to lung injury by uncontrolled CD8+-T-cell activity. Therefore, multiple myeloma patients may be at increased risk of a more fulminant course of H1N1 viral pneumonia.

1612

DISSEMINATTED CRYPTOCOCOSIS RESSEMBLING DISEASE PROGRESSION IN A PATIENT WITH A HLTV-1 ASSOCIATED ADULT-T-CELL LEUKEMIA-LYMPHOMA IN THE COURSE OF CHEMOTHERAPY ADMINISTRATION

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Background. HTLV-1 retroviral infection is endemic in African coun-

tries, Japan and Caribbean region. Adult- T- Cell leukemia-lymphoma (ATLL) has been described in this population. ATLL is characterised by blood lymphoma signs, tumoral nodes and extranodal lesions, hypercalcemia and positive HTLV-1 serology. Cryptococcal infections have been described in immunocompromised hosts in associate to HIV, transplantation, lymphoma and LLC. Aims We report a case of an adult African patient with ATLL who developed a systemic opportunistic infection due to Cryptococcus neoformans in the course of chemotherapy administration, mimicking lymphomatous disease progression. *Case Report.* A 40 years-old healthy male, born in Ghana, was diagnosed of ATLL in July 2010, showing as main features: remarkable malaise with bone marrow and peripheral blood infiltration by typical flower- shaped lymphocytes, bilateral pulmonary nodules, hepatosplenomegaly, generalized adenophaties and high LDH. Systemic chemotherapy with Ifosfamide, Etoposide, Metilprednisolone, Doxorubicin, high dose Methotrexate and Dexametasone was started. Alpha Interferon and Zidovudine were added after positive HTLV 1 serology result. An initial favourable clinical response was achieved with improvement of peripheral blood infiltration, pulmonary disease and general condition. After five cycles of chemotherapy; thoracic CT Scan revealed a well- defined growing pulmonary mass (4,5x4,7 cm) located in upper right lobe. In the mean time, renal function deterioration and hypercalcaemia (14, 6 mg/dl), was developed. Under suspicion of unconfirmed lymphomatous disease progression, a fine needle aspiration was programmed, which disclosed typical encapsulated Cryptococcal yeasts with Indian ink technique. The patient developed cephalea and vomiting, with normal cerebral scan, however, cryptococcal yeasts were present in CSF and serum cryptococal antigen was 1/32. Combination treatment with liposomal Amphotericin B and Flucytosine was applied for the opportunist infection with a good evolution. *Conclusions*. Under the presence of hypocalcaemia and pulmonary nodules in patients with HTLV-1 infection, under chemotherapy and antiretroviral treatment are mandatory exclude opportunist infections.

1613

EARLY REACTIVATION OF HERPESVIRUSES IS ASSOCIATED WITH COMPLICATIONS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Cytostatic therapy preceding HSCT causes common infectious complications, including reactivation of viral and fungal pathogens. Aims. The aim of our study was to study possible associations between herpesvirus reactivation and common complications following HSCT. Patients and methods. A group of 143 patients at the age of 2 to 61 years old (a median of 18.5 years) with various oncohematological disorders underwent allogeneic HSCT. Ratio of marrow-to-peripheral stem cell transplants was 35:65; unrelated HSCT, 64% of cases; myeloablative conditioning was chosen in 58%. Common complications, i.e., acute GvHD, mucositis, cystitis, pneumonias, neurological disorders, severe general infections were registered post-HSCT. The study included a sub-group of 54 children and young patients up to 21 years old with ALL and AML who were subject to HSCT with myeloablative (n=31) or non-myeloablative conditioning regimens (n=23). DNA testing for cytomegalovirus (CMV), herpes simplex virus (HSV), Epstein-Barr virus (EBV), Candida albicans, and Toxoplasma gondii was performed in leukocytes by gene-specific PCR at a weekly basis, up to day +100. Results. In general group of the patients, the incidence of HSV, CMV and EBV after HSCT was, respectively, 51%, 57% и 45%. Meanwhile, frequency of viral reactivation proved to be age-dependent, i.e., HSV and CMV positivity rates were lower in younger children (1 to 4 years old), followed by increased viral reactivation rates at 10-20 years. Strong correlation was found between CMV and HSV, as well as CMV and EBV reactivation, thus suggesting mixed infectious states. We have not revealed any differences in viral reactivation among patients who underwent conditioning therapy of different intensity. However, a higher percentage of infectious complications was found among young HSV-positive patients vs HSV-negative cases (resp., 88% and 39%, p=0,057). In younger patients (<21 years), a correlation was found between HSV persistence and neurological symptoms (P=0.002). Skin mucositis severity and duration was connected with HSV and CMV reactivation (P=0.02, or 0.008). Risk of acute intestinal GvHD and hemorrhagic cystitis correlated with EBV reactivation (P=0.01). Polyomavirus DNA (BK, JC) was detected in urine sediments, and positive findings, generally, reflected clinical signs of cystitis. Conclusions. Post-HSCT reactivation of HSV in younger patients is significantly associated with higher incidence of early complications, including bacterial infections, skin mucositis and neurological complications.

1614

GRANULOCYTE TRANSFUSIONS AS ADJUNCTIVE TREATMENT OF INFECTIONS IN NEUTROPENIC PATIENTS

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Background: The degree and duration of neutropenia have been recognized as crucial prognostic factors in hematological patients with invasive infections. Since the introduction of granulocyte colony stimulating factor (G-CSF), there has been a renewal of interest in granulocyte transfusions (GTX). However there is a large variability in the transfusion practice, and uncertainty about the beneficial effects of GTX as adjunctive measure to antimicrobial therapy. Aims: We designated a retrospective analysis to evaluate feasibility, efficacy and safety of GTX as adjunctive treatment for neutropenic fever unresponsive to antimicrobial therapy. *Methods:* We conducted a retrospective analysis about adult patients with hematological malignancies (HM) and fever during neutropenia (ANC<500x10^6/l and anticipated duration >7days) who received GTX after no clinical response to antimicrobial therapy. Volunteer donors received G-CSF 12h before the first of two consecutive collection procedures (5μg/kg). All of them had signed an informed consent for G-CSF administration and leukapheresis. Results: During a 7-year period (2004-10) 46 courses of GTX were administered to patients with HM and fever, after no clinical response to antimicrobial therapy. Patients were suffering from acute leukemia (30 myeloid and 5 lymphoid), lymphoma (9), multiple myeloma (2). Overall, 209 GTX were administered, with a median of 4 GTX per episode of infection (range 1-20). Infections causing fever were identified in 41 episodes: 17 bacterial sepsis, 23 invasive fungal diseases (IFDs) and 1 mixed bacterial/fungal sepsis. Remaining 5 cases were classified as fever of unknown origin (FUO). IFDs included 16 cases of pulmonary aspergillosis (proven/probable), 5 candidemia, 1 invasive zygomycosis, 1 invasive fusariosis and 1 infection due to Blastoschizomices capitatus. Donors' mean white blood cell (WBC) count at first leukapheresis was 27 x 10^9/l (range 13-45); at second procedure WBC count was lower (15 x 10^9/l, range 8-33), as expected. The mean yield was 25.6 x 10^9 PMN (range 3.5-75.8) at first procedure and 11.1 x 10^9 PMN at the second one (range 0.6-42.4). Mean transfused dose was 3.7 x 10^9/kg at first day (range 0.6-9.6) and 1.4 x 10^9/kg at second day (range 0.1-4.7). The combination of antimicrobial therapy with GTX led to a favourable clinical response in 33 of 46 valuable patients (72%); the acute infection-attributable mortality rate at 30th day after the last GTX was 29% for sepsis, 22% for IFD and 40% for FUO. *Conclusions:* at preliminary analysis GTX may be safe and efficacious in HM with severe infection to bridge the period of deep neutropenia, when antimicrobial therapy has failed. Controlled studies are needed to confirm this datum, and to define the proper role of this procedure and the optimal schedule for HM.

1615

PIPERACILLIN/TAZOBACTAM + AMIKACIN VS CEFEPIME + AMIKACIN AS EMPIRICAL ANTIBIOTIC THERAPY IN NEUTROPENIC PATIENTS. A SINGLE CENTRE RETROSPECTIVE ANALYSIS

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Background: Hematologic patients (pts) are at high risk for infections because of the disease and the chemotherapy that could induce a prolonged and deep neutropenia. *Aims:* The aim is to evaluate the efficacy of two different empirical antibiotic regimens. *Methods:* Between 2001 and 2006, 316 consecutive pts were included. At the beginning of chemotherapy they were randomly assigned to receive piperacilline/tazobactam (162 pts, Group 1) versus cefepime (154 pts, Group 2) + amikacin. All received levofloxacin as prophylaxis. In case of fever after blood cultures levofloxacin was discontinued and each patient received the assigned regimen. The 2 groups were similar for gender, age and disease's phase. The diagnoses were for each group respectively: leukemia 74 and 63; lymphoma 27 and 37, myeloma 56 and 41; the remaining diseases (aplastic anemia, multiple sclerosis, solid tumors) were similar in the 2 groups. 140 pts received a SCT (108 autologous and 32 allogeneic); the transplants were homogeneously distributed in the 2 groups. A CVC was present in 113 pts (70%) of group 1 and in 110 pts (72%) of group 2. The days with PMNs <0.1 x 10^9/L were 10 (range 1-

40) for each group. Results: 92/162 (group 1) and 87/154 pts (group 2) had fever and received the assigned treatment. The mean days with fever was 8.4 for group 1 and 8 for group 2. The diagnoses of the febrile episodes were for group 1 and 2: FUO 28 vs 26, clinically documented 16 vs 16, microbiologically documented with bacteremia 47 vs 44, neoplastic 4 vs 5 and drug-related 5 vs 9. The gram positive bacilli were 32 and 28 for each group (85% of them were coagulase-negative staphylococci). The gram negative were 17 (9 pseudomonas, 5 escherichia, 1 stenotrophomonas maltophilia, 2 klebsiella) and 14 (7 pseudomonas, 5 escherichia, 2 enterobacter) for each group. The clinically documented infections were all pneumonia and were considered fungal infections so the pts received antifungal therapy. The duration of antibiotic therapy was respectively 9.8 and 10.3 days. In case of isolation of methycillin-resistant bacteria the pts received vancomycin. 13 and 11 pts needed a second antibiotic course in group 1 and group 2 respectively (they received carbapenems). Excluding the clinically documented episodes, antifungal therapy was administered in 12 of group 1 and in 5 phases of group 2 for suspected or documented breakthrough fungal infections. All the febrile episodes except 6 resolved: of the 6 deaths, 4 were in the first group and 2 in the second one. The infection-related mortality in the microbiologically documented infections cohort was 5 and 4 cases in group 1 and 2 respectively. Of them 2 pts in group 1 and 2 pts in group 2 dead for breakthrough fungal infection; the other pts dead for bacterial infections. *Conclusions:* the response in the 2 groups is similar in terms of days of fever, need for further antibiotic courses, need for antifungal therapy and resolution of fever even if further and larger studises are necessary to assess if one regimen is superior.

1616

A MILIARY TUBERCULOSIS CASE ACCOMPANIED BY HEMOPHAGOCYTOSIS AND PANCYTOPENIA

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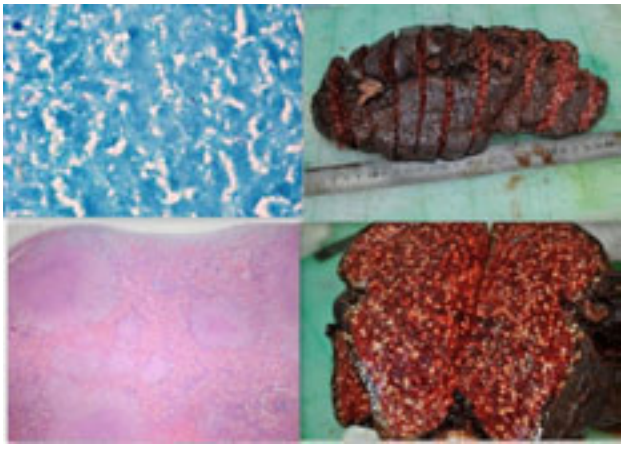
Background:Miliary tuberculosis occurs through uncontrollable spread of mycobacterium tuberculosis via lympho-hematogenous route. The symptoms of tuberculosis are quite variable; primary infection tends to be acute whereas late miliary tuberculosis is frequently subacute or chronic. The disease could go unnoticed because of the non-specific symptoms such that in USA, it was reported that % 20 of cases of miliary tuberculosis had a post-mortem diagnosis (2). Methods:A 25-year-old male patient sought medical help for fever, fatigue, weight loss and night sweats for three months; his fever was uncontrolled despite antibiotic and antifungal therapy. Hepatosplenomegaly and multiple cervical lymphadenopathies were detected in physical examination. His hemogram was Hg: 6.9g/dl, Hct: 20.6%, Plt: 125.000/μL, WBC: 4000/μL, Neu: 3600/μL, and in peripheral smear; there were marked anisopoicylocytosis, slight rouleaux formation, and 6 % of fragmented erythrocytes. In bone marrow aspiration; apparent dismyelopoiesis, hemophagocytosis and megaloblastic changes, an increase in eosinophilic series, in number of megakaryocytes, in blastic cells (4-5%) were seen, also atypical plasma cells (plasma cell proportion < 5%, few atypical mast cells were observed. Flowcytometry was nonspecific. Bone marrow biopsy was normocellular; a slight increase in precursor erythroid series, the cluster of megakaryocytes, and a relative decrease in granulocytes were observed. Bone marrow aspirates were also obtained for bacterial, micobacterial and parasite analysis. Protein electrophoresis was normal. The patient's temperature occasionally rose to 40°C during daytime and blood cultures which were taken during fever period were negative. Sputum ARB was negative. Salmonella, Brucella, Syphilis, Plasmodium, Leishmania, Toxoplasma, Echinococcus, CMV, EBV, HSV, HBV, HCV and HIV serologies were negative. Imaginig were obtained by CT and ultrasonography which showed hepatosplenomegaly (liver 207 mm and spleen 180 mm), multiple cervical and bilateral hiler lymphadenopathy. Excisional biopsy of lymp node was concordant with necrotizing lymphadenitis and medium-small vessel vasculitis. ANA was homogeneous granular pattern positive whereas other autoimmunity markers were negative. As LAP biopsy highlighted possible Wegener's Granulomatosis, paranasal sinus CT and cerebral MRI obtained but they were normal so as upper airway exam; then we performed renal biopsy and no signs of vasculitis were found. Bronchoscopy and brush biopsy were normal Transthoracic Echocardiogram was normal. In follow-up, pancytopenia developed; Hemoglobin: 5.8 g/dl, WBC: 1700/μL, Neutrophile: 1100/μL, PLT: 59000/μL. Besides, liver function tests followed a high and fluctuating course; AST: 110 U/L, ALT: 129 U/L. Results:We decided to perform splenectomy, liver wedge biopsy and peritoneal biopsy concurrently to the patient who had still fever to establish diagnosis. Tuberculosis bacilli were seen in the spleen microscopy(picture 1). The patient was diagnosed to have miliary tuberculosis and antituberculosis medications were started. In the liver and peritoneum biopsies, tuberculosis bacilli and granulomatous inflammation were seen. Later on, mycobacterium tuberculosis complex were also cultured in the bone marrow as well. Summary/Conclusion: Diagnosis of military tuberculosis is difficult because of nonspecific and unpredicted clinical course. It is unlikely that tuberculosis is accompanied by especially severe hemophagocytosis and pancytopenia. We have reported this case since it was difficult to diagnose and miliary tuberculosis was accompanied by severe hemophagocytosis and pancytopenia



1617

IMPORTANCE OF PRE-MEDICATION IN DECREASING THE INCIDENCE OF INFUSION-RELATED REACTION BEFORE USE OF AMPHOTERICIN B COLLOIDAL DISPERSION IN PATIENTS WITH ACUTE LEUKEMIA

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Background: Fungal infections cause significant morbidity and mortality in patients with acute leukemia (AL). Conventional amphotericin B deoxycholate (cAmB) has been successfully used for treatment of these infections. Nevertheless, the side effects and particularly nephrotoxicity of cAmB limits its use. Amphotericin B colloidal dispersion (ABCD) has decreased the rate of nephrotoxicity, but it seams that the frequency rate of infusion-related reaction (IRR) is still high in comparison to other forms of AmB, despite the pre-medication received before its use. The most commonly reported IRRs were chills, rigor, fever, nausea and vomiting. Aims: The aims of this single-center study were to determine the type and the incidence of IRR in patients with AL who were receiving ABCD according to the type of pre-medication used as well as ABCD's doses. Methods: In this study 32 patients with AL, receiving ABCD, were included. Indications for ABCD use were empirical in prolonged febrile neutropenia or therapeutical in documented fungal infection. Patients were divided into 2 groups according to the 2 types of pre-medication applied. Each group involved 16 patients. One group received type 1 pre-medication: methylprednisolone 300 mg + metamizol 2.5 mg intravenous (i.v.) 30 minutes before infusion of ABCD. The other group received type 2 pre-medication: methylprednisolone 40 mg i.v. + loratidine 10 mg per os (p.o.) + paracetamol 1000 mg p.o. 30 minutes before infusion of ABCD. Applied doses of ABCD were 2.5-5 mg/kg. Duration of IRR in days (started after first infusion of ABCD) were recorded. The median duration of ABCD's treatment was 9 days. The risk factors were identified using the univariate and multivariate

analysis. Results: The median age of a patient was 43 years, range 19-65 years. The average dose of applied ABCD was 3.25 mg/kg and the average duration of IRR was 2 days (range 1-8 days). Recorded IRR concerted the following symptoms: chills, rigor and fever. The other types of side effects and toxicity were not detected in patients involved in this study. IRR was recorded in 47% of patients. In the group who received pre-medication of type 1, IRR was recorded in 18.75%, while in the group who received pre-medication of type 2 IRR was recorded in 75% of patients. Thus, the patients, who received type 2 pre-medication, had IRR significantly more frequent compared with the patients who received type 1 pre-medication (p=0.004). The patients treated with the dose of ABCD >3 mg/kg had IRR significantly more frequent than those treated with the dose ≤3 mg/kg (p=0.033). The multivariate analysis indicated the type of pre-medication as the most significant risk factor for IRR: p=0.022, relative risk (RR)=0.231 (95% CI 0.066-0.810). The significant risk factors for prolonged duration of IRR were type 2 pre-medication (p=0.002) and the dose of ABCD >3mg/kg (p=0.005). The most significant risk factor for prolonged duration of IRR was the applied dose of ABCD >3mg/kg: p=0.003, RR=2.325 (95% CI 0.835-3.815). Conclusions: The pre-medication with high doses of methylprednisolone with metamizol significantly decreased the rate and duration of IRR in AML patients receiving ABCD.

1618

VOLUME, CONDUCTIVITY AND SCATTER PROPERTIES OF LEUKOCYTES (VCS TECHNOLOGY) IS A HIGHLY SENSITIVE AND SPECIFIC PREDICTOR OF BLOOD CULTURE PROVEN NEONATAL SEPSIS

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Background. Neonatal septicemia remains as the major cause of mortality in newborns. As per WHO estimates, more than 1/3rd of the estimated 4 million neonatal deaths around the world are caused by severe infections and a quarter, around 1 million, are due to neonatal sepsis/pneumonia alone. In India, in a community based study, septicemia and pneumonia accounted for 52% of neonatal deaths. In a hospital-based data have been reported 18.6% neonatal deaths to be due to sepsis and 37.6%. in extra-mural babies sepsis. The existing tests for sepsis screen include blood culture (gold standard), CBC, Immature neutrophils (smear), immature to total neutrophil (IT) ratio and C-reactive protein (CRP). *Aims.* We evaluated the new automated WBC morphology: data on individual cell volume (V), conductivity (C) and laser light scatter (S) (VCS technology, generating what is called Cell Population Data (@CPD), that are reported as numerical values (Mean and Standard Deviations (SD) for every type of leukocyte. Examples of these CPD data are: Mean Neutrophil Volume that has previously been reported as a good test for Sepsis and Bacterial infections. *Methods.* We have included 133 consecutive neonates (0-28 days of life) admitted to neonatal intensive care unit for suspected sepsis were enrolled in the study together with 36 gestation-matched controls. The blood samples were run on the Beckman Coulter® LH750 and LH755 hematology analyzers to provide CBC, WBC Diff, retics and CPD. Were also evaluated blood culture and CRP. Peripheral blood smears were made on all cases. In this study we analysed MNV, CRP and IT ratio as a single parameters and combinations of them to detect sepsis. Results. Searching for the best combination of parameters, we found that combining MNV and CRP, we obtain: sensitivity: 100.0%, specificity 85.7% cut-off>154.8, AUC: 0.968. and we found a discriminant function for sepsis (DFS=1.12 - 0.004* NE# - 0.065* HGB + 0.013*MNV - 0.019*MNS + 0.025SD -S-Ly) with 100% sensitivity,100% specificity, AUC=1 (Table 1) When we compare (proven sepsis + probable sepsis) and controls, DFS have shown 82.1% sensitivity and 100% specificity with AUC 0,889, at a cut-off >0.3242 (Table 2). Immature neutrophils (AUC 0.634) had a lower performance (P = 0.0044) than Mean Neutrophil Volume (CPD) (AUC: 0.922) even if they look for similar aspects of neutrophils, because is well known that Immature Neutrophils are bigger than segmented neutrophils (Woessner et al.). *Summary.* These findings demonstrate that the automated leukocyte morphology (CPD) and the combination of CPD and other parameters may dramatically improve the early detection of neonatal sepsis. Further studies could evaluate IL-6 to see if IL-6 could further enhance the predictive value. Also, prospective protocols on a larger number of cases and/or randomized co-operative studies could be designed to prove the clinical efficacy of the present findings so as to incorporate them in daily clinical practice.

Table 1. Sepsis vs Normals

	Hgb	CRP	MNV	MNV+CRP	DFS
Sample size (Positive / Negative)	47 (19/28)	46 (19/27)	50 (22/28)	50 (22/28)	47 (19/28)
Sensitivity	73.7	78.9	95.5	100	100
Specificity	89.3	96.3	82.1	85.7	100
Criterion	<=11.6	>7	>154.2	>154.8	>0.3242
AUC	0.857	0.891	0.925	0.968	1.000
p (area=0.5)	<0.0001	0.0001	0.0001	0.0001	0

Table 2. (Sepsis and probable sepsis) vs Normals

	Hgb	CRP	MNV	MNV+CRP	DFS
Sample size (Positive / Negative)	67 (39/28)	61 (34/27)	67 (39/28)	61 (34/27)	67 (39/28)
Sensitivity	82.1	70.6	69.2	79.4	82.1
Specificity	60.7	96.3	89.3	96.3	100
Criterion	<14.2	>7	>155.3	>161	>0.3242
AUC	0.77	0.855	0.764	0.849	0.889
p (area=0.5)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

1619

A SURVEY ON CMV REACTIVATION IN 102 PATIENTS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: EFFECTIVENESS OF PREEMPTIVE THERAPY

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Background: Cytomegalovirus (CMV) continues to cause major complications after allogeneic stem cell transplantation (allo-SCT). Materials and methods: In 2009 and 2010, 102 pts (median age 49; 68 males) received transplant for high risk haematological malignancies in our centre: 51 AML, 14 ALL, 2 biphenotypic AL, 6 MDS, 10 HD, 6 NHL, 3 CML, 3 MFI, 7 other diseases. Allo-SCT were performed in 96/102 from PBSC source; 10 from HLA identical sibling (Sib), 21 from unrelated volunteer (MUD), 65 from family haploidentical donor (Haplo) without ex-vivo T-cell depletion, 6 cord blood (CB). Pts were evaluated for CMV quantitative DNA PCR twice weekly within the first 3 months after allo-SCT. Donor/host serostatus was: -/- 8 (8 %), +/+ 52 (51%), -/+ 33 (32%), +/- 9 (9%). All pts received acyclovir as viral prophylaxis. Results: We observed CMV reactivation in 59/102 pts (58%), median time of onset 31.5 (3-91) days post Allo-SCT, median number of CMV PCR 1377 (93-346480) cp/mL. The donor/host (D/H) serostatus in these pts was +/+ 33 (56%), -/+ 23 (39%), +/- 3 (5%); 32 were Haplo, 5 CB, 15 MUD, 7 Sib allo-SCT. Preemptive therapy was administered in 39 pts; 13 pts received oral valganciclovir (VCGV) as first line preemptive therapy, 17 ganciclovir (GCV), 9 foscarnet (FCV). Median time of CMV infection was 28 days (5-407) days and we observed a median of 1 CMV further reactivation in the first year post transplant; these events were similar in all pts of this group. A total of 13 pts required drug cross-over; D/H serostatus was neg/pos in 9 pts, pos/pos in 4. Only 2 cases of CMV organ involvement were observed after MUD allo-SCT: in one the site of CMV disease was colon in the other lung. In these pts who experienced a CMV reactivation 37 are still alive, 22 were dead. No CMV reactivation was reported in 43/102 pts (42%). The donor/host (D/H) serostatus in these pts was -/- 8 (19%), +/+ 19 (44%), -/+ 10 (23%), +/- 6 (14%); 33 were Haplo, 1 CB, 6 MUD, 3 Sib allo-SCT. In these pts who not experienced a CMV reactivation 21 are still alive, 22 were dead. Conclusions: These data suggest the efficacy of CMV preventionin HCT recipients from all donor sources.

1620

INCIDENCE OF INFUSION-RELATED REACTIONS IN PREMEDICATED PATIENTS RECEIVING AMPHOCIL®: A SINGLE CENTER EXPERIENCE

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Background: Higher rate of infusion-related reactions (IRRs) occuring with Amhotericin B Cholesteryl Sulfate Complex for Injection (ABCD; Amphotec®/Amphocyl®) vs. other forms of amphotericin B are report-

ed. It has been recently shown that premedication prior to ABCD decreases the incidence of IRRs. Aim: To determine the IRRs incidence associated with Amphocyl® therapy in the context of the premedication. Patients and Methods: Patients beginning treatment with Amphocyl® were eligible. They were treated as follows: 1mg/kg first day and then 3-4mg/kg (maximum 6mg/kg); i.v infusion. At least two premedication drugs were recommended (ad libitum). The patients has received the same premedication combinations during the treatment. In the case of IRRs the infusions were stopped and Acetaminophen and/or Meperidine was administered. Corticosteroids were reserved for grade III of IRRs. Data on ABCD doses and premedications have been collected during the whole treatment period. Data collected included: demographics, co-infections, history of prior antifungals, routine laboratory values, type of fungal infection, premedication type, daily dose of ABCD and IRRs occurence/grade/type. Informations regarding the patients response to ABCD were collected. Mann-Whitney test was used to compare the rates of IRRs between various premedication regimens. Results: 39 adult patients (M:F-25/14; median age 40 yrs, range 18-65; median body weight 79 kg, range,48-108 were treated with Amphocyl® from March 2007-June 2008. The majority had acute leukemia 28/39; 22/39 received prior antifungals (convential amphotericin B 14, azoles 8). Two patients had definite, 6 probable and 17 possible fungal infections, respectively. Lungs were the most frequent infection site (21); 14 pts were treated with Amphocyl® empirically. There were 351 infusions in total, range 1-35; average daily dose was 3.46 mg/kg, range 1.7-5.80, average cumulative dose was 2613 mg, range 100-10.300; average therapy duration was 9 days, range 1-35. All the patients were premedicated- 19 received two, 20 three drugs. The premedications used were as follows: Acetaminophen, Loratadine, Meperidine, Dexamethasone, Methylprednisolone, Metamizol. The most frequent combinations of drugs in premedications were: Acetaminophen + Loratadine in 9 and Acetaminophen + Loratadine + one corticosteroid in 11 pts. 20/39 pts. experienced IRRs while IRRs were reported following 48 infusions (13.7%). The most frequent IRRs were: chills 40/48, fever 42/48 and rigors 16/48. All IRRs were low severity. The incidence if IRRs was highest in the first five days of therapy (d1: 17, d2: 13, d3: 7, d4: 4, d5: 2, d6: 1). Loratadine was associated with a statistically significant lower rate of IRRs vs. other types of premedication (p? 0.03). Reasons for Amphocil® discontinuation were as follows: recovery (17), death (10), identification of fungal organism (6), infusion intolerability (3) and infusion intolerability associated with recovery (3). No significant nephrotoxicity was registered. After 12 weeks of folow-up 27 pts were alive, all positively responding on Amphocyl®. *Conclusions*: We demonstrated lower rates of IRRs compared to historical rates which, is in agreement with the results of PRoACT. The incidence of IRRs decreased significantly from days 1-5 and desappeared after day 7. Premedication with Loratadaine was associated with significantly lower IRRs rate comparing with other type of drugs.

1621

INFLUENZA A-H1N1 VIRUS INFECTION IN PATIENTS UNDERGOING HSCT

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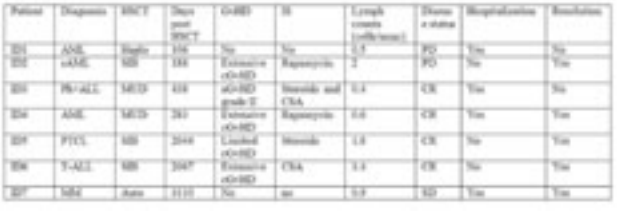
Background. Hematological patients (pts) under hematopoietic stem cell transplantations (HSCT) are at a high risk for severe viral infections. We analyzed the clinical impact of influenza A infection (H1N1) outbreak in our patients population. Material and Methods. From September 2009 to January 2011 we reported 7 cases of H1N1 infections in 7 HSCT recipients. Patients' characteristics are shown in table 1. Diagnosis of H1N1 was made by real-time PCR assay in nasopharyngeal samples. Results. Six pts were not vaccinated, one developed the first symptoms of influenza 19 days after the first dose of vaccine. The most frequent symptoms were: fever (100%), cough (86%), dyspnea (43%), rhinorrhea (28%), odynophagia (28%), headache (28%) and arthralgia (14%). All cases were confirmed by PCR in nasopharyngeal or bronchoalveolar lavage samples. Five pts had pneumonia; CT-scan showed multiple pulmonary nodules with ground glass appearance. Chest X-ray results were not diagnostic. All patients were treated with oseltamivir 75 mg bid for a median of 7 days (7-19), except for one who received oseltamivir 150 mg bid for the severity of the infection. Oseltamivir was started within 24 hours from the onset of symptoms. All pts received concomitant antimicrobial therapy for a median of 17 days (7-40). No major adverse events related to anti-influenza treatment were reported. We observed a decrease of platelet counts in four pts with pancytopenia at the time of infection, 3/4 pts required transfusion support. Resolution of symptoms was achieved in a median of 14 days (8-30) in 5/7 pts. Four pts needed hospitalization for a median of 15 days (10-18); 3/4 pts needed ventilatory support (C-PAP for ID1, C-PAP plus intubation for ID3, high-flow O2 therapy for ID4). All pts developed lymphopenia (<1000/mmc). Two pts died from respiratory failure: one was in progression disease, one other patient was in complete remission but profoundly immunosuppressed (high dose steroids plus Cyclosporin as GvHD therapy). Conclusions: H1N1 infections in HCST recipients can result in a severe and fatal syndrome. Since symptoms of a H1N1 infection are unspecific, early testing for H1N1 virus in hematological pts is mandatory. CT scan is the diagnostic investigation of choice to rule out pneumonia. Clinical courses of H1N1 infections in HCST recipients range individually. The early use of oseltamivir may help determinig the good outcome of the infection, but prognosis seems to be strongly related to the disease status and to the degree of immunosuppression. In our experience lymphopenia correlates with the outcome.

Table 1



1622

HUMAN HERPESVIRUS-6 (HHV-6) REACTIVATION IN HEMATOLOGICAL PATIENTS

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Background: Human Herpesvirus-6 (HHV-6) can reactivate and sustain various clinical manifestations in immunocompromised patients (pts). Materials and methods: We retrospectively evaluated hematological pts who developed positivity to HHV-6, measured by quantitative PCR. Results: From January 2009 to January 2011 we observed HHV-6 positivity in 49 pts (median age 54; 30 males), 39 of them received allogeneic stem cell transplantation (allo-SCT), 2 autologous transplantation, 8 chemotherapy. At the time of reactivation all pts were receiving acyclovir as viral prophylaxis except 5 (3 pts off antiviral therapy, 2 on ganciclovir). A concomitant CMV positivity was detected in 11/49 pts, while a severe neutropenia in 23/49 pts. Allo-SCT was performed in 38/39 pts from PBSC source; 5 from HLA identical sibling, 3 from unrelated volunteer, 29 from haploidentical donor, 2 cord blood. Among allo-SCT pts 15 had GvHD (13/15 with grade III-IV aGvHD), and 32 were profoundly immunosuppressed with variable association of 2-4 drugs. Median time from allo-SCT to HHV-6 reactivation was 41 days (7-625). In 25 pts HHV-6 was detected in plasma, with a median number of 19937 cp/mL (34-4524600); 18/25 pts had fever (9 bacterial and 1 fungal infection), 8/25 skin rash, 4/25 worsening of liver function, 5/25 cytopenia. In 9 cases HHV-6 was present in bone marrow samples and 5 of them had concomitant HHV-6 plasma positivity; the median viral load was 25123 cp/mL (568-904000) and 3 pts developed cytopenia. In 11 pts HHV-6 was observed in bronchoalveolar lavage samples with a median of 502 cp/mL (57-50211); 9/11 pts had fever (5 bacterial and 1 fungal infection). In 16 pts (15 of them after allo-SCT, 9 with previous gut aGvHD) HHV-6 was also present in gastrointestinal biopsy (13 colorectal, 3 gastric) with a median of 5550 cp/mL (120-163800); in 4 cases HHV-6 was also found on plasma; 11/16 pts had diarrhoea. HHV-6 was found in cerebrospinal fluid in 3 pts (all within 30 days post allo-SCT); in 2/3 virus was also detected in peripheral blood; the median viral load was 19454 cp/mL (4508-39250); 3/3 pts experienced encephalitis showing confusion and anxiety, 1/3 seizure and 2/3 abnormal findings on brain MRI; all pts had fever and 1 skin rash. In all these cases HHV-6 was treated only when associated with potentially related clinical manifestations. Antiviral therapy was necessary in 23 pts (all received foscarnet

except 3) and 13 of them solved the event. Among pts who experienced HHV-6 reactivation, 26/49 pts (53%) died. Conclusions: HHV6 reactivation is associated with high morbidity and mortality in hematological pts undergoing intensive treatment. Particularly, in pts who underwent allo-SCT HHV6 reactivation is associated with a poor outcome. A regular DNA monitoring is prospectively performed and a pre-emptive treatment is implemented in the setting of allo-SCT.

1623

CHARACTERISTICS AND OUTCOMES OF ADULT HAEMATOLOGY PATIENTS ADMITTED TO THE INTENSIVE CARE UNIT (ICU)

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Background. The role of intensive care support for haematology patients has historically been contentious. *Aims.* To profile patient characteristics and assess outcomes of adult haematology patients admitted to ICU. Methods: Retrospective audit of medical notes, laboratory records and Intensive Care National Audit and Research Centre (ICNARC) data for all adult haematology patients admitted to Belfast City Hospital ICU in 2009. Results: Twenty one patients were admitted to ICU; mean age was 56-years (SD 12.5), 52% were male and 19 (82%) had a malignant diagnosis. The main indication for admission was neutropenic sepsis with associated organ impairment (n=18, 85%). ICU mortality was 43%. ICU survivors had lower acute physiology and chronic health evaluation (APACHE II) scores, and decreased requirements for invasive ventilation and inotropic support. Three and six-month mortality rates were 62% and 67% respectively. Of the post-six month survivors, one had relapsed, one had responding disease and five remained in remission. Two patients have subsequently undergone a reduced intensity conditioning transplant. Conclusion: A third of patients survived >6 months indicating that critically ill haematology patients may benefit from ICU admission, allowing progression to potentially curative therapies.

Table 1. Characteristics of ICU survivors and non survivors.

	ICU survivors (n = 12)	ICU non-survivors (n = 9)
APACHE II score, (mean, SD)	20 (5)	28 (6.6)
Invasive ventilation (n %)	6 (50%)	8 (90%)
Continuous renal replacement therapy	4 (33%)	3 (33%)
Inotropic support (n %)	3 (25%)	7 (78%)
Maximum no. of organs supported (mean, SD)	2.25 (1.1)	3 (0.7)
Days of ICU stay (median, IQR)	4.3 (2, 8)	4 (1, 7)

1624

BONE MARROW ASPIRATION DISCLOSES DISSEMINATED HISTOPLAMOSIS IN AN APPARENTLY IMMUNOCOMPETENT PATIENT

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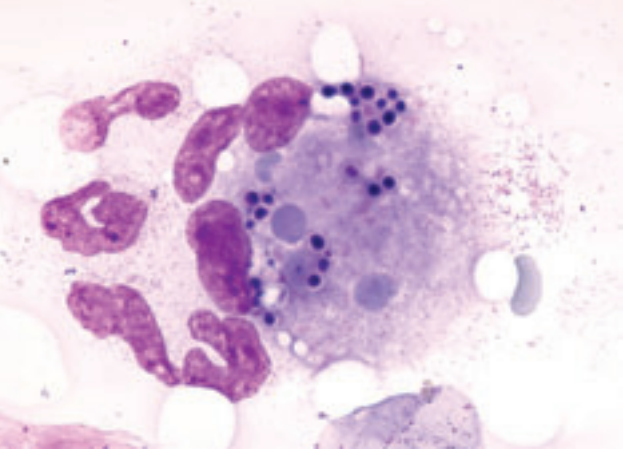
Background. Histoplasma capsulatum is a dimorphic fungus endemic in the American continent, Africa and Asia. People acquire histoplasma through inhalation of spores from soils contaminated by bat or bird excrements. In Europe cases are usually imported and non frequent. Disseminated histoplasmosis normally affects patients with immunodeficiency. We report on a non-immunocompromised patient who developed a disseminated histoplasmosis due to a reactivation of a latent infection acquired during a previous travel to America more than 10 years earlier. Case report: A 39 year-old woman, with no prior medical history of interest, HIV negative, developed twelve days before admission, fever (39.6 °C), vomiting, diarrhoea and nocturnal cough. Blood tests showed thrombocytopaenia (platelets 59x10^9/L) and mild transaminase elevation as the only pathological findings. After admission, her condition worsened with the appearance of bilateral basal pulmonary infiltrates and persistent fever, irresponsive to treatment with Levofloxacin and Metronidazole. An abdominal ultrasound scan showed hepatosplenomegaly only. She was moved to the intensive care unit due to progressive respiratory failure that required intubation with mechanical ventilation and noradrenaline perfusion. A bronchoalveolar lavage was performed, with negative results for mycobacteria, fungi, viruses, legionella and other bacteria. Doxycycline and Meropenem were added to the treatment. All microbiological tests were repeatedly negative (blood cultures, coprocultures, parasites in stools, serological tests for HIV, brucella, Epstein Barr virus, cytomegalovirus, coxiella burnetti, s. pneumoniae, hepatitis, salmonella, chlamydia and mycoplasma, H1N1 virus, clostridium difficile toxin and Mantoux test). Some days later, the patient's clinical condition progressively improved, allowing the withdrawal of respiratory support, but the fever reappeared. The patient was transferred to an internal medicine ward, with progressive pancytopaenia (hemoglobin 7 gr/dl, leukocytes 3.7x10^9/L and platelets 37x10^9/L). A bone marrow aspirate showed an increased number of macrophages, with the presence of round PAS-positive pseudo-capsulated intracellular microorganisms, suggestive of infection by histoplasma capsulatum. A bone marrow biopsy showed the same microorganisms and a PCR for histoplasma capsulatum confirmed the diagnosis. Bone marrow cultures for bacteria, fungi and mycobacteria were negative. The patient was initially treated with Itraconazole, but due to sub-optimal response the treatment was changed to Amphotericin B (lipidic complex) for three weeks, followed by oral Itraconazole. At discharge, the patient was afebrile, asymptomatic, and the histoplasma capsulatum PCR in peripheral blood was negative. The only pathological finding in the blood count was an inflammatory anaemia that subsided after a month. Although the patient had not travelled recently to endemic areas, she had been in Costa Rica 12 years before this episode. Conclusions: this case highlights the utility of bone marrow aspiration/biopsy in the diagnosis of disseminated histoplasmosis. It emphasizes the importance of a detailed inspection of bone marrow aspirates, with special focus on macrophages if an infection is suspected.



1625

PREDICTION OF PROGNOSIS FOR CHILDREN CARED IN INTENSIVE CARE UNIT AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION.

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Background. The pediatric index of mortality 2 (PIM-2) is a useful scoring system for prediction of prognosis in children with ICU support. The oncological pediatric risk of mortality score (O-PRISM) was also using for intensive cared children with hematopoietic stem cell transplantation (HSCT). Aims: Using both O-PRISM and PIM-2, we investigated risk factors, prognostic prediction tool, and survival for early detection of admission to intensive care unit (ICU). Methods: We reviewed retrospectively medical records of children cared in our institution ICU after HSCT between 2004 and 2010. There were excluded in patients died within 2 hours after moving to ICU. We used O-PRISM and PIM-2 for prediction of prognosis. We analyzed worst parameters in ICU by t-test, ANOVA, Cox-regression, multiple logistic regression, and receiver operating characteristics curve (ROC). Results: Fifty five out of 54 children were admitted to ICU on post-HSCT period. Non-malignant disease was 8 patients, and 16 children were transplanted with high risk disease

status. The source of stem cells were 14 matched sibling donor, 22 unrelated, 19 cord blood. The median duration in ICU was 9 days (0~110). The reasons of admission to ICU were 32 pulmonary, 14 neurologic, and 9 hemodynamic events. Twenty seven children did not take care of mechanical ventilation. Six patients (11.1%) were survived after intensive care. The factors with discharge with survival were mental status (P=0.04), although there were FiO2, prothrombin time, potassium, pupil reflex in univariate analysis. In multiple logistic regression, there were significant factors with PaCO2 (P=0.028), O-PRISM (P=0.039), and PIM-2 (P=0.004) for prognosis. For prediction of prognosis, O-PRISM (P=0.019) was superior to PIM-2 (P=0.435) in intensive cared children after HSCT. Conclusion: O-PRISM following HSCT is more predictable scoring system in children with ICU support, and Glasgow coma scale and PaCO2 were more reliable prognostic factors for ICU admission.

1626

CENTRAL VENOUS CATHETER INFECTIONS IN HEMATOLOGICAL PATIENTS: REPORT FROM A SINGLE INSTITUTION

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Central venous catheters (CVCs) are currently used in hematological settings for intravenous therapy, stem cell transplant (SCT), blood products and fluids infusions. However, catheter-related bloodstream infections (CRBSIs) are an important cause of morbidity and mortality. Aims: The study aimed at retrospectively analyzing infectious complications in hematological patients. Patients and methods: One hundred fifty-five CVCs (100 Port-a-Cath, 57 external CVC), inserted from March 2001 to June 2010, have been analyzed. Patients with external CVC received chemotherapy for acute leukemia or autologous SCT for myeloma or lymphoma. Patients with port-a-cath systems, affected by lymphoma or myeloma, underwent less intensive chemotherapy. Results: Out of 57 external CVCs, 6 experienced infections: 4 sepsis (P.aeruginosa), 1 skin tunnel infection (P.aeruginosa and S.epidermidis), 1 endocarditis (Streptococcus oralis). Three fatal cases occurred in leukemic patients treated with intensive chemotherapy. Infections did not affect catheter survival. Performance status did not influence catheter infections, neither fever nor infections at the time of procedure. Percutaneous technique was significantly associated to CRBSI (p=0.023). CVC infections were more frequently related to left side insertion and to percutaneous technique (p=0.023). CRBSI was not statistically related to the type of immunosoppressive therapy (p= 0.72). The underlying disease (p=0.03) and the type of therapy (p=0.029) were significantly associated with development of P.aeruginosa infections. Patients with a neutrophil counts < 500/? L for more than 10 days more frequently developed Pseudomonas infections (p=0.002). Out of 100 port-a-cath, sepsis occurred in 3 port-a-cath, 2 by S.epidermidis, and one by fungal infection. Overall, the infection rate per 1000cvc days was 0.71 in external CVCs, and 0.65 in ports. Cumulative survival was mainly influenced by infectious complications (Log Rank and Breslow p<0.0001). Although not statistically different, patients who developed a CR-BSI had lower neutrophil counts at the time of insertion. Less intensive chemotherapy in patients with port did not influence CRBSIs. Summary/conclusions: Some studies reported that CRBSIs are more frequent in patients with malnutrition and unfavorable performance status. However, the presence of infections and unfavorable performance status did not influence CRBSIs in our patients with external CVC and port-a-cath. There are conflicting data from the literature upon the importance of neutrophil counts at the time of insertion: according to some authors, neutropenia is a risk factor for CVC-related infections. Our study did not report a correlation between neutrophil counts and infection development neither in external CVCs or in ports. Diagnosis and therapy did not influence CRBSIs rate because of heterogeneity and/or low number of patients. In this retrospective study were confirmed the data reported by others that the side and type of venous access represent risk factors for CRBSIs. Materials and type of device may promote colonization and biofilm formation, with higher infection risk, but we were not able to find this association in 157 CVCs. The present study contributes to underline the complex management of hematological patients and their particular susceptibility to CVC infections.

1627

PREVALENCE OF HEPATITIS C AMONG MULTI-TRANSFUSED THALASSEMIA PATIENTS IN THE SULTANATE OF OMAN- SINGLE CENTRE STUDY

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Background. Regular blood transfusion in thalassemia major is essential to maintain life. Risk of viral infections, especially hepatotropic viruses associated with blood transfusion, is a major concern. Screening of blood products for HIV, hepatitis B and C using the most sensitive methods have markedly decreased the risk of viral transmission. *Aims*. To evaluate the prevalence of hepatitis C in multi-transfused homozygous thalassemia patients in the Sultanate of Oman and to identify possible related risk factors. *Methods*. This is a retrospective chart review of all thalassemia major and intermedia patients (n=200) treated at the thalassemia Unit at Sultan Qaboos University Hospital in Oman. Relevant demographic and clinical characteristics were collected along with liver function tests, anti-HCV, and HCV RNA. Analyses were conducted using descriptive statistics. *Results*. Mean age of the patients was 23 ± 7 years ranging from 7 to 50 years. Eighty-one (41%) thalassemia patients were found to be HCV-Ab positive. HCV-Ab positive patients were significantly older compared to their HCV-Ab negative counterparts (28 versus 20 years; p<0.001). 11 patients (5.5%) have become sero-positive (PCR positive) after institution of donor screening. HCV-Ab positive patients were significantly more likely to be diabetic than HCV-Ab negative patients (26% versus 8%; p<0.001). HCV-RNA was performed in 62 patients out of the eighty-one patients positive for anti-HCV and found to be positive in 41% of the patients (33/62). Eighteen of the 33 patients (55%) had genotype 1, six (18%) had genotype 2 & 3 and five (15%) had genotype 4. Conclusions: High prevalence of hepatitis C among multi-transfused thalassemic patients in Oman is likely due to transfusion of blood before the implementation of hepatitis C screening. Blood screening initiated after 1991 in Oman has significantly reduced the risk of hepatitis C associated with blood transfusion. Nevertheless, despite this screening, a number of patients have become HCV PCR positive. Hepatitis C infection remains a major clinical complication in this group of patients.

1628

TWO YEARS WITH PANDEMIC H1N1 2009 INFLUENZA A IN A TERTIARY HEMATOLOGY DEPARTMENT

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Background. Pandemic influenza A H1N1 2009 has been the prevailing influenza strain in Czech republic from autumn 2009 till March 2011. Severly immunocompromised patients with hematological malignacies and after stem cell transplantation are at increased risk of severe and devastating complications of this infection. *Aims*. To evaluate the morbidity and mortality from H1N1 influenza A among immunocompromised patiens and the importance of aggressive approach in management in hematology department. *Methods*. We have retrospectivelly evaluated the cases of influenza H1N1 (PCR proven) among patients hospitalized in our 35 bed haematology and bone marrow transplantation department in Prague, Czech republic during two influenza seasons, 2009/2010 and 2010/2011. In total we identified 17 cases (13 patients with acute leukemia, 2 with non-hodgkin lymphoma, 1 with chronic myelocytic leukemia and one with thrombotic trombocytopenic purpura) 6 of the patients were after alogeneic haematopoietic stem cell transplantation. We changed the attitude to prophylaxis and management of the individual cases before 2010/2011 influenza season. In the 2010/2011 season we increased the percentage of vaccinated personnel, we instituted oseltamivir prophylaxis to all hospitalized patients (considering them close contacts) at the time of the first detection positivity of H1N1 in hospitalized patient. All immunocompromised H1N1 positive patients received combination treatment - oseltamivir 300 mg p.o. per day and zenamivir inhalation 20 mg per day. Results: There were 9 H1N1 positive hospitalized patients in 2009/2010 season, 6 of them had pulmonary involvement and 3 of them died (33%). There were 8 H1N1 positive cases in 2010/2011, 6 of them had pneumonia (not all cases of pneumonia in H1N1 positive patiens were confirmed by bronchoalveolar lavage) and none of them died. *Conclusions*. The consequences of H1N1 infection could be devastating among immunocompromised patients. Our experience strongly supports very aggressive surveilance, prophylaxis

and therapeutic approaches in hematology department. Immediate institution of aggressive treatment to early diagnosed cases in 2010/2011 season enabled recovery in all cases which contrasts with our early experiences from 2009/2010 season.

1629

CHARACTERISTICS AND RISK FACTORS FOR DEEP TISSUE ABSCESSES IN A CONSECUTIVE COHORT OF PATIENTS WITH ACUTE MYELOID LEUKEMIA: A NATIONAL POPULATION STUDY IN TAIWAN

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Background. Abscess may occur as an infectious manifestation in acute myeloid leukaemia (AML) patients. However, deep tissue abscess is more complicated since bleeding tendency was a major obstacle for diagnostic approach or surgical drainage in leukaemic patients. *Aims*. We focused on the prevalence of deep tissue abscess in patients with AML and the clinical characteristics and risk factors of AML patients developing deep tissue abscess. *Methods*. 5066 newly diagnosed patients from Jan 1997 to Jun 2006 were retrospectively analyzed from the Taiwan National Health Insurance Research Database in Taiwan. Deep tissue abscess was defined as abscess occurred at gastrointestinal tract, genitourinary tract, liver, deep neck, eye, central nervous system, mediastinal or pulmonary, and oral cavity in all AML patients after the diagnosis of AML. Eligible patients were sub-grouped as abscess group (n=768) and non-abscess group (n=4298). We determined the factors potentially associated with deep tissue abscess incidence. *Results*. The prevalence of all kinds of deep tissue abscesses in AML patients is 15.2%. The most predominant site is gastrointestinal tract abscess (n = 290). The median time from AML diagnosis to deep tissue abscess is 131 days. 197 (25.6%) patients with deep tissue abscess have systemic infection within 30 days prior to the abscess diagnosis. Three independent risk factors were identified in predicting deep tissue abscess development in AML patients within 36 months after AML diagnosis. They are age less than 60 year-old (p<0.001, HR=1.871), male gender (p=0.004, HR ì=1.249), and secondary AML (p=0.01, HR=1.365). Risk stratification system was categorized according to the numbers of independent risk factors (0, 1, and more than 2 risk factors). The cumulative incidence of abscess within 36 months after AML diagnosis according to the risk stratification system was 0%, 9.4%, and 66.5% (0 factors vs. 1 factor vs. more than 2 factors, respectively, p < 0.001). *Conclusions*. Deep tissue abscess are not uncommon in AML patients. Patients who are man, older than 60 year-old and secondary AML warrant special attention since they are prone to have abscess after the diagnosis of AML.

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CIRRHOSIS ASSOCIATED WITH POOR OUTCOME IN ACUTE MYELOID LEUKAEMIA PATIENTS DEVELOPED LIVER ABSCESS: A NATIONAL POPULATION STUDY IN TAIWAN

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Background. Liver abscess following prolonged neutropenic periods during disease course has emerged as a major infectious complication for patients with acute myeloid leukaemia (AML). Previous report has showed that bleeding tendency was a major obstacle for diagnostic approach and surgical drainage in leukaemic patients. *Aims*. We focused on whether the surgical drainage procedure can prolong survival and determined factors associated with survival in AML patients developing liver abscess. *Methods.* We retrospectively reviewed data drawn from the Taiwan National Health Insurance Research Database which cover the entire civil of Taiwan. Cases of liver abscess (ICD-9 code 572.0) were defined as an intrahepatic infection with abscess formation occurring in all patients after the diagnosis of AML between Jan 1997 to Jun 2006. Amoebic liver abscess is excluded. We computed the impact of surgical drainage on 30 days survival after the diagnosis of liver abscess. We further examined whether other comorbidities increased the relative risk of death of 30 days after the diagnosis of abscess among patients with AML. RESTULS: The prevalence of liver abscess in all AML patients is 2.6% (n=132, 132/5066). Only 8 patients (6.1%) received surgical drainage. The surgical drainage did not improve the 30 days survival after abscess diagnosis (p = 0.328). Liver cirrhosis (p = 0.032, HR = 3.661) and ischemic heart disease (p = 0.041, HR = 3.080) independently predict the 30 days post-abscess survival. Patients with either comorbidities has significantly lower survival 30 days after liver abscess diagnosis (p = 0.016). *Conclusions*. Surgical drainage cannot improve the 30 days post-abscess survival in AML patients. AML patients with liver cirrhosis or ischemic heart disease have increased relative risk of death 30 days after liver abscess diagnosis.

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COMPARATIVE EVALUATION OF CELL DIFFERENTIAL IN BODY FLUIDS USING AUTOMATED HEMATOLOGY ANALYZER AND VISUAL MICROSCOPY

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Backround. The new generation automated hematology analyzer Sysmex XE-5000 provides rapid body fluid analysis, including the differentiation of polymorphonuclear and mononuclear cells. The technology used is based on fluorescence flow cytometry. The precision is ensured due to extended counting in contrast with the visual microscopy. It is important to determine whether the fluid is a transudate or an exudate because it can be helpful in diagnosing the disease or condition present. Aim. The aim of this study was to evaluate the accuracy of Sysmex-5000 in determination of the cell differential in common body fluids. Methods. Thirty Body fluid (BF) samples were prospectively evaluated: pleural fluid, ascitic fluid, continuous ambulatory peritoneal dialysis fluid, and synovial fluid. All fluids were collected in dipotassium ethylenediaminetetraacetic acid (K2EDTA) anticoagulated tubes. Differential counts were made by classical method, counting 200 cells under light microscopy on slides stained with May-Grünwald-Giemsa, and automatically with Sysmex-5000 without pre-treatment of the samples. Mesothelial, polymorphonuclear and mononuclear cells were differentiated. Results. Results for conventional cell categories compared excellently between Sysmex XE-5000 and visual microscopy. Strong correlation was observed between the manual and the automated method as for mesothelial, neutrophils and mononuclear cell differentiation (r=0.888, p<0.001, r=0,985, p<0.001, r=0,960, p<0.001, respectively). No extreme discrepancies, comparing the two methods, were noticed during measurement. Only in one sample the manual examination of 400 cells was needed in order to confirm the correspondence with the analyzer. Conclusion. In summary, our investigation revealed a excellent agreement between manual and automated differentiation of body fluid cells, indicating the reliability of the automated analyzer and therefore its utility in daily practice.

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FUNGAL INFECTION PROPHYLAXIS WITH POSACONAZOL IN ACUTE MYELOID LEUKEMIA

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Invasive Fungal Infection (IFI) is a poor prognosis complication in Acute Myeloid Leukemia (AML). Patients with hematological malignancies and prolonged neutropenia are at high risk of IFI. Posaconazol is a 2nd generation triazole indicated in IFI prophylaxis (IDSA NCCN and ECIL guides). Aims: To evaluate effectiveness of IFI prophylaxis with Posaconazol in profound neutropenia episodes in AML patients on intensive chemotherapy treatment. Methods: A retrospective study was carried out in two Spanish sites including AML patients treated with AraC based intensive chemotherapy receiving posaconazol as IFI prophylaxis. Effectiveness was defined as the occurrence of probable/proven IFI (EORTC criteria) and initiation of antifungal preventive therapy. Toxicity was evaluated with CTCAE v3.0 of NCI criteria. Results: In the period between January 2007 and December 2010, a total of 35 AML patients initiated IFI prophylaxis with oral Posaconazol 200 mg/8h. Mean age 57.3 years (26-76), male/female 19/16. Biweekly galactomannan antigenemia (AGA) was analyzed. All patients received support with G-CSF. There were 84 profound neutropenia episodes, with a mean duration of 19 days (10-38). The mean days of febrile neutropenia was 3 (0-7). Effectiveness: In 4 out of 84 episodes antifungal treatment with liposomal amphotericine was required: In 1 episode empiric therapy was initiated because of persistent fever with negative AGAs and no findings in HTCAT; in 2 episodes were initiated because of positive AGAs and pathologic HTCAT; and 1 episode had pathologic HTCAT without AGA. Toxicity: In 5 episodes Posaconazole prophylaxis was discontinued due to impossibility of oral administration: mucositis grade III-IV in

4 cases, and gastric hemorrhage in 1 case. No relevant renal or hepatic toxicity was reported. Conclusions: This retrospective study, with an incidence of probable/proven IFI of 2.3%, confirms Posaconazol as a high effective and well tolerated option for IFI prophylaxis, in patients with AML at high risk of fungal infection.

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GLYCOGEN PHOSPHORYLASE BB AS A POTENTIAL MARKER OF CARDIAC TOXICITY IN PATIENTS TREATED WITH ANTHRACYCLINES FOR ACUTE LEUKEMIA

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Background. Chemotherapy-induced cardiotoxicity remains an unresolved problem strongly impacting the quality of life and the overall survival of cancer patients. Anthracyclines represent the greatest risk for development of cardiotoxicity. From cardiac biomarkers, cardiac troponins have been recommended for monitoring of cardiotoxicity in oncology. Glycogen phosphorylase BB (GPBB) is among proposed biomarkers of cardiac injury with very limited experience in this context. Aims: To assess plasma GPBB concentrations in acute leukemia patients treated with anthracycline-based chemotherapy to detect acute and chronic cardiotoxicity, and to compare plasma GPBB concentrations in acute leukemia patients with healthy blood donors. Methods: A total of 24 adult acute leukemia patients treated with 3 - 6 cycles of chemotherapy containing anthracyclines were studied. All patients had normal liver and renal functions during the study. Plasma concentrations of GPBB were measured at the diagnosis (before chemotherapy), after first chemotherapy with anthracyclines and circa 6 months after completion of treatment. The cut-off value for GPBB positivity was 10.00 µg/L as recommended by the manufacturer (Diagenics, Germany). Twenty-four healthy blood donors were used as a control group. Results: Before chemotherapy, mean plasma GPBB concentration was 5.25 ± 3.81 µg/L, increased above the cut-off in 1 patient (4.2 %). After first chemotherapy, mean GPBB was 6.61 ± 5.54 µg/L, positive in 7 (29.2 %) patients. Six moths after treatment, mean GPBB was 10.06 ± 11.41 µg/L, positive in 8 (33.3 %) patients. The difference between GPBB concentrations before chemotherapy and 6 months after treatment were statistical significant ($p < 0.01$). The patient with GPBB positivity before chemotherapy (18.55 µg/L) had higher GPBB positivity in the subsequent samples (20.53 and 32.16 µg/L). Mean GPBB concentration in 24 healthy blood donors was 2.14 ± 0.28 µg/L (range 1.81 - 3.05), negative in all subjects. The differences in plasma GPBB concentrations between healthy blood donors and patients treated for acute leukemia were statistical significant ($p < 0.01$ in all cases). Conclusions: Our results suggest that GPBB could become a potential biomarker for detection of acute and chronic cardiotoxicity associated with anthracycline chemotherapy. Plasma GPBB concentrations within 6 months after treatment were significantly higher in comparison with baseline values. The predictive value for development of treatment-related cardiomyopathy in the future is not known and will be evaluated during the follow-up, as well as correlation with established biomarkers of cardiac toxicity and echocardiography. A larger prospective and multicenter study will be needed to define the potential role of GPBB and other proposed biomarkers of cardiac injury in the assessment of chemotherapy-induced cardiotoxicity.

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FIND - 'FUNGAL INFECTION DATABASE' - RETROSPECTIVE ANALYSIS OF INVASIVE ASPERGILLOSIS IN HEMATOONCOLOGICAL DEPARTMENTS IN CZECH AND SLOVAK REPUBLIC BETWEEN 2001-2009

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Background. "Fungal infection database" (FIND) represents international database of invasive fungal infections in Czech and Slovak hematooncological departments. FIND - aspergillus covers all case of invasive aspergillosis (IA) in participating centers since 2001. Methods: The goal of our retrospective analysis was to evaluate incidence, early diagnostic procedures and effect of antifungal therapy in proven and probable IA that occurred in 9 institutions participating in FIND database between 2001-2009. We followed EORTC/MSG 2002 criteria in evaluation of IA diagnosis and therapy response. Results: 162 probable and 36 proven IA (91% isolated pulmonary IA, IPA) have been documented. Prolonged and profound neutropenia (63%) and long-term use of corticosteroids (31%) were identified as the major risk factors of IA. 161 cases (89%) had abnormality on pulmonary CT, however with non-specific infiltrates as the most frequent finding (49%). 74% pts. had consecutive positivity of serum-galactomannan (S-GM) (OD index > 0.5). 81% pts. with IPA and bronchoalveolar lavage (BAL) had positive GM in BAL fluid (OD index > 0.5). In pts. with IPA only 9% BAL fluids and 21% sputum samples had positive microscopic result for filamentous fungi and 16% BAL fluids and 61% sputum samples had positive culture for Aspergillus spp. Primary mold active antifungal prophylaxis was used in 23% pts. - 13% itraconazole, 4% voriconazole (VORI), 3% posaconazole and 3% others. Empiric antifungal therapy was used in 43% pts. with median of 5 days of administration before IA diagnosis (range: 2-44 days) - amphotericine B deoxycholate (C-AMB) was used in 31% of patients with empirical antifungal therapy, lipid-based AMB (LBA) in 24%, VORI in 18% and echinocandins (ECHINO) in 15%, respectively. The primary antifungal therapy of IA represents: in 38% of cases VORI, in 6% ECHINO, in 26% VORI+ECHINO, in 11% C-AMB and in 8% LBA. Overall RR to primary therapy of IA was 62% - VORI 62%, VORI+ECHINO 60%, C-AMB 32%, LBA 53%, ECHINO 20%. There was a statistically significant difference in overall RR to targeted therapy in pts. with neutophil count < 0,1 and > 1,0 $x10^9$/l at the end of therapy (21% vs. 71%). The overall mortality rate was 57%, with 42% attributable to IA. Conclusions: On the basis of our analysis we confirm typical risk factors for IA and critical role of S-GM and CT for early diagnosis and prompt start of antifungal therapy of IA. A reasonable treatment response was achieved using VORI, VORI+ECHINO or LBA in primary therapy of IA. We have confirmed neutropenia at the end of antifungal therapy as the major predictive factor for therapeutic response.

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CLINICAL CHARACTERISTICS AND OUTCOME OF INFLUENZA A (H1N1) INFECTION IN HEMATOLOGICAL PATIENTS DURING TWO CONSECUTIVE WINTER-SEASONS

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Background. Influenza A (H1N1) (IA-H1N1) infection usually results in mild respiratory symptoms in immunocompetent patients, whereas in immunocompromised patients might lead to severe complications and even related-mortality. Clinical characteristics and outcome of IA-H1N1 infection in patients with hematological neoplasms are not well described and need further investigation. *Methods*. Data from patients and infectious episodes of IA-H1N1 in hematological patients from a single institution were prospectively collected from November 2009 to February 2011 (two winter seasons). All patients with clinical suspicion of respiratory viral infection underwent H1N1 real-time PCR analysis of nasopharyngeal aspirate or nasal swab. *Results*. A total of 25 patients were diagnosed with IA-H1N1 infection during the study period (11 in the 2009-10 season and 14 in the 2010-11 season). Median age was 66 years (range 17-89) Most frequent underlying diseases were non-Hodgkin lymphoma (n=9) and chronic lymphocytic leukemia (n=4). Five patients had received hematopoietic cell transplantation (2 allogenic). All patients but one (pneumonia) presented with upper respiratory tract infection. Main clinical symptoms were fever (n=24, 96%), cough (n=16,

64%) and rhinorrhea (n=8, 32%). All patients were treated with oseltamivir 150-75mg bid for a median of 5 days (range 5-20). A second RT-PCR determination of IA-H1N1 was performed in 9 patients with persistent clinical symptoms at the fifth day of treatment. Of them, four patients had persistent H1N1 positivity and were treated until negativization. Seventeen (68%) patients required hospitalization for a median of 5 days (range 1-40). Risk factors for hospitalization were low hemoglobin and platelet count, and low oxygen saturation measured by pulse oximetry at diagnosis. We did not observe IA-H1N1-related deaths. Five patients (20%) had received prior IA-H1N1 vaccine. No differences in the severity of the infection (oxygen saturation at diagnosis, duration of respiratory symptoms, or need for hospital admission) were observed between vaccinated and non-vaccinated patients. *Conclusions*. In patients with hematological diseases, clinical presentation and outcome of IA-H1N1 infection were similar to that described in immunocompetent patients. IA-H1N1 infection may occur in vaccinated patients and presents similar characteristics and outcome than in non-vaccinated patients.

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NEW TECHNOLOGIES TO DEVELOP LONG-ACTING FILGRASTIM: GLYCO-PEGYLATION OF R-METHUG-CSF AND ALBUMIN-FUSION OF NATURAL G-CSF SERVE A ONCE-PER-CYCLE FIXED DOSE STRATEGY TO PREVENT FEBRILE NEUTROPENIA

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Background. Long-acting filgrastim offers the advantage of less dosing intervals when used for reduction of neutropenia and incidence of febrile neutropenia (FN) in patients treated with cytotoxic chemotherapy for malignancy. The target is a once-per-cycle fixed dose administration to prevent FN and being convenient for patients and their treating physicians. *Aims*. GlycoPEGylation and albumin-fusion were evaluated to serve as platform technologies to design a once-per-cycle fixed dose filgrastim.Methods Glyco-PEGylated r-metHuG-CSF (GPG) was designed by attaching a 20 kD PEG to glycan at natural O-glycosylation site. A completely recombinant albumin fusion protein was developed by fusing natural G-CSF to human serum albumin through recombinant DNA technology. Pharmacokinetic and pharmacodynamic non-clinical and clinical studies were performed for both products. Once-per-cycle fixed dose approach for the prevention of chemotherapy induced FN has been investigated in clinical studies with pharmacokinetic, pharmacodynamic, efficacy and safety endpoints. Results. Compared to unmodified natural G-CSF, both glyco-PEGylated r-metHuG-CSF (GPG) and albumin-fused hu-G-CSF (AFG) have a prolonged circulating half-life. GPG and AFG show the expected pharmacologic activity of G-CSF in vivo, i.e. they stimulate neutrophil recovery in a dose-dependent way measured by an increase of absolute neutrophile count due to the induction of neutrophile pre-cursors proliferation. Efficacy and safety data obtained in clinical studies showed the expected profiles for a long-acting filgrastim. Summary / Conclusions. The platform technologies of glyco-PEGylation and albumin-fusion can be used effectively to obtain long-acting filgrastims and many other proteins. Pharmacokinetic and pharmacodynamic characteristics of GPG and of AFG show that these long-acting filgrastims are suitable for a once-per-cycle fixed dose use to prevent FN in patients treated with cytotoxic chemotherapy for their malignant tumor disease. The results for pharmacokinetics and pharmacodynamics for both products as well as the current efficacy and safety endpoints are as to be expected for a long-acting filgrastim supporting an efficient and safe once-per-cycle fixed dose administration.

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THE EMERGENCE OF MUCORMYCOSES IN PATIENTS WITH HEMATO-ONCOLOGIC DISEASES

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Background: Mucormycoses in patients with hemato-oncologic diseases are increasing in past few years, representing the third leading cause of invasive fungal infection in this patients following Aspergillus and Candida species. This increase may correspond to changes related to antifungal prophylaxis, particularly with the azoles, and the widespread use of antifungal agents. Historically, Mucormycoses presents itself as a fatal fungal infection, reaching in some series 90% mortality. Aims and Methods: We proceeded to the retrospective analysis of Mucormycoses at our centre in the last six years. Diagnosis was defined as probable or definite according to the EORTC/MSG criteria. Results: Mucormycoses were diagnostic in 5 patients, median age 54 years (range, 18-64 years) with Acute Myeloid Leukemia (4/5) and bone marrow aplasia (1/5). The diagnosis was verified in neutropenia (median 20 days, range 3-57 days) during induction (2/5), consolidation (2/5) and salvage therapy (1/5) of current chemotherapy protocols. Regarding the mode of presentation, 2/5 of cases were with pulmonary involvement and 3/5 with rhinosinusal. Computed tomography was performed in all patients after prolonged febrile syndrome with symptoms suggestive of fungal infection and liposomal amphotericin B 5mg/kg/day was started empirically. Patients with rhinosinusal involvement underwent surgical debridement and those with lung involvement underwent thoracic surgery (right lower lobe and upper left lobectomy). One patient required a second surgery with lobectomy and partial hepatectomy for liver involvement and diaphragmatic extension of fungal process. The histological study of surgical specimens confirmed the diagnosis of suspected Mucormycoses in all patients. Mycological culture was negative in all cases. Liposomal amphotericin B was maintain for a median of 50 days (13-80 days), followed by a period of secondary prophylaxis also with Liposomal amphotericin B in tapered low dose scheme, for a median of 3 months (range, 1-6 months). No mortality was attributable to fungal complication, the only death verified was due progression of underlying disease. Remaining patients had resolution of Mucormycoses and 3/4 completed the chemotherapy protocol designed to reach complete remission of hemato-oncologic disease. Conclusions: Although we had a mortality rate lower than that reported in other series of patients, this experience shows the challenge that Mucormycoses represents in patients with hemato-oncological diseases. Clinical presentation is unspecific and diagnosis is difficult to establish. Early suspicion associated with high-dose antifungal therapy and aggressive surgical approach is the best option for treating these infections often fatal.

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DEVELOPMENT OF RESISTANT INTESTINAL BACTERIA IN HEMATOLOGICAL PATIENTS WITH PROLONGED NEUTROPENIA WHILE ON LEVOFLOXACIN PROPHYLAXIS

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Background: Levofloxacin prophylaxis (LP) is used to reduce the number of febrile episodes in neutropenic patients, although bacterial resistance is a matter of concern. We carried out a prospective study to know the impact of levofloxacin profilaxis (500 mg o.d) over the intestinal flora with special emphasis in extended spectrum? ? -lactamase Enterobacteriaceae (ESBL-E) and ampicillin-resistant Enterococcus faecium (ARE). Material and methods: 17 patients diagnosed of leukaemia, lymphoma or multiple myeloma who were receiving conventional chemotherapy or conditioning chemoradiotherapy before transplant, with an expected neutropenia time > 10 days, were enrolled in this study. Patients received levofloxacin until the development of fever, when a beta-lactamic was added empirically and levofloxacin withold. Faecal samples at admission, during LP and while on beta-lactamic therapy were seeded in ampicillin (10mg/L) m- Enterococcus agar for the screening of ampicillin resistant E.faecium (ARE) and in ciprofloxacin (0.1mg/L) and cefotaxime (1mg/L) MacConkey agars for ciprofloxacin resistant (CIP-R-E), extended spectrum B-lactamase (ESBL-E) and/or carbapenemase producing (CP-E) Enterobacteriaceae.

% of patients with Extended spectrum β-lactamase *Enterobacteriaceae* and Ampicillin-Resistant *Enterococcus faecium* isolates in the intestinal flora

	Admission	Under LP	After LP*	At any moment
Enterobacteriaceae:				
Rp resistant	12%	29%	59%	75%
ESBL producing strains	6%	0%	24%	41%
ARE	29%	65%	53%	70%

* Patients under treatment with β-lactamic as empiric neutropenic therapy

Colonies were screened for ESBLs and carbapenemases. Clonal relatedness was studied by PFGE. Blood and urine cultures, obtained while the patient was febrile, were studied according to standard methods. Results: 220 faecal samples in 28 different neutropenia episodes were studied.

Mean duration of levofloxacin treatment was 10 days per episode. Percentage of patients colonized in the different study periods is shown in Table 1. Clonal analysis led to: -ESBL-E: The detection of four strains of E. coli Fq-resistant in the blood cultures of two patients, one of them being an exact clone of the one isolated in that same patient's feces. No carbapenemase producing strain was detected. -ARE: ARE bacteremia was found in three patients (18%), all of them due to clone B (ST117) (the most prevalent and persistent clone, detected in 59% of the patients and 72% of the total of isolations). 75% of the isolations were resistant to Levofloxacin, 88% exposed high level resistance to streptomycin and all of them remained susceptible to vancomycin, teicoplanin, daptomycin and linezolid. Conclusions: In the neutropenic patient under LP, a high faecal colonization by Fq-resistant Enterobacteriaceae and ARE was found, whilst ESBL-E was less noticeable. The levofloxacin-selected clones may cause ulterior bacteriemia.

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NOSOCOMIAL INFECTIONS IN ADULT HEMATOLOGY/ONCOLOGY PATIENTS

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Background: Hematology patients present an increased risk of nosocomial infections that vary in different populations and different institutions with considerable morbidity and mortality. Aims: The purpose of our study was to evaluate the frequency and patterns of nosocomial infections in 50 hematology patients and to determine the prevalence of causative organisms and their antimicrobial sensitivity. Methods: A retrospective analysis was made in the patients admitted between April 2010 and January 2011 to the Department of Haematology of the First Propedeutic Department of Internal Medicine of University Hospital AHEPA. The 50 patients showed 240 admissions and 4002 inpatient days. The Centers for Disease Control and Prevention criteria were used as a standard definition for nosocomial infections. *Results*. The overall rate of nosocomial infections in hematology patients was 9.7 and in neutropenic patients 22.9 per 1000 patient-days respectively. Nosocomial fever of unknown origin constituted 62.9% of cases. The frequent sites of nosocomial infections were respiratory system (42.7%), the blood stream (25.3%), the urinary system (22.2%) and others (9.8%). The incidence of nosocomial infections was significantly higher during neutropenic days (P<0.001). Gram-negative organisms represented 71,2 % of pathogens (Klebsiella 48.6%, Pseudomonas 35.7%, Acinetobacter 7,8%, E. coli 7,8%, and C. albicans 5.5%). Gram-positive organisms represented 28,8.% of pathogens (Staphylococci 81.5%, Streptococci 18,5%). Positive cultures were more frequent in winter (November to March). Susceptibility of isolated organisms was relatively low (ampicilline/sulbactam 49.9%, amikacin 35.9%, imipenem 34.4%). Methicillin-resistant S. aureus and extended spectrum beta lactamase represented 30% and 65% of isolated S. aureus and Gram-negative organisms respectively. Carbapenem-resistant strains of Klebsiella and Pseudomonas were isolated from the patients. KPC-2 carbapenemase-producing Klebsiella strains were isolated from the patients. *Conclusions*. Respiratory infection and fever of unknown origin are the most common nosocomial infections in adult hematology patients with a higher risk during neutropenic days. Isolated organisms are multi-drug resistant, predominantly Gram-negative pathogens with a high incidence of extended spectrum beta lactamase, methicillin-resistant S. aureus, and carbapenem resistant organisms.

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RISK ASSESSMENT FOR BLOOD TRANSFUSION REQUIREMENT OF AT LEAST 3 UNITS DURING THE APLASTIC PHASE FOLLOWING CHEMOTHERAPY

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Background. Faced with the ongoing reduction in the number of blood donors, the clinical hematologist must often ask patient families for help in finding suitable donors. Another benefit of the predictive models are improved communication with the blood bank, allowing the clinical hematologist to order the appropriate amount of blood units necessary for the whole duration of the aplasia. Our passed experience was creating a predictive model for blood transfusion requirement in a patient with acute leukemia in aplasia following chemotherapy using a linear model (multiple regression). The variables representing the best predictors of blood units number transfused to an acute leukemia patient in an aplastic phase following chemotherapy were: hemoglobin levels at chemotherapy start, disease stage at the beginning of chemotherapy regimen, hemorrhagic episodes during the next aplastic period and hemolytic transfusion reaction. *Aims*. Risk assessment for a blood transfusion requirement of at least 3 units of blood during the aplastic phase following chemotherapy administrered to acute leukemia patients Methods: Study type: analytical, cohort, retrospective. We studied 246 patients with acute leukemia admitted in the Hematology Clinic in Cluj-Napoca during 1995-2008 and treated according to international protocols (exclusion of palliative care therapy, deceased or transferred patients). 860 aplasia episodes secondary to chemotherapy were included in the study. All the patients signed the informed consent. The study has the agreement of Ethic Committee of Medicine University Cluj-Napoca, Romania. Statistical analysis was performed using a probabilistic model (logistic regression). Independent risk factors were identified using the probabilistic model of logistic regression using *amount of blood* as the dependent variable. The *amount of blood* is a dichotomous qualitative variable - large (the number of transfusions is equal to or greater than 3) or small (the number of transfusions is less than 3). Statistical analysis was performed using SPSS, Statistica and Excel. *Results*. 1Independent risk factors for performing at least 3 transfusions are: a.Hemorrhages during aplastic period (Minor hemorrhages: OR=6.60, 95% CI, 3.55-12.27, p=0.00<0.05Major hemorrhages: OR=91.01, 95% CI, 22.09-374.95, p<0.05) b. Haemolytic transfusion reaction (Haemolytic transfusion reaction: OR=7.92, 95% CI, 3.03-20.73, p<0.05) c.Disease stage at the beginning of chemotherapy (Disease stage- diagnosis: OR=7.62, 95% CI, 3.65-15.89, p<0.05; Disease stage- partial remission: OR=0.98, 95% CI, 0.65-1.49, p<0.05; Disease stage- complete remission: OR=0.50, 95% CI, 0.37-0.67, p<0.05; Disease stage- no response: OR=2.73, 95% CI, 1.57-4.73, p<0.05). 2.Probability of needing at least 3 transfusions: where e ≈ 2,71= Euler's number, x1, x2, ..., xn are independent variables and b1,..., bn are regression coefficients. The described model is appropriate for experimental data (according to Lemeshow-Hosmer test, p=0.76>0.05, rejecting the null hypothesis of the regression model being unfit to the studied data) and has about 83.5% correct prediction rate. Conclusions: Independent risk factors for performing at least 3 transfusions for acute leukemia patient in aplasia following chemotherapy are: hemorrhages during aplastic period, haemolytic transfusion reaction, disease stage at the beginning of chemotherapy.

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CLINICAL SIGNIFICANCE OF RED CELL ALLOIMMUNIZATION IN MULTITRANSFUSED PATIENTS

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Background. Developing alloantibodies after being transfused with red blood cells is a major problem, especially for multitransfused patients, because it can cause episodes of alloimmune hemolytic anaemia. AIM: In this study we try to detect the alloantibodies developed after repeatitive red blood cell transfusions, and estimate the frequency of these antibodies among the multitransfused patients of our hospital, so as to avoid hemolytic episodes during next transfusions. *Methods.* Multitransfused patients of our hospital, suffering with solid tumors or haematological malignancies, are included in this study. What we routinely do with such patients in our department, is performing screening test (indirect antihuman globulin test) in order to detect unexpected alloantibodies, whenever these patients need to be transfused or whenever any reaction during or after the transfusion is reported to our department by the clinical doctors. Samples with positive screening tests are being tested (panel test) in order to identify the unexpected antibodies. Both screening and panel tests are being performed with an enzyme test by glass beads centrifugation method (microtyping system, Biovue Ortho Diagnostics). *Results.* A screening test was performed in 4739 samples of 4542 patients (49 patients were tested more than twice). 198 of these samples, referring to 91 patients (32 males and 59 females) were positive. Alloantibody identification revealed the following: 68 (74.7%) patients had a single, defined alloantibody. 9 (9.9%) patients had two or more defined alloantibodies. 14 (15.4%) patients had a combination of alloantibodies with undefined specificity. 12 (13.2%) patients had autoantibodies as well, as it was revealed by the positive direct antiglobulin test. The most frequent alloantibodies were: anti-Kell (28), anti-D (11), anti-E (9), anti-e (7), anti-C (6), anti-Jka (6), anti-Fya (5), anti-M (5), anti-P1 (5), anti-Lea (5), anti-Fyb (4),

anti-Leb (4), anti-Cw (4), anti-c (3), anti-Jkb (3). None of the patients above was reported to develop any kind of immediate or later hemolytic reaction, despite the repeatitive transfusions. *Conclusions*. The most frequent alloantibodies which are detected in the multitransfused patients of our hospital, are mainly versus the Kell and the Rhesus antigenic systems of the red blood cells, and less versus the Kidd, Duffy, MNS, P and Lewis antigenic systems. Continual and persistent searching and early detection and identification of these alloantibodies, and transfusion with red blood cell units which are negative for the analogue antigen, can offer in the prevention of severe reactions like the hemolytic episodes which worsen even more the bad general condition of the patients.

1642

THE USEFULNESS AND DEMERIT OF THE EXPECTED ELEVATION VALUE OF SERUM ALBUMIN BEFORE REPLACEMENT THERAPY

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Background and Aims: Pharmaceutical albumin has been used excessively in Japan. In the 1980s, Japan consumed one third of the world supply. Although aiming to decrease dependence on albumin based treatments, Japan's consumption of pharmaceutical albumin was 57,541 kg in 1999, which was 1.6 and 6.2 times more than that of the U.S.A. and U.K., respectively. Since then, the target elevation value of serum albumin was adopted in the Japanese Guideline for Blood Transfusion to determine the necessary dosage of pharmaceutical albumin for patients. The dosage is calculated by the formula; the necessary dosage (g) = the expected elevation value (g/dl) x amounts of whole plasma (dl) / 0.4. It contributed considerably to decrease consumption to 36,816 kg in 2009. On the other hand, the measurement of serum albumin is recently shifting from bromcresol green (BCG) assay to bromcresol purple (BCP) assay because the latter has less contamination other than pure albumin. In this study, the clinical importance of the target value of serum albumin was reevaluated for the BCP assay. Methods and Results: We analyzed the serum albumin value by BCG and BCP analyses in 251 cases, and examined the correlation between them. Y (BCP) = 1.09X (BCG) - 0.52 served as the standard curve and total correlation of coefficient value (r) was 0.99. We changed the target elevation value in our institute according to the standard curve from 2.5 g/dl to 2.3 g/dl in chronic diseases. However, the consumption of pharmaceutical albumin increased 23 %. Extensive examination revealed the poorer correlation between them 6 months later. We analyzed a further 28 cases whose serum albumin levels were less than 3.0 g/dl with BCG assay. Y = 0.95 X - 0.29 served as the new standard curve and the correlation of coefficient value (r) was 0.87. After we modified the target elevation value to 2.0 g/dl for chronic disease, the consumption of pharmaceutical albumin returned to the previous level of 6 month ago. We also analyzed the purpose of pharmaceutical albumin treatment for each patient, and the background of each case. A hundred and nineteen bedside cases treated with albumin replacement therapy were investigated. We divided these cases into 3 groups according to serum albumin level (z); 2.5 g/dl ≤ z < 3.0 g/dl, 2.0 g/dl ≤ z < 2.5 g/dl, and z < 2.0 g/dl. The coefficient value (r) was significantly poorer in lower serum albumin levels, and the serum albumin value did not elevate more than expected after pharmaceutical albumin treatment in such cases ($p<0.05$). *Conclusions*. The indication of pharmaceutical albumin varies and is complex especially in cases whose serum albumin level is lower. Target elevation value of serum albumin might be continuously effective in reducing the consumption of albumin. However, we have to understand the difficulty in estimating the required clinical dosage of pharmaceutical albumin from laboratory data even if we adopted more advanced assay. Simultaneously, we need more information about efficacy on each background disease accompanying hypoalbuminemia.

1643

HOME CARE MANAGEMENT OF TRANSFUSIONS IN HEMATOLOGICAL PATIENTS

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Background. Red blood cell (RBC) and platelets (PLT) transfusion is one of the most challenging tasks in the home care management of patients affected by hematological disease (HD). Indeed the treatment of anemia or thrombocytopenia are an essential part of the global management of most HD patients. In erythropoietin-failed patients or in those unsuitable for this option, RBC transfusions remain the only available measure and PLT transfusions, although less frequently required, are the unique effective treatment for symptomatic thrombocytopenia. *Aims*. To evaluate the management of RBC and PLT transfusions at home during the last two years (2009-2010). Methods. There were 266 pts (125 male) with a median age of 81 (20 - 98) years. Diagnosis was as follows : acute leukemia 51, multiple myeloma 34, lymphoma 35, myeloproliferative disease 30, myelodisplastic syndrome 68, solid tumor 23, other diagnosis 25. Patients were followed at home for a mean of 8,1 (1 - 24) months. Therapy with erythropoietin stimulating agents was used in 100 pts (38%). Results. Overall, 163 (61%) and 12 (5%) patients required RBC and PLT transfusions, respectively for a total of 2197 and 361 RBC and PLT units, respectively; RBC and PLT units in transfused pts were a median of 5 (1-60) and 13 (5-96), respectively. RBC and PLT units monthly requirement in transfused pts were a median of 0.83 (0.04-8.7) and 1,83 (0.4-13.8), respectively. AL and MDS diagnosis correlated with RBC units requirement; AL diagnosis correlated with PLT units requirement. All transfusions were safely administered at home without any untoward effect. Conclusions. QoL is a particularly important issue for hematological patients. With this regard, management of pts requiring multiple and repeated admissions to receive RBC or PLT transfusions may be a concern for the affected individual and for its family. Our experience demonstrated that the administration of RBC and PLT transfusion at home is a feasible, reliable and effective in our patient, avoiding social and economic costs due to an inappropriate removal from his domestic environment. In conclusion, in our experience domiciliary management of transfusions represented an important added value to home care program, allowing the best humanization of this procedure for our patients.

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EVALUATION OF NIGALE NGL XJC 2000'S PERFORMANCE IN P RODUCTIVE PLASMAPHERESIS AND QUALITY CONTROLS ON PLASMA (TYPE A) COLLECTED

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Background. The Nigale NGL XJC 2000, distributed by Hemotrans (Pomezia, Italy), is a new cellular separator exclusively dedicated to plasma production by apheresis. This instrument, characterized by a discontinuous blood flow with a single venous access, adopts the technology developed by Latham, namely the separation of blood components according to their density gradient by applying an appropriate centrifugal force. In the Nigale's kit, a blow moldeld bowl is included. It permits to obtain a plasma with a lower cellular contamination. AIMS. In our Transfusion Centre, we evaluated, in terms of effectiveness and efficiency, the qualitative and quantitative performances of this equipment. *Methods*. Between November and December 2010, one hundred plasmapheresis were performed with this new instrument, of course after informed consent, on 100 periodic blood donors with at least one previous experience in apheresis donation. In order to evaluate the operability and manageability of this cellular separator, the quality of its kits and the compliance of donors, we have recorded all adverse reactions to donation, any interruptions in the procedure due to malfunctions of instrument or abnormalities of the kit. *Results*. Only in 17 out of 100 plasmapheresis were recorded incidents. We have observed 15 mild adverse reactions due to citrate toxicity, resolved by changing the pump speed or with a little break, but completing the procedure; in one case the donation was interrupted by a fainting, an adverse reaction of moderate grade; while in the last case the apheresis failed due to a prolonged low-pressure in the collection, but without consequences for the donor. Serious reactions were not observed, thus no one procedure has been interrupted for malfunctions of instrument or for defects or disruptions of the kits. Mean factor VIII on collected plasma units was 90,35%. Mean fibrinogen was 332 mg/dL. All sterility controls resulted negative. Cellular contamination was not significant (mean erythrocytes=0,01 $x10^3$/microL; mean leukocytes = $0,1x10^3$/microL; mean thrombocytes = $11x10^3$/microL, with a minimum count of 2 and a maximum of 31). *Conclusions*. The availability of a new cell separator dedicated to plasmapheresis may allow a greater number of targeted donations. The Nigale

NGL XJC 2000, on the basis of our experience, is characterized by extreme simplicity in the mounting kit and by a simple and intuitive management software. The donation can be customized according to the parameters of the donor (sampling and re-infusion speed) and the amount of plasma to produce. The safety in the process is guaranteed thanks to the various sensors connected to alarms, moreover it is possible to re-infuse saline solution at will to achieve an optimal hemodynamic compensation. In this context we have also evaluated qualitative performance of this new instrument and our data (on factor VIII and fibrinogen dosage, microbial and cellular contamination) show that plasma collected by Nigale NGL XJC 2000 respect both Italian and European quality parameters. At the end the availability of another cell separator dedicated to plasmapheresis, also because offered at extremely attractive costs, may allow a greater number of targeted donations.

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TRANSFUSION-RELATED ACUTE LUNG INJURY IN HEMATOLOGIC PATIENTS

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Background : Transfusion-related acute lung injury (TRALI) is an underreported complication of transfusion therapy. It is the third most common cause of transfusion-associated death. TRALI is characterized by noncardiogenic pulmonary edema. The presence of HLA antibodies in the donor or recipient plasma supports the diagnosis of TRALI. There is no specific treatment other than supportive measures. *Case reports*: Case 1: A 46 year old male with Acute Myeloid Leukemia treated with "3x7"scheme with pancytopenia and without blasts in the bone marrow received platelets and two packed red cell units transfusion on the 21st day of treatment. 4 hours after the transfusion, he presented acute respiratory distress and was transferred to the ICU. All other causes of pulmonary edema were ruled out and TRALI was suspected. The patient died shortly afterwards. HLA antibodies in the donor resulted positive. Case 2: A 81 year old female with myelodisplastic syndrome and severe aortic stenosis was admitted because of cardiac failure. She required five packed red cell units over the following five days. On the sixth admission day, platelets were transfused because of severe thrombocytopenia. Two hours after transfusion, she had chills, fever, hypotension, dyspnea, bibasal crackles and tachycardia. She was transferred to the ICU, starting oxygen by mask, steroids, and diuretics. The patient recovered over the next 72 hours. Positive anti-HLA antibodies were present in the donor. *Comments.*: Endothelial cell damage and increased capillary permeability in TRALI are possibly caused by antibodies generated by the host against leukocyte antigens present in the blood products transfused, or by other substances that modify the biological response of the pulmonary bed neutrophils. The inflammatory reaction leads to pulmonary edema and respiratory failure. Antibodies against leukocyte antigens are positive in 60-90 % of patients with TRALI. There are wide variations in the reported incidence of TRALI. These discrepancies are probably explained by underdiagnosis, difference in diagnostic criteria and lack of standardized reporting. Joint efforts by international experts to standardize case definition may contribute to improve diagnosis. *Conclusion:* Our two cases illustrate TRALI as a relevant differential diagnosis in patients with sudden respiratory failure within six hours of transfusion. It is essential to notify immediately to the blood bank all potential TRALI cases, to ensure proper investigation of donor risk factors and to test for HLA antibodies.

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HOME BLOOD TRANSFUSIONS: THREE YEARS OF EXPERIENCE IN MYELODYSPLASTIC SYNDROMES

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Background. Transfusion-dependence is a common issue in myelodysplastic syndromes (MDS) including low risk MDS after EPO failure or supportive care in high risk MDS. *Purpose.* Red blood cells (RBC) transfusions are usually performed at hospital. Alternative solutions are available through the development of home blood transfusion services in collaboration with the general practitioner (1,2). We report our experience of home blood transfusions in MDS patients. *Patients and methods*. since 2009 our hospital developed home blood transfusion services. More than 220 red blood cell transfusions were made at home. We studied indications, safety, cost and feasibility of this modality of transfusion in comparison with standard procedure i.e. in hospitalization. Results: 116 transfusions were made in 25 patients. Mean age was 78.6 years (53-96). 16/25 patients (64%) suffered from myelodysplastic syndromes (MDS) or secondary acute myeloid leukemia (AML). Patients experienced no major adverse. In 10 cases transfusion was not possible due to nurse or general practitioner inaccessibility. No patient refused this modality of transfusion. Economic costs were evaluated: home transfusion appeared to be less expansive. Conclusion: home transfusion is safe and feasible. To receive treatment at home is a common patient's preference and increase quality of life particularly in chronic disease which affects elderly people. Home supportive care represent an alternative solution if both patient and gener practitioner are agree, that could improve hospital accessibility for more severe procedures.

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ENDOCRINE DYSFUNCTIONS IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS

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Background. Patients affected by thalassemia major frequently have one or more endocrine dysfunctions. Despite use of intensive chelation therapy, it is difficult to prevent this condition, and this condition usually leads to a decrease in quality of life. *Methods*. Endocrinological evaluation of 36 thalassemia patients (22 female and 14 male) receiving deferasirox was done. The age range was 7.5-35 years; 29 patients; > 18 years, 6 patients; 10-18 years and 1 patient <10 years. Presence of growth failure, carbohydrate metabolism disturbance, hypoparathyroidism, thyroid dysfunction, hypogonadism and osteoporosis and the correlation of endocrine dysfunctions with age, sex, ferritin levels were investigated. *Results*. All patients had at least one endocrine disorder. Thirteen (36%) had growth failure, 15 (41%) had carbohydrate metabolism disturbance, 3(8.3%) had hypoparathyroidism, 2 (5.5%) had subclinical hypothyroidism, 2 (5.5%) had hypocortisolemia and 19 (52%) had hypogonadotropic hypogonadism. Thirteen of the 22 female patients (59%) had amenorrhea, 7 of the 14 male patients (%50) had low testosterone levels. Nineteen patients (52%) had osteoporosis. The frequency of growth retardation was not different between girls and boys, but carbohydrate metabolism disorders, hypogonadism and osteoporosis was found to be a little more frequent in girls. Ferritin levels were found to be directly correlated to growth retardation and hypogonadism, but there was no direct relationship between ferritin levels and osteoporosis and disorders of carbohydrate metabolism. In addition, these disorders are also found in those with ferritin levels below 500 ng/ml. Growth retardation, and carbohydrate metabolism disorder was found to have an earlier onset, seen starting from the age of 10. Hipogonadism and osteoporosis become more evident over the age of 18. *Conclusions*. Despite low levels of ferritin and intensive chelation therapy, many endocrine dysfunctions can occur in thalassemia major patients. It may be more effective to aim at lower levels of ferritin. Endocrine disorders arise from the age of ten. Close follow up for endocrine dysfunction and treatment will improve the quality of life in thalassemic patients.

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COMPARATIVE EVALUATION OF THERAPEUTIC EFFICACY OF PHOTOCHEMICALY TREATED AND GAMMA-IRRADIATED PLATELET CONCENTRATES TRANSFUSED TO PATIENTS AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. The maintaining of functional properties of blood components for transfusion is one of the important requirement for the processing methods. Gamma irradiation (GI) of apheresis platelet concentrates (A-PLT) is used for inactivation of allogenic T-lymphocytes to prevent

transfusion-associated graft versus host disease (TA-GvHD) in susceptible recipients. Photochemical treatment (PCT) of A-PLT with amotosalen and long-wavelength ultraviolet A (UVA) light has been developed to prevent transfusion complications, associated with pathogens and donors leukocytes. *Aims*. The aim is to compare GIed and PCTed A-PLT on the base of clinical markers of their functionality and viability. Methods: There were selected 30 A-PLT transfusions (15 transfusions of GIed and 15 - of PCTed A-PLT) to be characterized on the following criteria: 1h posttransfusion count increment (1h CI) and 1h corrected count increment (1h CCI), 24h posttransfusion count increment (24h CI) and 24h corrected count increment (24h CCI), hemostatic efficacy and acute transfusion reactions. A-PLT were collected in 35% plasma and 65% platelet additive solution. PCT of A-PLT was performed with 150 μM amotosalen and 3,6 J/cm2 UVA light. GI dose of A-PLT was 25 Gy. The platelet content per transfusion dose was 2,5 to 3,5 x 1011. Quality control of all physicochemically treated A-PLT samples was performed by flow cytometry on the base of activation (CD62P) and apoptosis (Annexin V binding) markers. All treated A-PLT were ABO compatible and were transfused to thrombocytopenic patients after autologous haematopoietic stem cell transplantation during the first 48 hours after collection. Results: After informed written consent was obtained, 12 patients were included in the study, each patient received 1 to 4 A-PLT transfusions. The mean pretransfusion platelet count was 19,25±5,2×10^9/L. The average 1h CI in the group received PCTed A-PLT was 25,7±7,49×10^9/L. There were no observed differences in patients groups with transfusions of PCTed and with GIed A-PLT: 1h CI in the group received GIed A-PLT was 23,85±5,22×10^9/L. The mean 1h CCI was similar in both groups: 19,4±7,2×10^3 for the group received PCTed A-PLT and 15,3±5,6×10^3 for the group received GIed A-PLT. The mean 24h CI was 12,4±5,6×10^9/L in PCT group, 24h CI for GI group was on the same level - 12,7±5,1×10^9/L. There were no differences in comparative assessment of average 24h CCI for PCT and GI groups: 24h CCI was 9,24±4,6 and 8,21±3,6×10^3 respectively. All transfusions had adequate hemostatic efficacy. No episode of posttransfusion reactions were observed in these groups. Summary/conclusions: The results of 1h CI and CCI, 24h CI and CCI were acceptable according to the European recommendations and to international guidelines. The physicochemically treatment of A-PLT was not lead to decreasing of therapeutic efficacy of A-PLT. PCTed and GIed A-PLT were demonstrated the identic clinical markers of their functionality and viability. The PCT of A-PLT prevent a broad spectrum of transfusions associated risks that is why this method is preferable for patients after autologous haematopoietic stem cell transplantation.

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DIFFERENT METHODS FOR DETERMINATION OF LOW PLATELET COUNTS: THE REASON FOR POSSIBLE INAPPROPRIATE PLATELET TRANSFUSION

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Background. It is known that the use of available hematology analyzers is based on two basic principles: electronic impedance (IMP) and optical light scatter analysis (OPT). Both methods may result in some inaccuracies in PLT measurement, which can be caused by interference from cells or materials of a similar size to the PLTs. Therefore, immunoplatelet method is proposed as a new reference method, using monoclonal antibody specific to a cluster of differentiation common to all platelets. *Aims*. We decided to evaluate low PLT counts by IMP and OPT methods and to compare them with the immuno plt CD61 measurement by flow- cytometry. We also examined the possibility of inappropriate PLT transfusion resulting from an inaccurate PLT count. *Methods*. We analyzed consecutive blood samples of patients with acute myeloid leukemia and OPT PLT counts of less than 50x10^9/L. Also, we compared the number of prophylactic PLT transfusion indications (threshold of 20 x 10^9/L) according to the PLT counts determined by the OPT and IMP methods with the number of prophylactic PLT transfusion indications according to CD61 method. In our study we used Cell-Dyn Sapphire (Abbott Diagnostics) hematological analyzer. This instrument measured the PLT count by three methods: OPT, IMP, and CD61 methods using monoclonal antibody directed against glycoprotein IIIa (CD61). *Results*. We collected 44 samples. The mean ± SD values of the PLT counts were 17x10^9/L, 16x10^9/L, and 21x10^9/L for the CD61, OPT, and IMP methods, respectively. The correlation of the OPT method when compared with the CD61method was very strong (r=0.872; p<0.001) and R2 was 0.761. The correlation of the IMP method when compared with the CD61 method was weaker (r = 0.686; p<0.001) and the R2 was 0.470. In the bias analysis, the IMP method (but not OPT) showed higher PLT counts when compared with the CD61 method (mean of difference 4.37x10^9/L; p=0,001 and 0.72x10^9/L, p>0,05, respectively). We saw overtransfusion in 29.5% of cases and undertransfusion in 2.3% of cases (p=0.002; McNemar's test) when we selected a threshold of 20x10^9/L with the IMP method. *Conclusions*. PLT counts determined by the IMP methods showed some disagreement when compared with the CD61 and OPT method. This disagreement caused both PLT undertransfusion and overtransfusion.

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POSITIONAL PARAMETERS FOR THE DETECTION OF VITAMIN B12 AND FOLATE DEFICIENCIES

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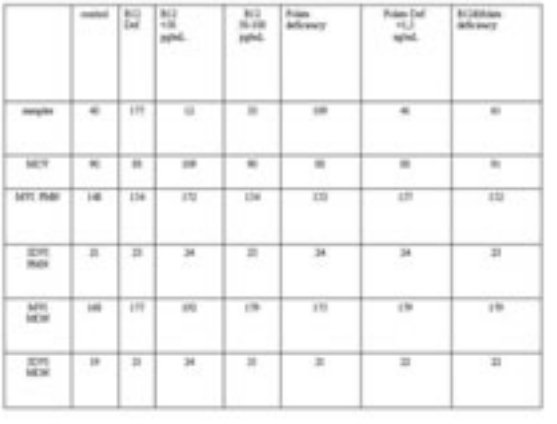
Background. Classical flow charts used widely for the diagnostic approach of anemia due to Vitamin B12 (B12) deficiency or anemia due folate deficiency includes the Mean cell Volume of the red blood cells (MCV) as one of the key tests for the suspicion of these diseases and differential diagnosis of anemia. Only around half of the patients with B12 deficiency or folate deficiency have high MCV, in many of the situations because the coexistence of other causes of anemia. The Coulter LH 780 hematology analyzer (Beckman Coulter) has the ability to measure specific parameters of neutrophil and monocyte populations like mean and standard deviation (SD) of cell volume (MVI,SDVI), conductivity (MCI,SDCI), and light scatter (MSI, SDSI). These so-called positional parameters (PP) can detect morphologic changes in neutrophil and monocyte population. Using VCS parameters we investigated the correlation between megaloblastic neutrophils and monocytes in B12 and/or folate deficiencies.



Aims. Correlation of B12 and/or folate deficiencies with neutrophil and monocyte PP. METHODS Study population included 427 patients. 177 had B12<145pg/Ml, 189 patients had folate <2.33ng/mL There were 61 cases with both serum folate and B12 deficiency. Anemia was defined according the WHO anemia criteria (Hb<12 g/dL women, Hb<13 g/dL men). We collected blood samples from 43 healthy control subjects. VCS parameters and full blood count was obtained by the Coulter LH 780 hematology analyzer. B12, folate and ferritin values were obtained using paramagnetic particle chemiluminescent immunoassay . P value less than 0, 05 were considered significant. Anemia (mean Hb 11 g/dL) was found when B12 values were either less than <50pg/mL or between 50 to 100 pg/mL, while ferritin and folate mean values were normal at these groups. Statistically significant difference was observed for MCV only when we had B12 values lower than <50pg/mL(P<0.0001), all the other cases we studied P was equal to 0.4. PP correlation between controls and the other groups showed statistical significance (P<0.0001). SUMMARY/CONCLUSIONS. MVI and SDVI of neutrophils and monocytes may be used for the detection of megaloblastic neutrophils and monocytes. Megaloblastic neutrophils and megaloblastic monocytes may be seen in B12 and/or folate deficiency. Positional parameters have significant statistical role in the detection of those deficiencies in contradiction with the widely used MCV, because they are not affected by the presence at the same time of iron deficiency or other reasons of anemia. Although plausible, this hypothesis needs to be sustained clinically by a prospective study.

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THE STRUCTURAL PARAMETERS NEUT-X AND NEUT-Y IN MEGALOBLASTIC ANEMIA

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Background. NEUT-X and NEUT-Y are new structural parameters determined by the Sysmex XT-2000i analyzer (provided by service data). NEUT-X is the mean value of the side scatter diffraction of the neutrophil population and represents the structure of the neutrophils, while NEUT-Y is the mean value of the fluorescence measurement. Low values of NEUT-X and NEUT-Y reflect neutrophil dysplasia in myelodysplastic syndromes. *Aims*. The aim of this study is to evaluate these new parameters in patients with megaloblastic anemia (MA) due to vitamin B12 deficiency. *Methods*. Blood samples from 62 normal healthy subjects - controls (normal results of blood count and blood smear) and from 40 patients with MA (hemoglobin < 12.0 g/dl, serum vitamin B12 levels < 120 pg/ml, determined by Access, Beckman Coulter analyzer, and microscopical hypersegmentation of neutrophils) were performed with Sysmex XT-2000i analyzer. Statistical analysis: Student's t-test and Pearson correlation were applied. Values of $P < 0.05$ were considered to indicate statistical significance. Results: Patients with MA have statistically significant higher values of NEUT-X than normal controls, but there was no difference in NEUT-Y values (Table). In patients with MA, NEUT-X values correlate negatively, in a statistically significant degree, with vitamin B12 values (r=-0.577, P=0.001). *Conclusions*. The increased NEUT-X value (an index of the structure of neutrophils) observed in patients with MA, reflects the presence of hypersegmented neutrophils and could help with the diagnosis of megaloblastic anemia. The laboratory cytologist should consider above which threshold of NEUT-X value, a microscopical blood film review is routinely necessary.

Parameter	Normal controls, n = 62	Patients (MA), n = 40	*P* value
NEUT-X, mean ± SD	1384 ± 28	1412 ± 56	0.006
NEUT-Y, mean ± SD	390 ± 19	390 ± 27	0.860
Vitamin B12 (pg/ml)	325 ± 110	88 ± 29	0.000

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EXTENDED LEUKOCYTE DIFFERENTIAL OF LEUKOPENIC SAMPLES USING COMBINATION OF AUTOMATIC CELL ANALYZER AND 5-COLOR 6-ANTIBODIES FLOW CYTOMETRY

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Background. The performance of the manual differential on leukopenic samples has very poor precision due to insufficient number of counted cells. Frequently, it is very difficult to classify cells and time consuming also. Recently, the HematoFlow method has been introduced as a combination of automatic cell analyzer and CytoDiff ® 5-colors 6-antibodies flow cytometry. Aims. We evaluated the usefulness of HematoFlow method in leukocyte differential of leukopenic samples. Methods. Leukopenic blood samples(249) showing WBC count of 500-2,000/uL were selected. The CBC samples were analyzed by flow cytometry using premixed reagent for leukocyte differential and no wash mode(CytoDiff, Beckman Coulter). HematoFlow results were selected or calculated using automatic blood cell analyzer(DxH800, Beckman Coulter) results and CytoDiff results. Manual differential counting was duplicated including a hematopathologist(reference count) and 50 to 100 cells were counted. Immature granulocytes(IG) of manual count was sum of metamylocytes, myelocytes and promyelocytes. IG was calculated by subtracting the DxH800 eosinophil count from sum of CytoDiff IG and eosinophil count. If basophil and nonB nonT blasts populations were not well separated, differential was performed using median fluorescence level of 3.5 using CD2+CRTH2. Statistical analysis was performed using Fisher's F test for analysis of precision and Pearson correlation test for correlation analysis. The study was approved by IRB. Results. The proportion of adjusting gates in CytoDiff was 19 out of 247 cases(7.7%) due to huge debris contamination(2 cases) and incomplete separation of basophils and blasts. The precision of HematoFlow was superior to manual differential in counting of 5 leukocyte subpopulations, IG and blasts(Table 1). Especially, the neutrophil count by duplicated manual count revealed 5.4±5.8% difference and by HematoFlow revealed only 1.24±2.70% difference. The blast was found in 46 samples and 17 samples showed blasts only in one manual count(37%), but all cases showed blasts in both HematoFlow counts. The manual blast count showed 1.7±6.6% difference and by HematoFlow only 0.65±1.99% difference. The eosinophil count of DxH800 showed best correlation to reference count. All data analysis is summarized in Table 1. Conclusion Leukocyte differential in leukopenic samples using HematoFlow is much more reproducible and accurate than manual differential. Especially, detection of leukemic blasts is much more reliable than manual differential. In conclusion, HematoFlow is very useful in differential counting of leukopenic samples.

Table 1. Results of HematoFlow differential to manual differential count

Cell population	Difference of repeated assay		Correlation to reference manual differential	
	Manual	CytoDiff		
Neutrophil	5.4±5.8	1.24±2.70 (*P*<0.001)	Manual1	0.9610(<0.0001)
			DxH800	0.9463(<0.0001)
			CytoDiff	0.9704(<0.0001)
Lymphocyte	6.1±5.6	0.98±1.53 (*P*<0.001)	Manual1	0.9633(<0.0001)
			DxH800	0.9618(<0.0001)
			CytoDiff	0.9724(<0.0001)
Monocyte	3.4±4.7	0.85±1.19 (*P*<0.001)	Manual1	0.8512(<0.0001)
			DxH800	0.5817(<0.0001)
			CytoDiff	0.8981(<0.0001)
Eosinophil	0.7±1.4	0.48±2.33 (*P*<0.001)	Manual1	0.8222(<0.0001)
			DxH800	0.8766(<0.0001)
			CytoDiff	0.6671(<0.0001)
Basophil	0.6±1.6	0.32±0.61 (*P*<0.001)	Manual1	0.7886(<0.0001)
			DxH800*	0.0389(0.5424)
			CytoDiff*	0.8727(<0.0001)
IG*	0.8±3.4	0.71±2.16 (*P*<0.001)	Manual1	0.2170(<0.0007)
			CytoDiff	0.3576(<0.0001)
			Hematoflow	0.3639(<0.0001)
Blast	1.7±6.6	0.65±1.99 (*P*<0.001)	Manual1	0.8627(<0.0001)
			CytoDiff	0.8325(<0.0001)

*IG: immature granulocytes – (promyelocytes, myelocytes, metamyelocytes)

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OUTCOME AND RELAPSE RISKS OF THROMBOTIC THROMBOCYTOPENIC PURPURA: AN EGYPTIAN EXPERIENCE

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Background: Thrombotic thrombocytopenic purpura [TTP] whether idiopathic or secondary is a rare but life threatening condition. Plasma exchange [Px] has decreased significantly the mortality from the disease but a substantial proportion of patients relapse. We describe the clinical spectrum and response to treatment and explore the risks for relapse in our cohort of patients. Patients and Methods: Patients treated for TTP at the Clinical Hematology Unit of the Department of Internal Medicine of Cairo University Egypt, between 2000 and 2008 were identified. Complete demographic and clinical data, laboratory results, treatment modalities and outcome data were collected and analyzed. The follow-up duration was 24 months. ADAMTS13 and its antibody were assayed for idiopathic patients admitted after June, 2007. Results: A total of 30 patients; 13 males (43%) and 17 females (57%) with a median age of 42 years were treated for 46 episodes of TTP. The median duration of disease onset-to-diagnosis for the first episode was 7 days. 23 patients (76.66%) were diagnosed as idiopathic primary and 7 patients (23.33%) were secondary TTP. Four patients died during the first 24 hours. Out of the 26 patients followed beyond 24 hours, 22 patients (84.6%) achieved remission with an average of 7.55 Px sessions, 13 of whom achieved a sustained remission (50%) whereas 4 patients (15.3%) were refractory. Nine patients relapsed thereafter (total 25 relapses) (mean 2.7 per patient) with an average of 9 Px sessions to achieve a subsequent remission. The 24 months overall survival was 80%. Initial low platelet count and high LDH were the only two statistically significant relapse predictors. Conculsions: The current results are conforming to the reported literature of the outcome of TTP. The very early mortality due to late referral highlights the need of education and awareness about the disease among primary health care providers.

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MYH9-RELATED PLATELET DISORDERS: A CHALLENGE IN LABORATORY MEDICINE- CASE REPORT

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Myosin heavy chain 9 (MYH9) - related platelet disorders belong to group of inherited thrombocytopenias. As a result of MYH9 gene mutation premature release of platelets from bone marrow is happening. Macrothrombocytopenia is present at birth and the only therapy that increases platelet count is transfusion of platelet concentrates. Considering the main characteristics of MYH9- syndrome, thrombocytopenia with very high MPV, laboratories should give precise platelet count, in order to avoid risk of inappropriate treatment. Impedance cell counters do not recognize giant platelets and, therefore, underrate both platelet count and platelet volume, so manual assessment is required. Mean of this work is to establish a modern approach of platelet counting, using immuno-determination of superficial antigen CD61, as the most accurate diagnostic tool. The patient, 25-year old male, with MYH9 syndrome, confirmed clinically by presence of macrothrombocytopenia and inclusion Döhle's bodies in granulocytes, was admitted to clinic for digestive surgery due to appendectomy. During routine laboratory investigation platelet counts, done by flow-cytometer Sapphire, Abbott Diagnostics, showed discrepancy in impedance and optical values (21 and 37 x 10^9/L, respectively). Because of very large MPV (15,1 fL) and the lack of experience in such atypical thrombocytopenias, presumption was that CD61 will give the most precise platelet count and this count was the highest (49x10^9/L), which was confirmed microscopically. Not underestimated the vigor of impedance principle, immuno-platelet counting, as a reference method, even in this rare clinical cases proves its strength for the best platelet enumeration.

1655

THROMBOTIC COMPLICATIONS IN PATIENTS WITH ADULT IMMUNE THROMBOCYTOPENIC PURPURA

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Background. Immune thrombocytopenic purpura (ITP) is autoimmune disease characterized by platelet destruction and decreased platelet production. Occasionally in ITP a life-threatening hypercoagulable state develops after splenectomy. Also there are reports of thrombotic events after management of ITP with intravenous immunoglobulin (IVIg). We present two patients, the first with thrombotic complications after splenectomy as a result continuous platelet activation and normalization of platelet number and the second patient developed acute myocardial infarction 5days after finishing 5-day therapy with IVIg. Case 1. A 53-year-old woman was diagnosed with ITP in February 2007. She had not other thrombophilic risk factors. She was treated with corticosteroids with moderate response. In August 2008. she developed bronchopneumonia with formation of pulmonal abscess. After recovery from infection she was splenectomized in November 2008.g. After splenectomy the number of platelets normalized but on 21st day after splenectomy a portal vein thrombosis developed. The patient was reoperated and gangrenae of small intestine in 60 cm long was found, which was resected. The patient recovered from surgical procedure but again on 31st December 2008 she got left-sided hemiparesis and epi convulsion. Endocranial MRI revealed a thrombosis of superior sagital sinuses. She was on LMW heparin and afterwards on oral anticoagulant therapy. Now she is well with moderate thrombocytopenia. Case 2. A 49-year-old woman suffering from ITP for 13 years, without response to splenectomy were on several type of treatments and during the last year she was on cellcept(micophenolat mofetil). After viral infection her platelet count dropped to 1x10^9/l with diffuse haemorrhagic syndrome. She was admitted to hospital and she received IVIg 0.400 mg/kg/day for 5 consecutive days. She responded to the therapy with normalization of her platelet count but after 5 days she developed acute anterolateral myocardial infarction with creatin kinase 2260, troponin 27,8. She was treated conservatively with IV heparin and she completely recovered. During treatment with heparin the number of platelets dropped to 52x10^9/l and heparin was stopped. *Conclusions*. In first patient because platelet count did not increased significantly after splenectomy, factors other than platelet count are responsible for thrombotic events. We suggest that previous infection may have provoked life-threatening hypercoagulable state. We advise to be cautious after splenectomy for possible post-splenectomy thrombotic complications in the subset of ITP patients who may have persisting platelet activation. In second patient IVIg have promoted thrombosis by increasing blood viscosity, activating platelets, or causing vasospasm.

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SMOKING: AN UNDERESTIMATED CAUSE OF THROMBOCYTOSIS

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Background: while smoking is a well-known cause of secondary polycythemia and neutrophilia, an association between smoking and an increased platelet count has not been reported. *Aims*: to identify and to characterize smokers developing, in addition to smoking-induced leukocytosis, an unexplained chronic thrombocytosis. *Methods*: a cohort of smoking patients with both a high leukocyte count and thrombocytosis observed during the last 18 years in Jolimont hospital (index cohort) was compared to similar patients from UCL St Luc Brussels(control cohort) observed during the last 16 years. Inclusion criteria included an unexplained thrombocytosis (no evidence of myeloproliferative neoplasm and of secondary thrombocytosis) confirmed at least 3 months apart, associated with tobacco-induced leukocytosis.

	Index cohort	Control cohort	p (test)
Patients with tobacco-induced leukocytosis (n)	168	68	
Patients with leukocytosis and unexplained chronic thrombocytosis (%)	13 (7.7%)	8 (11.7%)	.4 (Fisher)
Male/Female	4/9	4/4	.6 (Fisher)
Median age (range)	45 (31-71)	46 (23-57)	.8 (M-W)*
Median platelet count x 10^9/L (range)**	487 (404-680)	416 (358-562)	.04 (M-W)
Median leukocyte count x 10^9/L (range)	13 (11.4-17.3)	15 (10.9-18.3)	.3 (M-W)

*M-W: Mann-Whitney
** normal values were not identical in both cohorts (index cohort: <400, control cohort <350)

Results: in the index cohort, out of 168 smokers with chronic leukocytosis, 13 presented, in addition, a high platelet count while in the control cohort, out of 68 smokers with a high leukocyte count, 8 presented with thrombocytosis. There were no major differences between both cohorts (Table). In particular, the incidence of thrombocytosis in patients with tobacco-induced leukocytosis was similar (7.7 vs 11.7%, p=.4). The slightly lower platelet count in the control cohort may be related to lower normal values in the *control* laboratory. Overall, the thrombocytosis was modest, did not vary substantially during a protracted follow-up (median FU: 72 months), and was not associated with major arterial or cardiac events. *Conclusions*: although this study provides no direct evidence that thrombocytosis was induced by smoking, its unexpectedly high prevalence in persons with smoking-associated leukocytosis suggests a causal relationship with smoking, especially since similar incidence and characteristics were observed in 2 independent cohorts. Smoking-associated thrombocytosis should be considered in patients with otherwise unexplained long-standing, moderate, and stable thrombocytosis, associated with leukocytosis. The clinical course of this condition is benign. Such patients should not be submitted to aggressive or expensive investigations.

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CHRONIC ITP - CASE REPORTS OF THE TREATMENT BY TROMBOPOETIN ANALOGUES IN TWO CHILDREN

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Background. Immune thrombocytopenic purpura (ITP) is a common acquired bleeding disorder in children characterized by skin and mucosal bleeding. There is an isolated thrombocytopenia caused by antithrombocytic antibodies. The standard treatment is corticosteroids and intravenous immunoglobulin. The alternative treatment can also be anti -

CD20 antibodies and other immune suppression regimens. The splenectomy should be considered if the above-mentioned treatment fails, although we always try to avoid splenectomy in very young children because of possible severe complications such as post-splenectomy sepsis. This was the reason for using thrombopoetin analogues in two girls under the age of eight years, presented on this poster Aims. To assess the efficacy of treatment with romiplostim in two children with chronic ITP Methods and Results In the first patient neither intravenous immunoglobulin nor corticosteroids in dose up to 2mg/kg lead to a sufficient and lasting response. The treatment with antiCD 20 antibodies induced remission for just 5 months. Then platelets decreased again after a viral infection. In the second patient, corticosteroids induced remission only for a few days and after intravenous immunoglobulin the remission was lasting only for 5 weeks. Moreover the patient had repeated severe allergic reaction to intravenous immunoglobulin. Both of our patients fulfilled the criteria of chronic ITP and we offered them and their families treatment with romiplostim with the dose scheme based on data published by Buchanan et al (ASH abstract 680, 2009). Since our patients have been commenced on romiplostim, the platelet count is stable between 100-200x10^9/l. Conclusion Although the prognosis in the patient with chronic ITP is uncertain and the treatment options may not guarantee permanent remission, the alternative treatment with thrombopoetin analogues induced long-term remission in our patients. This treatment also might help to postpone or avoid splenectomy in our two patients and thus improve their quality of life.

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CASE STUDY OF SEVERE THROMBOCYTOPENIA AND ASSOCIATION WITH THE COURSE AND FINAL OUTCOME OF THE DISEASEAT A RURAL HOSPITAL OF GREECE

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Background-Aims. To study the severe thrombocytopenia (PLT <50.000) which is a frequent problem in hospitalized patients, a causal association with various diseases and demonstrate the important role it plays in the disease course and outcome. *Material-Methods*. We studied retrospectively 1250 records of patients. *Results*. There were 32 patients (19 men and 13 women) with a platelet count 4000-50000. Of these 23 (72%) experienced major bleeding disorders. The average age for men was 66 years for women 71. Causes identified: - In 12 cases of malignant disease with chemotherapy onward (37.5%) In 6 cases of malignant hematological diseases (18.8%) In 6 cases of hepatic cirrhosis (18.8%) In 4 cases of septicemia (12.5%) - In 3 cases of idiopathic thrombocytopenic purpura (9.4%) - In 1 case (3%) reptile bites Despite appropriate therapeutic treatment in 7 cases (22%) death occurred as a consequence of disseminated intravascular coagulation. Summary-Conclusions: 1)Most common cause of thrombocytopenia is a malignant disease after chemotherapy, followed by malignant hematological disease and followed by hepatic cirrhosis. 2)The profound thrombocytopenia is a serious prognostic sign and threatening situation for life itself, despite the appropriate measures. 3)Bleeding disorders occur at a low price in a large percentage of PLT. Attention, therefore, in these cases to the attention of all.

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RELAPSES AT THE PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA (ITP) TREATED AS FIRST-LINE THERAPY WITH HIGH DOSE DEXAMETHASONE VERSUS STANDARD STEROID THERAPY

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Background. Immune Thrombocytopenic Purpura (ITP) is an acquired disease characterized by an immunological peripheral platelet destruction , with a chronic evolution at adult patients. Glucocorticoids are first-choice therapy, but relapses appear in the third of cases. Aim of study: to evaluate if there is a significant difference of relapses at the patients with ITP treated with high dose dexamethasone (HD-DXM) versus standard steroid therapy. *Methods*: we studied 23 patients with ITP, median age 43 years, hospitalised in the Clinic of Hematology from Craiova (Romania), between 2007-2010. All patients were diagnosed with ITP, other causes of thrombocytopenia having been ruled out by bone marrow aspiration. All patients were treated with glucocorticoids : 11 patients with 1mg/kgc/day for 4 weeks, followed by a degression , and 12 treated with high dose of dexamethasone, 40 mg/day during 4 consecutive days, repeated every 21 days for six total courses. The response was evaluated considering complete response (CR) if platelet count was higher than 10 x 10^9/L, partial response (PR) if platelet count was higher than 5 x 10^9/L and failure if platelet count was lower than 5 x 10^9/L. At the end of glucocorticoids therapy, 15 patients had CR, 4 patients PR and 4 patients presented failure response, needed other therapies (vincristine, splenectomy). The median period of observation at responding patients was of 9,8 months. Seven patients of 19 responders relapsed: 4 of them initial treated with standard steroid therapy and 3 treated with HD-DXM needed other therapy (2 vincristine, 4 splenectomy, 1 eltrombopag) . Conclusion: our study didn't reveal major differences between relapses at the patients with ITP treated with HD-DXM versus standard steroid therapy.

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HEREDITARY PLATELET FUNCTION DISORDERS INVESTIGATION; ONE YEAR REFERRALS IN THE HAEMOPHILIA CENTRE OF NORTHERN GREECE

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Hereditary platelet function disorders constitute a rare cause of symptomatic bleeding. These disorders are heterogeneous in clinical expression and also in laboratory evaluation. The accurate measurement of the platelet function and the identification of a congenital platelet disorder is complex, includes different assays and the repetition of testing. 55 patients (42 women/13 men) were referred for platelet function evaluation due to personal and/ or family bleeding tendency (51) and 4 to check the antiplatelet therapy. They all manifested symptoms of excessive mucocutaneous bleeding such as bruising or excessive bleeding following surgical or dental procedures. Patients with prolongation of PT, aPTTT and TT were excluded and they were investigated for coagulation factors deficiencies. All patients were tested for Von Willebrand's Disease. The platelet function investigation consisted of 1) the platelet count number and the 2) blood film report for white cell inclusions and platelet size, 3)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 4)global test of haemostasis by PFA-100. The LTG was found normal in 34 patients (61,2%) and 21 patients (38,8%) had abnormal LTG. 14 patients among them (66,6%) were women as expected, since these disorders are more commonly diagnosed in women because of the menstrual cycle and the haemostatic challenge of childbirths. 13 were diagnosed as disorders of platelet secretion and signal transduction (8 women/5 men), 1 as thrombasthenia Glanzmann, 1 as aspirin like defect, 4 due to antiplatelet therapy, one had abnormal aggregation to collagen and one abnormal aggregation to all agonists. The use of PFA-100 in our patients showed that among 16 patients tested, 8 had normal values but 5 of them had abnormal aggregation tests and diagnosed as disorders of platelet secretion and signal transduction. Among the other 8 patients with abnormal prolonged PFA-100, 4 were found to have normal aggregation tests, 3 were diagnosed as disorders of platelet secretion and signal transduction and one was due to antiplatelet therapy. The abnormal platelet morphology was found in 4 out of the 13 patients diagnosed as disorders of platelet secretion and signal transduction. Platelet nucleotides release measurement was not feasible. The hemorrhagic history of the 13 patients who were diagnosed as disorders of platelet secretion and signal transduction was serious menhorhagia and bruises in the 8 women and the excessive bleeding following surgical and dental procedures in the 5 male patients. The aggregation tests give a lot of information about various platelet disorders as seen in our patients. Normal values of PFA-100 cannot exclude the diagnosis of a platelet function disorder as found in 5 of our patients, though the number of patients is limited. In literature and clinical practice there is a lack of consensus about the diagnostic tests concerning platelet function disorders. According to our findings, the aggregation tests seem to remain the gold standard in the evaluation of platelet disorders.

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EFFICACY AND SAFETY OF SIX DOSES OF RITUXIMAB (375MG/M^2) IN TREATMENT OF CHRONIC REFRACTORY IMMUNE THROMBOCYTOPENIA AN EXPERIENCE FROM QATAR

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Background. ITP is an autoimmune disorder leading to premature platelet destruction and persistent thrombocytopenia. Treatment is generally not recommended until the platelet count is <30,000/μL, bleeding occurs, or there are other predisposing co morbid conditions.The goal of treatment is to raise the platelet count to a hemostatically safe level. Different doses of Rituximab were tried 1 Gram fixed dose day 1 and day 15 (Rheumatoid arthritis) like regimen , or 375mg/m2/ week for four weeks and 100mg/week for four weeks but nobody has tried 375mg /m2 /week for six weeks. *Aims*: to evaluate the efficacy and safety of 6 doses of Rituximab 375 mg/m^2, in treatment of patients with chronic refractory thrombocytopenia. *Patients and Methods*. From our retrospectively collected data of 14 patients diagnosed with chronic refractory thrombocytopenia between the January 2007 and January 2011 in AL-Amal hematology /oncology centre with mean follow up of 27 months all of them failed, steroid as first line therapy as well as IVIG, and 4 of them underwent splenctomy with no response, 8 were males and 6 were females with a mean age of 32 year, all of them had platlets count of less than 10.000 on presentation. Each of them received Rhituximab 375mg /m2 weekly for six doses and followed initial with weekly CBC for 3months and then every two months. Results: two patients did not achieve any response one is male and the other is female. Two patients achieved partial response platelets count more than 50.000 and less than 100.000 for more than six month and 12 achieved complete response defined as platelets count more than 100.000 for more than one year, with mean complete response of 28 months, none of them developed grade 2 or more infection as per WHO toxicity scale, and none of them developed progressive multifocal leucoencephalopathy. Conclusion: We concluded that six doses rituximab are safe effective and durable in patients with chronic refractory ITP keeping in mind the small number of patients further studies are needed to confirm these results.

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TIME-RELATED CHANGES OF THE SENSITIVITY TO ANTI-PLATELET THERAPY IN PATIENTS WITH ACUTE CORONARY SYNDROME AFTER PERCUTANEOUS CORONRY INTERVENTION

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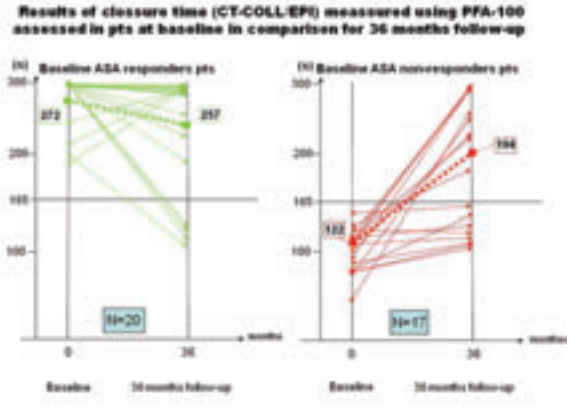
Study population. 37 patients (pts), 26 male, one month after an episode of acute myocardial infarction treated by percutaneous coronary intervention (PCI) with stent placement. All pts were receiving dual antiplatelet therapy (ASA - 75 mg/pd and clopidogrel - initial 600 mg, next 75 mg/pd) for at least one month before entering into the study. During the second and third year of observantion all pts were on ASA 75-150 mg/pd. *Methods*: The platelet reactivity was assessed on a basic of closure time (CT) in platelet function analyz er PFA100 (Dade Behring). CT was measured on the 30 th day and 36 months after PCI. ASA resistance was defined as a normal collagen/epinephrine CT (<165 s) despite ASA treatment (compliance proved by a diminished intraplatelet mylonyldialdehyde [MDA] concentration).



Results. During 3 - years follow-up changes of ASA resistance status were observed in 14 pts - 10 from "responsive"to "resistant"and in 4 pts from "resistant"to "responsive"status. In 23 pts there were no changes of ASA resistance status. The results of CT in 30 th day and 36 months after PCI were presented on the figure. *Conclusions.*During 36 months follow-up the changes of ASA resistance status (assessed using PFA-100) were observed in 38% of pts treated with antiplatelet drugs after percutaneous coronary intervention (PCI) with stent placement.

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PRELIMINARY DATA ON THROMBOPOIETIN RECEPTOR (CMPL) EXPRESSION IN DIFFERENT PLATELET DISORDERS

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Background. Interaction between thrombopoietin (TPO) and cMPL appears to be critical in the platelet production, promoting CD34+ differentiation, megakaryocytes (MK) proliferation and maturation via cytoplasmatic down-stream signalling. Another consequence of TPO-cMPL interaction is the regulation of TPO level. It is know that TPO is high in thrombocytopenia secondary to MK hypoplasia but TPO levels are low in immune thrombocytopenia (ITP) as well as in inherited macrothrombocytopenia and variable in essential trombocythemia (ET). The aim of these study is to investigate cMPL expression in different cases of thrombocytopenia to better understand the therapeutic use of TPO mimetics. Patients and methods We enrolled 78 patients suffering from inherited and acquired platelet disorder: 20 ITP patients, 18 with an inherited macrothrombocytopenia(FAM) (MYH9 related disorder, Bernard Soulier classical, N41H or type2), 25 patients with a thrombocytopenia secondary to megacaryocyte hypoplasia(MK-Hyp) and 15 ET patients. We enrolled also 25 healthy subjects as control group. cMPL (82 kD, glycosylated) expression has been evaluated by Western-Blot on 2 x 107platelets lysates using1 mg/ml rabbit polyclonal anti-Human cMPL antibody. Anti CD41-clone SZ22 monoclonal antibody against platelet GpIIb complex was used as control on the same membranes, after stripping anti-cMPL antibody. Expression of both cMPL and anti CD41 was quantitated by densitometry analysis of Western Blot. The results are given as mean± SEM of cMPL/CD41 expression ratio for each group of patients. Serum thrombopoietin levels have been evaluated by an ELISA assay, using a trade kit (Quantikine, R & D System). Results ITP (0.17±0.04), FAM (0.19 ± 0.06) and Mk-Hyp (0.18±0.04) patients have higher cMPL expression than controls (0.04±0.01) and ET (0.0±0.01). There were no differences between controls and ET. TPO (pg/mL) in ITP (149±22) is higher (p=0.001) than controls (67±14); Mk-HYP have TPO levels (833±301) higher than controls (p=0.008) and ITP (p=0.029). All three form of thrombocytopenia have TPO level higher (p<0.05) than ET (respectively ITP 149±22, Mk-HYP 833±301, FAM 98±15 pg/mL vs ET 55±8 pg/mL). No correlation between TPO and cMPL has been demonstrated in our groups. *Conclusions*. Our preliminary data demonstrate that cMPL is overexpressed in thrombocytopenia in spite of the type. On the contrary TPO is increased above all in patients with Mk-Hyp. These findings justified the use of TPO mimetics in ITP and FAM.

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COMPLETE ITP REMISSION IN ADULTS FOLLOWING THE SPLENECTOMY IN LATVIA

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Background. Immune thrombocytopenic purpura (ITP) manifests as decrease in platelet (PLT) count of various degrees, which can even cause fatal bleeding. Glucocorticoids (GC) and intravenous immunoglobulin (IgG) are used as the first line of treatment; splenectomy is "the second stage". *Aims*. Determine the rate of complete remission following the splenectomy patients with ITP. *Methods*. The 668 patients to 2.3 million people were cured with diagnosis ITP in the period from 2002 to 2010 at the Riga East University Hospital (REUH) clinic "Linezers"and State Hematology centre (SHC). The retrospective study included 13 patients which underwent surgery at the 8 years period. Demographic data, disease duration and previous therapy, full blood-count, spleen size, type of operation-laparoscopic (LS) or open (OS) splenectomy were analyzed. Indications for splenectomy were thrombocytopenia with bleeding signs and or lack of response to conservative treatment and low platelet count while on massive steroid. Processing of the data used nonparametric statistical methods. *Results*. The study includes 8 women and 5 men, the median age are 37 (IQR=18) years. The median duration of the disease prior to the surgery was 24 (IQR=80) months. Prior to the surgery 2 (15.4%) patients received only GC therapy, 5 patients (38.5%) received

GC and immune suppressive (IS) therapy, in 5 cases (38.5%) GC and IgG was applied. One patient, for whom the duration of the disease prior to the surgery was 7 months, had received GC, IS and IgG therapy (7.7%). All patients received pneumococcal vaccination prior the surgery. The median size of the spleen was 10.2 (IQR=2.1) cm detected by ultrasonography. In one US case and in one CT case accessory spleens were detected, which were removed during the surgery. 11 LS and 2 OS were done. The pre-operative median value of PLT 56 x10^9/L (IQR=93) had increased in a statistically significant way in accordance with Wilcoxon signed-rank test (z=2.76; p=0.006) compared to the median value of PLT 183 x10^9/L (IQR=220), which was detected during the last follow-up on median 36 (IQR=55) months after the surgery. Complete remission (PLT count >50x10^9/L, without additional therapy and bleeding signs) were observed in all patients at the median 36 (IQR=55) months observation period following the surgery. *Conclusions.* The splenectomy is safe and effective *second stage* procedure for patient with ITP in our study. Complete remission of ITP was observed in all cases.

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PLATELET GLYCOPROTEINS AND STICKY PLATELET SYNDROME

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Background/Aims. Sticky platelet syndrome (SPS) is a hereditary, autosomal dominant thrombophilia associated with an increased incidence of arterial and venous thrombosis. Light transmission aggregometry (LTA) is used to confirm a platelet hyperaggregation induced by very low concentration of platelet inducers - by adenosinediphosphate (ADP) and epinephrine (EPI). Etiology of SPS is unknown but some studies suggest that abnormalities of platelet surface glycoprotein (GP) receptors can lead to their hyperfunction. Aim of the study was to verify if there are some abnormalities in the expression of platelet membrane GP receptors in patients with SPS. *Materials and Methods*. Seventy-five patients with SPS were included into the study and examined by flow cytometry to assess the expression of platelet surface GP receptors: P-selectin (CD62P), CD63 and CD51. The control group included 30 healthy individuals. All patients and controls agreed with participation in the study and signed an informed consent. Results: The results of flow cytometric analysis of SPS patients have shown that there is a significantly higher expression of P-selectin (CD62P), CD63 and CD51 compared to healthy controls (P-selectin: p<0,001, CD63: p<0,05, CD51: p<0,05 respectively). These GP receptors are neoantigens expressed on the platelet surface only after platelet activation. *Summary/Conclusions*. On the basis of our measurements we can say that platelets in SPS patients are activated compared to the controls. We suggest that the increased expressions of CD62P, CD63 and CD51 may serve as predictors of thrombophilia in SPS patients.

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IDIOPATHIC THROMBOCYTOPENIC PURPURA IN ADULTS IN THE LAST 10 YEARS: SINGLE CENTRE EXPERIENCE

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Background. Adult idiopathic thrombocytopenic purpura (ITP) is a benign disease with low morbidity and mortality and frequent remissions that occur spontaneously or in response to first line treatment with steroids or splenectomy. *Aims*. The aim of this study is to analyze the clinical outcomes of 170 patients with ITP diagnosed and/or treated in our hospital in the period between 2000 and 2010. *Methods*. We retrospectively have analyzed the institution database for patients diagnosed and/or treated for ITP. All patients met the diagnostic criteria for ITP. *Results*. The median age at diagnosis was 47 years (range 14-83 years). Forty six (27%) were males and 73% were females. Forty three (25%) were asymptomatic, 65% had minor skin or mucosal bleeding and 10% had significant bleeding from gastrointestinal or genitourinary system. None of the patients had severe, life threatening bleeding symptoms. The median platelet count at diagnosis was 13x10^9/L (range: 0-98x10^9/L). Number of patients with severe thrombocytopenia (Plt <30x10^9/L) was 125/170 (73.5%), with moderate thrombocytopenia (Plt 30-50x10^9/L) was 14.1% and with mild (Plt 50-100x109/L) was 12.4%. Bleeding symptoms were more common in patients with severe thrombocytopenia 108/125 (86.4%) patients, comparing to 45.8% of patients with moderate and 38% with mild thrombocytopenia (p<0.001). Bone marrow examination was performed in 76% of patients. Anti-platelet antibodies were positive in 8/35 (23%) of patients. Direct antiglobuline test was positive in 7/135 (5.2%) patients and 2.9% patients had hemolytic anemia. Median follow up of all patients was 13 months. Ninety five patients had follow up longer than 12 months, with median 44 months (range 14-384). Corticosteroids were initial treatment for 95% patients, 38(22%) were splenectomized, 25(14.7%) were treated with intravenous gamma globulins, while 9 didn't received any specific treatment. Complete response to initial treatment (prednisone±splenectomy) was achieved in 55/161(34.2%), partial response in 55.9% and no response in 9.9% patients. The median platelet count at last control was 161x10^9/L (range: 2-704x10^9/L). At the last follow up, number of patients with severe thrombocytopenia was 12/170 (7.1%), with moderate was 12(7.1%), with mild 33(19.4%) and with platelet count >100x109/L was 66.4%. At the last control 46.5% patients were without therapy for ITP. In the group of patients with follow-up longer than 1 year; 28 (29%) patients had refractory ITP with median follow-up of 66 months. All patients with refractory ITP were treated with steroids, 11 were splenctomized, significantly more patients 12(43%) were treated with IVIG, p=0.005. Median age of 38 splenectomized patients was 28 years and it is significantly different from the other patients (p<0.001). There were no significant difference in other characteristic between splenctomized or refractory ITP and other patients at diagnosis. *Conclusions:* Results from our study are similar with previously reported in view of median age, sex distribution, hemorrhagic symptoms, response rate etc. Our results approved that most adults with ITP have a good outcome with low morbidity and mortality. But almost 30% of patients have refractory ITP, which represent significant medical problem due to a constant need of treatment.

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MEAN PLATELET VOLUME DECREASED AFTER IMMUNOAPHERESIS OR CASCADE PLASMA FILTRATION THERAPY IN FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction: Platelet size, measured as mean platelet volume (MPV), is associated with platelet reactivity. If MPV would drop after LDL-lowering therapy, decreased MPV could be one of the simple markers of successful therapy (decreased atherosclerosis activity). Methods and patients: MPV was investigated in patients with severe familial hypercholesterolemia (FH) long-term treated (3-12 years) by LDL-apheresis (immunoapheresis) or cascade filtration. Plasma was obtained by centrifugation. Adsorbers Lipopak 400® were used for immunoapheresis and filters Evaflux 4A® were used for cascade filtration. 95 pair samples were measured (before and after the procedures) - 8 times during 4 years in 12 patients. Results: Mean MPV before the procedures was 10.891 fl (CI 10.25-11.53) and decreased after the procedures: 10.478 fl (CI 09.84-11.11) - this difference is significant (p = 0.036). MPV did not correlate with age, sex, platelet count, duration of therapy.Discussion: Our study gives a good evidence of MCV drop after the described therapy; we did not find any other data about MCV changes in FH with extracorporeal elimination therapy in the literature. MPV is easily available and is often disregarded, but sometimes may indicate the need for a careful assessment in patients with FH: The association of the MPV with the risk of coronary and cerebrovascular diseases and atherosclerosis activity was reported. *Conclusions*. MPV could be one of the markers of therapeutic efficacy in patients with FH treated by extracorporeal elimination. MCV examination is a simple, inexpensive and easily accessible method.

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STUDY OF T-CELL IMMUNOGLOBULIN- AND MUCIN-DOMAIN-CONTAINING MOLECULE 3 POLYMORPHISMS IN PEDIATRIC EGYPTIAN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background. Idiopathic thrombocytopenic purpura (ITP) is an acquired autoimmune disease characterized by the production of autoantibodies that mediate platelet destruction. Dysfunctional T cell-mediated immunity plays an important role in the pathophysiology of ITP. In humans,

the T-cell immunoglobulin and mucin-domain (TIM) gene family, located on chromosome 5q33.2, consists of TIM-1, TIM-3, and TIM-4 genes, which encode cell-surface glycoproteins with similar structures. TIM-3 was reported as a central regulator of T-cell responses. Recently it was reported that TIM-3 mRNA expression in peripheral blood mononuclear cells was significantly lower in ITP patients than in healthy controls which indicated that TIM-3 may play an important role in the pathogenesis of ITP. One single study by Du et al (Human Immunology, 2009, 70:398-402) investigated the role of TIM-3 polymorphism in ITP in North China. Nonetheless, pharmacogenetic studies show marked ethnic variations. Also this study did not address the impact of the polymorphisms on severity and clinical course of the disease. *Aims*: (1) To study TIM-3 gene polymorphism as a risk factor for ITP in a cohort of Egyptian children. (2) To verify if any of the polymorphisms would have an impact on the severity or the clinical course of the disease. *Methods:* Under informed consent 100 patients (46 acute, 50 chronic and 4 persistent ITP cases) and 210 controls were tested for TIM-3 G1516T, T574G, and G4259T polymorphisms using PCR-RFLP. Allele frequencies were calculated for patients and controls. Patients were treated according to standard protocols. The impact of TIM-3 gene polymorphisms on severity and clinical outcome was studied. Results: No significant difference in the distribution of the wild, heterozygous and homozygous genotypes was encountered between cases and controls. Only TIM-3 T574G showed a near significant difference (p=0.058) where GG, TG and TT constituted 43.88%, 51.02% and 5.1% in cases as compared to 54.28%, 38.1% and 7.62% in the controls. T and G allele frequencies were 30.6% and 69.4% in cases as compared to 26.67% and 73.33% in the control. Neither was there significant association between any of the polymorphisms on one hand and the severity of disease or the clinical outcome on the other hand. However near significant association (p=0.08) was encountered between 4259TT and severity as represented by bleeding at presentation (61.3% vs. 38.7%). On the other hand 574GG showed near significant association with better quality of response (71.42% in responders vs. 42.1% in non-responders, p=0.07). Similarly, 4259TT showed near significant association with better quality of response (85.7% in responders vs. 57.8% in non-responders, p=0.09). *Conclusions:* This study confirms the previous report that TIM3 gene polymorphism might not play an important role as a genetic risk factor in the pathophysiology of ITP. Neither has it had a major impact on the severity or clinical course of the disease. Nevertheless studying a larger number of cases may further prove or disprove this notion.

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CHILDHOOD IMMUNE THROMBOCYTOPENIC PURPURA: A REGIONAL STUDY OF 140 CASES

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Introduction. Childhood immune thrombocytopenic purpura (ITP) is a rare disease. Physician management of childhood ITP is diverse. We report the clinical, therapeutic and outcome of childhood ITP in our hospital. *Patients and Methods*. We retrospectively analyzed cases of childhood ITP diagnosed in pediatrics and hematology departments of hospital Hedi Chaker Sfax, between January 1995 and December 2009. We analyzed the epidemiological, clinical, biological and evolution. Treatment response was defined as follows: complete response (CR), a plateled count ≥100000/mm^3 persisting for at least 2 months, partial response (PR): a platelet count between 50-100000/mm^3 and no response (NR): a platelet count <50000/mm^3. *Results:* During the study period, 140 cases of ITP were collected. The mean age at diagnosis was 6 years (3 months-15 years). The vast majority of children had mild bleeding symptoms: purpura and petechiae (100%), epistaxis (33%), gum bleeding (26%). Intracranial hemorrhage occurred in one child. Eighty four (60%) children were treated with corticosteroids alone and seventeen children (12%) with intravenous immunoglobulines (IVIG). One hundred forty two (90%) patients achieved good response (CR+PR) to corticosteroids. A chronic evolution was noted in 30 cases (21%). *Conclusions:* The data from our series revealed that clinical characteristics and outcome of childhood ITP are similar to those series reported in literature. Corticosteroids remain the first-choice treatment for childhood ITP resulting in a response in 90%.

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CHRONIC IMMUN THROMBOPENIC PURPURA: RETROSPECTIVE ANALYSIS OF 30 CHILDREN

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Childhood immune thrombocytopenic purpura (ITP) is an autoimmune acquired disorder. Approximately 20% to 25% of children manifest chronic ITP. We analyzed clinical characteristics and management of children with chronic ITP. *Patients and methods:* We retrospectively analyzed the clinical, therapeutic and evolution of children manifest chronic ITP diagnosed in pediatrics and hematology department of hospital Hedi Chaker Sfax, from January 1995 to December 2009. Treatment response was defined as follows: complete response (CR), a plateled count≥100000/mm^3 persisting for at least 2 months, partial response (PR): a platelet count between 50-100000/mm^3 and no response (NR): a platelet count <50000/mm^3. *Results:* During the period of study, 140 cases of PTI were collected in the department of haematology and paediatric. Twenty three children (21%) (22 girls and 8 boys) manifest chronic ITP at 6 months after the initial diagnosis of ITP. The mean age was 9 years. The distribution of cases according to age showed a maximum at 10-12 years. Twenty one children were treated in first line: with corticosteroid for 19 patients and intravenous immunoglobulin for 2 patients. Fourteen children, who were nonresponsive to first line treatment, 14 children were splenectomized and 2 children treated with immunosuppressive agents (vincristine, azathioprine). The follow up period after splenectomy ranged between 2 to 15 years. A complete remission (CR) was achieved in 78% cases without any relapse. There were no infections after splenectomy in any children.

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PLATELETS ASSOCIATED ANTIBODIES PRE AND POST SPLENECTOMY IN HEPATITIS C PATIENTS WITH THROMBOCYTOPENIA

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Background: The presence of platelet antibodies inducing thrombocytopenia in patients with HCV infection is a matter of great debate. *Subjects and methods*: The present study was conducted on 23 subjects categorized as follows: Group 1: included 23 cases diagnosed as chronic hepatitis C before splenectomy. Group 2: included the same 23 cases after splenectomy. For all subjects included in this study platelets counts was evaluated as well as platelets associated antibodies (IgM, IgG, IgA) Results All patients were thrombocytopenic before undergone splenectomy. Platelet counts (51.8±16.7). After splenectomy all patients were of normal platelet counts (174.2±35.8). The mean ±SD of platelets associated immunoglobulin were (64.2±9.6) for total Igs, (53.6±8.1)for IgG, (3.8±2.1) for IgM, (6.7±4.7)presplenectomy versus post-splenectomy for total Igs (13.4± 19.3), for IgG(5.4±1.8) , for IgM (1.9± 1.06), for IgA (2.1± 0.9) and the differences was statistically significant (P<0.001). The correlation studies between platelet count and PAIgs level in patients with chronic HCV infection pre-splenectomy revealed that there is significant correlation between platelets counts and total Ig (r -0.804, P= 0.000), (r -0.907 P= 0.000), (r - 0.467, P = 0.002),(r - 0.519, P 0.000). *Conclusions*: autoimmune mechanism plays an important role in the HCV associated thrombocytopenia and spleen is a major source of PAIgs.

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SERIOUS THROMBOCYTOPAENIA AS HEMATOLOGICAL MANIFESTATION OF NOONAN SYNDROME

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Bleeding anomalies have been reported in Noonan Syndrome although thrombocytopenia was not the most common, especially as first clinical manifestation. Abnormal platelet count or function could occur in this disorder. Boy, first child of nonconsanguineous parents, polyhydramnios diagnosed at 26 weeks of gestation. Birth weight was 2560 g (P5) and length was 45 cm (P<5), admitted for bleeding diathesis in the first day of life. On physical examination, petechial and purpuric rash, dysmorphic facies: low-set ears, high forehead, hypertelorism, and

bilateral cryptorchidism, syndactyly of four and five left foot fingers. There was no evidence of hepatosplenomegaly. Cardiologic investigations revealed an aortic valvar dysplasia with minimal insufficiency and atrial septal defect. An initial complete blood count showed a platelet count of 5.000 /ul. Evaluation of his neonatal thrombocytopenia included a platelet immune workup and a congenital infection workup; neither yielded positive results. Familial thrombocytopenia and the thrombocytopenia absent radius syndrome were ruled out. The patient was given several platelet transfusions and immune globulin over the first weeks of life because of repeated low platelet counts. The PT and PTT were within normal limits. Bone marrow aspiration was normal. Genetic analysis revealed the presence of a constitutive 218 C» G mutation in exon 3 of the PTPN11 gene that was associated with Noonan Syndrome. We present this case because Noonan Syndrome was rarely described as cause of serious inherited thrombocytopenia. This mutation was identified in several patients with juvenile myelomonocytic leukemia, hematological follow up is important in these patients.

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CARBAMAZEPINE-INDUCED IMMUNE THROMBOCYTOPENIA AND RELATED WITH TO PHENYTOIN

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Background. Phenytoin and carbamazepine are rarely associated with serious hematological side effects. Carbamazepine (CBZ) is an effective anticonvulsant for children with partially and secondarily generalized seizures. It is considered safe and relatively less toxic than other anticonvulsant drugs. The hematological toxicity of CBZ is well known and thrombocytopenia is a rare manifestation of CBZ-induced myelotoxicity. Thrombocytopenia is a rare manifestation of hematological toxicity of CBZ, and results from myelosuppression which occurs within four weeks of initiating the treatment. Case report: A 10-year-old boy was admitted with generalized, non-itchy erythematous body rash. There was no associated fever, conjunctival hyperemia, oral ulcers or other systemic manifestations. Three months before the presentation, he had had a generalized seizure and was started on 5mg/kg/day of phenytoin in two divided doses. He developed severe urticarial lesions. Di-phenylhydantoin was discontinued and the patient was switched over to CBZ. There was no previous history of bleeding diathesis or drug allergy. Examination revealed extensive purpura and petechiae mainly over the limbs and a few scattered lesions over the trunk. There was ecchymoses, wet bleeds and epistaxis. The child had no pallor, bone-tenderness or lymphadenopathy. Abdominal examination revealed no hepatomegaly and splenomegaly. Investigations revealed hemoglobin 12g/dl, total leukocyte count 8800/mm3 with 60% neutrophils, 32% lymphocytes, 2% eosinophils and 4% monocytes. Platelet count was 3.000/mm3. Anti-nuclear antibody was negative. Bone-marrow aspiration showed decreased megakaryocytes with no abnormal cells. CBZ was discontinued and mega-dose steroid treatment was started. There was a rapid recovery with platelet count rising to 15.000/mm3 on the second day and further to 518.000/mm3 on the fifth day of starting prednisolon. The patient has remained seizure-free and thrombocytopenia has not recurred did not occur during a follow-up of one month. Results: Thrombocytopenia is a rare manifestation of hematological toxicity of CBZ, and results from myelosuppression which occurs within four weeks of initiating the treatment. In our patient, ten days after beginning CBZ treatment, thrombocytopenia developed. Thrombocytopenia usually resolves once CBZ is discontinued. We used mega-dose steroid treatment for serious thrombocytopenia and epistaxis. Bone marrow examination is usually required to differentiate it from primary bone marrow disorders. Summary / Conclusions: Hematological monitoring is recommended the patient who developed phenytoin toxicity since it does not identify the patients at risk of serious blood dyscrasias. Physicians prescribing CBZ should be aware of phenytoin toxicity; we advise another anticonvulsant therapy that developed side effects of phenytoin.

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BENEFICIAL EFFECT OF EARLY ADMINISTRATION OF RITUXIMAB TREATMENT FOR ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA

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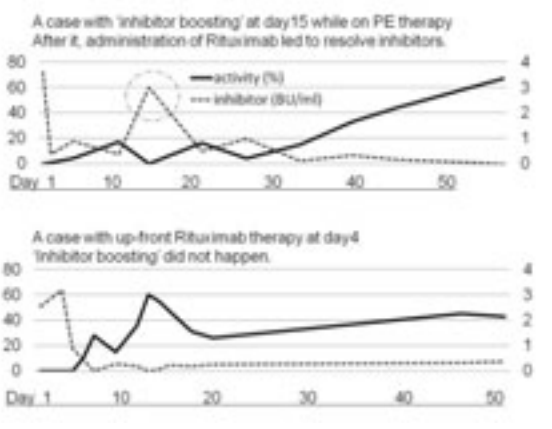
Background. Plasma exchange (PE) is first-line therapy for patients with acquired thrombotic thrombocytopenic purpura (TTP) because of the clearance of circulating ADAMTS13 inhibitors. However, there is a subset of patients who do not initially respond to PE treatment or subsequently deteriorate while on PE treatment around day 7-14. There are limited reports that describe detailed clinical and serial laboratory findings during this challenging process. The exact pathophysiology of this deterioration, we generally refer to as *inhibitor boosting*, remains still uncertain, furthermore, the appropriate management or prophylaxis is also unclear. Patients for refractoly TTP are at greater risk of complications and, therefore, early administration of Rituximab might be considerable option, which could reduce plasma requirement and avoid complications. Here we describe four patients with severe acquired TTP who were successfully treated with Rituximab. Aims: The purpose of presenting our cases is to highlight how early initiation of Rituximab could lead to a positive outcome, especially in a severely ill patient. We also present detailed laboratory analysis, including frequent ADAMTS13 measurements, during a phase of the 'inhibitor boosting'. Methods: We reviewed all the patients with acquired TTP treated with Rituximab at Tenri Hospital, 4 cases were identified from 2006 to 2010. Clinical and laboratory findings, including ADAMTS13 activity and inhibitors, were recorded and analyaed.



Results. All 4 patients (median age 67, range 40-78) showed severely decreased ADAMTS13 activity (<1.0%) with detectable inhibitor level (median 3.4 Bethesda U/ml, range 1.72-6.80). 3 patients presented during their first TTP episode while one patient had relapse of TTP after 10 months remission. We immediately treated all patients with PE at dianognosis with acquired TTP, however, in two cases of them, we subsequently administered Rituximab therapy because of deterioration while on PE treatment. At this time point (one patient at day 7, another at day 14), ADAMTS13 activity or inhibitor testing revealed considerable abnormal results. In another two high-risk cases, which were a recurennt case and a severe acute case of deep coma as the first episode, we administered up-front Rituximab thrapy after only a few PE treatments. After the addition of Rituximab (375mg/m2 each week for 4 week), all of the 4 patients achieved complete clinical and hematological remissions with rapid recovery of ADAMTS13 activity and resolution of detectable inhibitor. All patients required no additional plasma exchange after completion of rituximab and remained in sustained remissions with median follow-up of 30 months (range, 3-60 months). Disucussion: In refractory and recurrent cases, administration of Rituximab appears to be a reasonable therapeutic option. In our limited experiences, early up-front addition of Rituximab, before deterioration, may be also beneficial in avoiding exacerbation, decreasing the need for plasma exchange and sustaining long-terms remission. Prospective studies are needed to define the true efficacy and safety of up-front Rituximab therapy and timing of its initiation.

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RESPONSE TO THROMBOPOIETIN ANALOGUES (ROMIPLOSTIM) AS SECOND LINE TREATMENT IN SIX PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA REFRACTORY TO CORTICOSTEROIDS

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Background: Immune thrombocytopenic purpura (ITP) is an autoimmune disorder in which a high percentage of patients obtain complete remission after a first line therapy with corticosteroids. In other cases, recurrence or development of corticosteroid dependence forces us to choose a second line treatment. Although until now splenectomy appeared to be the first choice as a second line, the emergence of new therapeutic options (thrombopoietin analogues and rituximab), as well as patients refusal of surgery, have changed our clinical practice. Patients: We report 6 patients (2 males and 4 females) diagnosed with severe chronic ITP refractory to corticosteroid therapy, in whom we rejected splenectomy as second line for medical reasons. All of them initiated romiplostim as second line (one patient had previously received rituximab). Two patients started treatment with platelet counts (PC) > 100 x 10^9/L due to the effect of gamma globulin. The aim of treatment was to achieve a safe number of platelets, not to obtain complete remission. *Results:* All patients underwent dose escalation starting at 1 μg/kg/week. Rescue treatment with gamma globulin +/- prednisone was discontinued. Hemorrhagic symptoms disappeared in all cases except one, a female patient who did not respond despite escalation to the maximum dose of 10 μg/kg/week. Since her diagnosis was later changed to amegakaryocytic purpura (47, XX, +8), she was excluded from the response analysis. Four of the five remaining patients obtained a partial response (PC > 30 x10^9/L) within a week of treatment initiation (mean dose 1.5 μg/kg), and all of them achieved complete response (PC > 100 x10^9/L) after a mean of 31.4 days (mean dose 2.5 μg/kg)(table). All patients tolerated the treatment without clinically relevant side effects, except mild headache or bone pain, and were able to return to work, considerably improving their quality of life. Conclusions: Thrombopoietin analogues are a treatment option for patients with chronic ITP refractory to costicosteroid therapy, in whom other therapies such as splenectomy are contraindicated. In these patients, the administration of low doses of romiplostim, with the aim of restoring safe platelet counts, provides satisfactory responses from a clinical point of view, with safe platelet counts, decreased hemorrhagic symptoms and improved quality of life, with little or no adverse effects. Thus, thrombopoietin analogues are safe and effective as first choice for second line treatment of severe chronic ITP.

1676

EFFICACY AND SAFETY OF RITUXIMAB IN CHILDREN WITH CHRONIC IMMUNE THROMBOCYTOPENIA: A SINGLE INSTITUTION EXPERIENCE

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Background. Recent recommendations concerning primary immune thrombocytopenia (ITP) in childhood defines ITP as an acquired immune mediated disorder characterized by isolated thrombocytopenia, defined as a peripheral blood platelet count less than 100x10^9/L, and the absence of any obvious initiating and/or underlying cause of the thrombocytopenia. Furthermore, a recently approved and promising treatment option in chronic ITP in childhood is reported and concerns rituximab. Rituximab is a chimeric monoclonal antibody against the protein CD20, which is primarily found on the surface of B cells, inducing B cells destruction and autoantibodies depletion. On the other hand, although previous results of rituximab in adults are promising, treatment doses, efficacy and safety in the pediatric setting remains to be established. Aims. Evaluation of the efficacy and safety of rituximab as salvage treatment in children diagnoseed with chronic ITP. Materials and Methods. A prospective unicenter study regarding the primary outcome data of rituximab therapy in children with the diagnosis of chronic refractory ITP was performed. The treatment response was evaluated after the every course of rituximab treatment. Duration of response was considered from the day of the initial infusion to the first time of relapse or to time of analysis. Complete response (CR) was defined as PLT count >100x10^9/l, partial response (PR) was as any PLT counts between 30 and 100x10^9/l and no response (NR) below 30x10^9/l. Relapse was also defined as a decrease of the platelet count to below 30x10^9/l after CR or PR. The patients received no other treatment in addition to rituximab. Results: Eight children out of 13 with chronic ITP (53.85%) received rituximab - 85.7% females- with mean age 9.5±1.6 years, mean PLT count 19x10^9/L and median time from initial diagnosis till rituximab treatment 1.28 years. Previous treatment included for 5 children (71.4%) combined treatment with corticosteroids and IVIG, for 1 child (14.2%) corticosteroids, IVIG and anti D globulin, and for 1 (14.2%) corticosteroids, IVIG, anti D globulin and splenectomy. Rituximab was administered intravenously at a dose of 375 mg/m2 weekly, for a total of four infusions in 4 patients (71.5%) and in a single dose in 2 children. CR was recorded in 5/7 children (71.4%) with median PLT 149x10^9/L and median follow up time 25.3 months. PR and NR was recorded in 1 child and 1 child respectively. Immunophenotype analysis revealed no circulating B CD 19+ (<1%) in six children and no significant B cell depletion (>4%) in one child (poor responder). Side effects were mild and transient and were recorded in only 1 of 7 patients (14.2%) with fever and cough which was successfully treated with antimicrobial agents. Conclusions: Rituximab therapy is beneficial for some children with severe chronic ITP who are refractory to standard agents. The toxicity profile of rituximab is acceptable in most patients. Further studies are needed to determine the optimal treatment schedule, the real rates of efficacy before and after splenectomy, the long-term side effects, and the drug's mechanism of action.

1677

SPLENECTOMY IN THE TREATMENT OF IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background. The objective of this study is to report the results of splenectomy in the treatment of idiopathic thrombocytopenic purpura (ITP) in adults. *Materials and methods*: This is a retrospective study between January 1994 and December 2009 in the hematology department of the University Hospital of Sidi Bel Abbes. All patients had ITP and received initial treatment with corticosteroids.In case of steroid resistance or dependence, splenectomy was performed. *Results*. Of the 60 selected ITP, 18 splenectomy were performed (30%) among 15 women and 3 men with a mean age of 33 years. Before splenectomy, the average number of platelets was 34,000 with hemorrhagic manifestations in three quarter of cases. Two weeks after surgery, there was a complete remission (CR) in 13 cases (72%), while partial response (PR) was noted in 3 cases (17%) and failure in 2 (11%). Of the 13 patients in CR, one relapsed after a period of 12 months. The 3 patients with PR have relapsed after a median time of 24 months. Finally 12 patients (66%) are still in CR with a minimum follow-up of 18 months. two factors of response to splenectomy are noted: the age of the patient below 50 years and the initial response to corticosteroids. *Conclusions*. Splenectomy is an important therapeutic modality of ITP with immediate effect, but especially in the long term of at least 60% responses.

1678

NOVEL PLATELET INDICES IN PATIENTS WITH HOMOZYGOUS OR HETEROZYGOUS SICKLE CELL ANEMIA COMPARED TO IRON DEFICIENCY ANEMIA

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Background. Modern hematological analyzers have simplified registration of platelet indices, such as mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) and immature platelet fraction (IPF), which allows further study of these indices in disorders of hemopoiesis. Aims. The aim of this study was to compare the aforementioned platelet indices in patients with iron deficiency anemia and sickle cell anemia. Methods. We retrospectively studied patients with heterozygous sickle cell anemia (n=13, 7 female and 6 male), homozygous sickle cell anemia (n=92, 54 female and 38 male) and iron deficiency anemia (n=42, 26 female and 16 male). Hematological parameters were assessed by SYSMEX XE2100 hematological analyzer. SPSS Statistics for Windows, version 17.0 was used for statistical analysis. The significance level was defined as $p<0.05$. Results. The three patient groups were comparable as for age and sex. Mean PCT value was 0.2592± 0.118% for heterozygotes, 0.4147±0.1211% for homozygotes, and 0.2947±0.12174% for iron deficient patients. Mean PCT value differed

statistically significantly between homozygous and heterozygous sickle cell anemia patients (p<0.001), as well as between homozygous sickle cell anemia and iron deficiency anemia patients (p<0.001). Statistically significant positive correlations between IPF and MPV, as well as IPF and PDW were noted in homozygous sickle cell anemia patients (r=0.687, p<0.001, and r=0.651, p<0.001, respectively) and iron deficiency anemia patients (r=0.758, p<0.001, and r=0.763, p<0.001, respectively). *Conclusions*. Plateletcrit value was significantly higher in patients with homozygous sickle cell anemia, compared with heterozygous and patients with iron deficiency anemia, while no significant difference was observed between heterozygous and iron deficient patients. As for correlations among platelet indices, IPF was positively correlated with platelet indices MPV and PDW in homozygous patients with sickle cell anemia and in patients with iron deficiency anemia.

1679

A STUDY ON PLATELET INDICES IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background. Large platelets are more active and thus more thrombogenic than smaller ones, as they contain more dense granules, they produce greater amounts of vasoactive and prothrombotic factors and present an increased expression of adhesion molecules. Mean platelet volume (MPV) and platelet distribution width (PDW) are indicators of platelet function and activation and MPV has been reported to be influenced inversely by inflammation. Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder with various hematological manifestations. Altered platelet indices have been found in patients with RA and it remains intriguing to investigate the correlation of coagulation and inflammation in RA. Aims: The present study aims to examine the changes in platelet indices (platelet count, MPV and PDW) in patients with RA, and their interaction with biomarkers reflecting the inflammatory response [C-reactive protein (CRP), erythrocyte sedimentation rate (ESR)]. Methods: The study group consisted of 25 RA patients (males/females: 5/20, mean age: 47 years) at various disease phases and 30 age and sex-matched healthy controls. Platelet indices were measured with hematological analyser XE-5000 SYSMEX (ROCHE) with flow cytometry method within 1h of sampling in order to avoid the time-dependent swelling by EDTA. CRP was determined with the use of Immage 800 nephelometer (Beckman Coulter) and ESR with Ves-matic 20 (Diesse) analyser. Student parametric test (t test) and Pearson's correlation were used for the statistical analysis. P<0,05 was considered statistically significant. Results: The laboratory parameters are reported in Table 1. Platelet count, MPV and PDW have been found to be significantly higher (p<0,05) in RA. MPV values above the reference range were detected in the majority of patients tested (76%, 19/25). Platelet count was positively correlated with CRP and ESR in RA patients (p<0,05). The other platelet indices presented a positive but not statistically significant correlation with the examined inflammatory markers.

Table 1. Platelet parameters in RA patients and normal controls.

Laboratory parameters	RA patients (n=25)	Normal controls (n=30)	p-value
Platelet count (PLT. K/μL)	289.93±103.33	249.60±55.98	p<0.05
Mean platelet volume (MPV, fL)	11.15±1.09	9.79±0.44	p<0.05
Platelet distribution width (PDW, fL)	13.8±2.06	12.10±0.80	p<0.05

Summary/Conclusions: In RA patients alterations in platelet indices, indicative of platelet activation, were observed. Only thrombocyte count was correlated with the inflammatory markers tested. The association of platelet routine laboratory parameters with inflammation in patients with autoimmune inflammatory disorders and their clinical utility for the rapid assessment of the disease activity should be further investigated.

1680

SECONDARY POLYCYTHEMIA DURING THE COURSE OF IMMUNE THROMBOCYTOPENIC PURPURA (ITP) TREATMENT WITH ROMIPLOSTIM

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Background: Involvement of thrombopoietin (TPO) on erythropoiesis has been described in the literature (1). Then we can ask whether the polycythemia can occur as a side effect of TPO analogue, as Romiplostim. We report 2 cases of patients treated with Romiplostim and with increased hemoglobin level. Case 1: an asymptomatic thrombocytopenia (5.10^9 /L) found before a surgery lead to a diagnosis of ITP in June 2009, in a 40 years old man. Initial evolution was unfavorable with corticosteroids and intravenous immunoglobulin (IVIG) with the occurrence of relapses requiring repeated courses of IV Ig. Treatment with Danatrol and Rituximab were not efficient. Romiplostim was introduced in December 2009 with a good response obtained at a dosing of 3 mg/kg. In correlation with therapy initiation, appeared polycythemia with a hemoglobin level that reached 180 g / L in few days (13 g / L previously) without organic cause. Dose reduction was performed and provided a decrease on hemoglobin level. Case 2: a 74 years old man with the history of refractory ITP (including splenectomy) diagnosed in 1995, received in November 2009 a treatment with Romiplostim. Polycythemia appeared rapidly with a rise of hemoglobin level to 170g/L (versus 145 g/L before). A stable level of platelets was difficult to obtain requiring multiple variations of doses, which allows us to identify a positive relationship between the dose of Romiplostim and hemoglobin level. Conclusion: An increase in hemoglobin level was found in these 2 patients, and this soon after starting treatment with Romiplostim. This fact can suggest imputability of the drug in polycythemia, excluding other causes. Larger series will be necessary however to confirm this hypothesis.

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1681

PLASMA EXCHANGE IN THE TREATMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA - ONE CENTRE EXPERIENCE

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Background. Plasma exchange (PE) has changed significantly the prognosis of thrombotic thrombocytopenic purpura (TTP), so that remission occurs in more than 80% of patients, as is documented by the literature and our own 30-year experience. In spite of this several details remain to be cleared. *Aims.* We performed a retrospective analysis of therapy results of PE in TTP during the last 10 years when therapy at our workplace had been standardized. Methods: In the years 2000 - 2010 all patients sent for the therapy to our regional centre were evaluated. (The centre serves for a region of about 1.5 million inhabitants). All 40 adult patients (25 females, 15 men, mean age 43, range 20-79 years) had 66 episodes (initial treatment or relapses). PE was performed using Cobe Spectra separator (Caridian, Lakewood, Colorado, USA), software 7.0. Every day one plasma volume was replaced with fresh frozen plasma. ACD-A was used as anticoagulation fluid (Baxter, Munich, Germany). After the rise of thrombocytes above 150x10^9/L, PE was performed in gradually prolonged intervals. *Results:* In all 40 patients where TTP was confirmed (characteristic pentad of symptoms or at least unexplained thrombocytopenia + microangiopathic hemolytic anemia), PE was started within 4 hours. In initial therapy or in relapses 957 PE in total were performed (mean 14.5, range 3-59, median 9). Inherited TTP with

ADAMTS13 deficiency was confirmed in 3 patients, 12 cases were idiopathic, 7 patients had organ malignancies, 9 patients were after stem cell transplantation, myelodysplastic syndrome or myeloproliferative diseases had 3 patients, autoimmune diseases 3 patients, pregnancy 3 patients. Relapses occurred in 9 (22%) patients (mean number of relapses was 3.8, range 2 - 7, median 4). Within 30 days after initiation of PE 3 patients died. PE without effect was in all patients with malignancies and after stem cell transplantation and in 3 patients with idiopathic TTP. All patients received corticosteroids. Splenectomy was performed in 4 patients with relapse. 10.8% of side-effects were mostly allergic, none was clinically serious. Conclusions: Even with improved prognosis TTP remains a serious disease. All patients suspected of TTP should receive urgent PE which in our cohort was successful in 80 % of cases but never in organ malignancies ant after transplantation. PE is the key therapy in TTP - its early performance is a life-saving measure. PE is a safe method in hands of a skilled staff.

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IMPLEMENTATION OF THE NEW HEP SCALE FOR THE EVALUATION OF HEPARIN-INDUCED THROMBOCYTOPENIA

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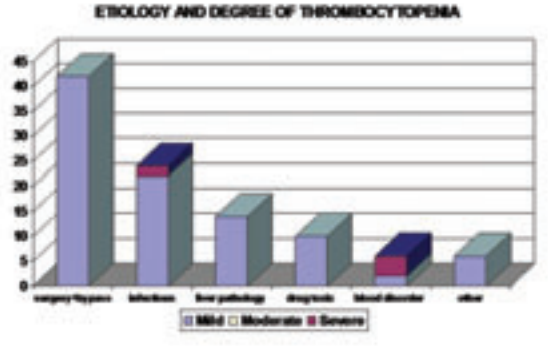
Alterations of hemostasis and blood counts are common in patients admitted to ICU. Thrombocytopenia increases the morbidity and mortality being its aetiology very varied. *Objectives.*: 1) Analyze the incidence of thrombocytopenia in patients admitted to ICU: clinical, laboratory and therapeutic factors. 2) Reclassify the patients undergoing cardiac surgery according to different scales that assess the likelihood of heparin-induced thrombocytopenia (HIT). *Methods and patients*. A retrospective study by reviewing medical records of patients with thrombocytopenia (platelets <125x10^9/L) admitted to four ICUs of our hospital during a 40 days period, classified as mild (100-50x10^9/L), moderate (50-20x10^9/L) and severe (<20x10^9/L). Evaluation of patients undergoing cardiovascular surgery with cardiopulmonary bypass, classifying them according to the new scale presented as pre-test model for Cuker *et al.* and the 4 T's model (Journal of Thrombosis and Haemostasis 2010; 8: 2642-2650) RESULTS: 237 patients admitted during this period, 50 showed thrombocytopenia (21%) Cumulative incidence: 19.8% in 40 days, excluding those who previously had his income. Men 56%, Women 44%, mean age 70.7 (extremes 19-88). Depending on different UCI: Coronary 9 patients, mean age 67.5, cardiovascular 25 patients, mean age 70.0; Polyvalent 11 patients, mean age: 65.8; Traumatology 6 patients, the mean age: 75.6. Reason for admission: 32% valve replacement, infection 14%, ischemic heart disease 14%, respiratory 8% hepatectomy 8%, coronary artery bypass surgery 8%, ruptured aneurysm 4%, bleeding 4%, decreased consciousness 4%, other 6 %. Mild thrombocytopenia: 94%, moderate: 0%, severe: 6%. Nadir figure rating: 90x10^9/L. Average time duration of thrombocytopenia: 4.2 days, median: 2.5 days. Etiology: Image 1. Distribution due to drug: abciximab 8%, tirofiban 2%. Incidence bleeding: 30%, with major bleeding (10%) located in: mediastinal 2%, hematoma in back with 2% drop in hematocrit, HDA 2%, brain 2% pulmonary and 2%, the rest were minor bleeds. Thrombotic phenomenon not observed.



Transfusion: CH 54%, CP 22%. Incidence of coagulopathy: 30%. Regarding the administered treatment: plasma 12%, vit K 8% FVIIr and other measures 2%, Vitamin K and plasma 2%. The attributable mortality: 10 patients (20%). Of 78 patients undergoing cardiac surgery 25 showed thrombocytopenia (32%) and all of them had a low probability of HIT in both the 4 T's scale as in the HEP scale, presenting both a score of <= 3 and <= - 3, respectively. *Conclusions*: 1) Surgery bypass is the most common cause of thrombocytopenia, because of our center nature, followed by infectious causes. 2) In most cases, thrombocytopenia is mild and transient, short-lived, and have not needed treatment. 3) The classification of patients with the proposed scales.

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SERENOA REPENS AS A CAUSE OF DRUG-INDUCED THROMBOCYTOPENIA. APROPOS OF TWO CASES

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Introduction. In the differential diagnosis of isolated thrombocytopenia should be considered drug induced. There are extensive listings of agents with causal relationship to the thrombocytopenia is likely although few are final. The diagnosis can only be done by the recovery of thrombocytopenia after discontinuation of the drug and confirmed his comeback to reintroduce it. Serenoa repens is a plant used for benign prostatic hyperplasia in herbal products and pharmaceutical specialties. CASE 1: 63 year old male visited the emergency department for hematuria, and spontaneous hematoma in the right hemithorax. He reported influenza vaccination for 15 days and a new treatment with an herbal product (Sabal extract) He had platelets/mm3 14000. Studies found no cause of thrombocytopenia. It began as emergency therapy steroids (1 mg / kg / day) and suspended Sabal extract. The patient's platelet count normalized in 7 days without subsequent relapse (follow-up two years). CASE 2: A 48-year-old, came to our Unit with cutaneous purpura mucosa detected platelets/mm3 3000. He complained of recent gastroenteritis and a contact with a young girl of his family suspected of having mononucleosis. He reported no chronic treatment. He started steroids (1 mg / kg / day), reaching normal levels of platelets in 15 days. Serology was found positive for C Hepatitis so he recieved treatment with interferon-ribavirin. Three years later the patient presented again with thrombocytopenia (5000 platelets / mm 3) and petechiae. Had no response to interferon-ribavirin but was stable in the liver. Receiving steroid treatment the platelet count normalized within a week. After insisting on the history the patient reported that days before the first episode had begun treatment with Neo-urgenin® (contains Serenoa repens) He discontinued the treatment on his own soon after starting steroids. Two weeks before the relapse he restarted the treatment because he had prostatic symptoms. *Discussion.*: The present cases suggest that thrombocytopenia in both patientes was induced by Serenoa repens. The drug was started a few days before the clinical detection of bleeding and thrombocytopenia. In both patients (and second in both events) thrombocytopenia disappeared quickly stop taking the drug. Although in the two patients steroids were empirically used that fact does not invalidate the diagnosis. The fact that in the second case to restart treatment with Serenoa repens caused a new episode of thrombocytopenia appears to confirm the involvement of this substance in the genesis of thrombocytopenia.

1684

ACQUIRED INHIBITOR OF FACTOR VIII ASSOCIATED WITH PROSTATE CANCER - A CASE REPORT

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Background. Acquired haemophilia (AH) is rare disease that occurs at a rate of approximately 1.0 per million each year, although it is likely that not all affected patients are reported. It is a severe bleeding diathesis that affects both males and females, caused by sudden appearing autoantibodies that interfere with coagulation factor VIII (FVIII) activity. In the approximately half of patients with inhibitors, no concomitant disease can be found, and in the conditions most commonly associated with factor VIII inhibitors include connective tissue disease, inflammatory bowel disease, some dermatologic disorders and malignancy (lymphoproliterative disorders and solid tumours, e.g. neoplasms of the colon, pancreas, kidney, prostate, testes, brain, and lung). Bleeding manifestations are often severe and may occur spontaneously or after minor trauma. In contrast to patients with congenital factor VIII deficiency those with acquired haemophilia principally experience soft tissue bleeding. Over-

all mortality associated with AH has been reported at between 8% to 42%. The aim. The aim of the presentation is a case of inquired haemophilia in the course of prostate cancer. Case report. A 71 -year-old man presented with sacrospinal, left forearm and brachial muscles non traumatic haematoma and spontaneous hematuria, but otherwise felt well. A coagulation screen was as follows: platelet count 140 G/L (range 150-400 G/l), prothrombin time was normal. Fibrinogen concentration was increased up 0.69 g/L (normal range 0.2-0.45 g/L). There was a prolongation of the activated partial thromboplastin time (aPTT) up to 82.1secs (normal range 26-40 secs), which did not correct following the in vitro addition of normal plasma. Acute disseminated intravascular coagulation (DIC) was excluded. The Factor VIII level was reduced to 0.6% (normal range 50% -150%). The Bethesda assay demonstrates an inhibitor 5.4 Bethesda Units (BU). He was screened for inflammatory and malignant disorder. Prostate specific antigen (PSA) rate was 400 ng/mL (normal range 0-4 ng/mL). Prostate cancer has been confirmed with multiple bone metastases. Treatment was aimed at stopping the acute bleeding, eliminating the inhibitor and curing the underlying disease. The patient was treated symptomatically with rFVIIa (Novo-Seven) 270µg/kg administrated every 3-5 days due to diathesis intensity. Efficacy was judged by decreasing bleeding episodes. Anticancer therapy was ordered by urologist (flutamide and triptoreline). For eradication of FVIII inhibitor after 10 days to that therapy cyclophosphamide 2mg/kg and prednisone 1mg/kg given every day orally was added. The acute bleeding stopped, but despite these therapy two weeks of immunosupressive therapy the FVIII level remained very low (0.1%) and the FVIII inhibitor level increased up to 14.7BU. Haematomas and acute mucosa bleeding reappeared. Bypass therapy with FEIBA 50U/kg administered every 12 h for 10 days had a small clinical effect, but after next three weeks bleeding symptoms diminished, and there was a notable increase in FVIII level up to 1.74% and significant decrease in FVIII inhibitor to 1.8 BU. Summary. In presented patient severe bleeding caused by factor VIII inhibitor associated with prostate cancer was controlled by a complex therapy.

1685

FACTOR XIII DEFICIENCY IN ADULTHOOD - INHERITED OR ACQUIRED?

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The inherited deficiency of factor XIII is very rare (1 case per 2 million population) and is transmitted as an autosomal recessive disease. The clinical manifestations depend on the amount of factor circulating, ranging from severe bleeding since birth to just slow healing of tissues. *Case report*. Male, 40 years old, without previous known diseases, suffered muscle rupture one month before admission, with consequent formation of gluteal hematoma, which kept gradually growing. In this first month he was observed in different hospitals and developed progressive disseminated intravascular coagulation (DIC). He also underwent needle aspiration of the hematoma with subsequent clinical deterioration. At admission in our hospital he was pale but hemodynamically stable, with a right gluteal hematoma extending to the dorsal region and knee. He had anemia (10,5 g/dL), thrombocytopenia (75 000x10^6/L), decreased haptoglobin (<8 mg/dL) and fibrinogen (94 mg/dL), D-dimer increased (12,66 ug/mL) and slightly prolonged PT and aPTT. We then proceeded to the determination of coagulation factors, including the rarest, which showed a deficiency of factor XIII (11%; normal range 75-150%). The patient received transfusion of concentrate of this factor, resulting in reduction of the hematoma, resolution of DIC, and increase of hemoglobin. We were told by the patient that he had slow healing of the skin and small late bleeding since childhood. He was also submitted to an appendectomy four years before, without major bleeding our other problems. In the outpatient clinic he remained without hemorrhage, with factor XIII between 50-70% without transfusion. *Conclusions*. The absence of both consanguinity in the family and prior major bleeding (including during surgery) suggested acquired deficiency. However, primary disease was excluded; also, the good response to the transfusion of the factor (suggesting inexistence of antibodies) and the presence of light but typical symptoms suggests inherited deficiency of factor XIII. In this case the deficiency was aggravated and only expressed in the context of DIC by trauma. The diagnosis of this entity allows the prevention of serious consequences, whether in the acute event or by performing prophylaxis. The transfusion of factor concentrate is effective and safe, without infectious complications.

1686

BLEEDING MAJOR SURVIVAL PREDICTOR IN PATIENTS OF VIRAL HEPATITIS INDUCED ACUTE LIVER FAILURE

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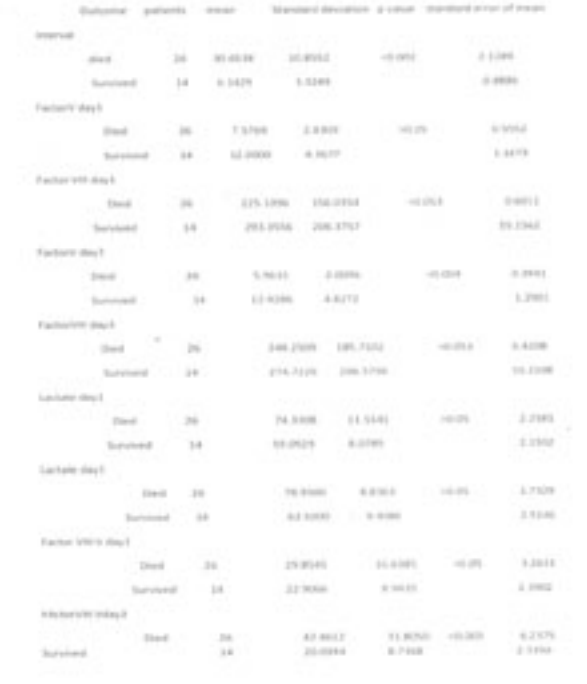
Background. In acute liver failure, variables favoring survival of patients is based on age, interval between onset of jaundice and encephalopathy, coagulation factor level, risk of bleeding but all these variables were concluded from the studies that were conducted in Western population where etiology most commonly is paracetamol poisoning and metabolic disorders like Wilson's disease; where as in Indian subcontinent set up major causative factor is viral hepatitis.



Thus we exploitedthe database collected by us in intensive care unit. 2.Aims-observational study to investigate major predictors of survival in viral hepatitis induced acute liver failure.Methods - observational study of 40 patients followed for 1 month to look for outcome- survival and death(7days) or less and measurement of metabolic parameters like lactate and arterial blood gases and assessment of bleeding risk by carrying out coagulation factor level V which is synthesized in liver and not affected by vitamin K and factor Viii which is not synthesised in liver. . Results-There were 12 patients who survived and had only 0-1 episode of mucosal bleeding. The analysis showed that patients who survived had factor V level on day 3 of diagnosis was in the range of 10%-25% and who died had factor V level below 10% ($p<0.004$)and had bleeding episodes of > 2 Conclusions-Factor V level on day 3 of diagnosis and episode of bleeding may help in predicting survival in patients of acute liver failure, thus help in triage for the need of orthotopic liver transplantation which is the only definite curative therapy in patients who have poor survival rates.

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ASSESSING THE ORAL CONTRACEPTIVES EFFECTS ON THE COAGULATION AND FIBRINOLYSIS

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More than 100 million women in the world make use of hormonal contraceptives and, from these, 93 million use the oral combined hormonal contraceptive. In spite of its desired effect on contraception, its metabolization occurs at liver, estimulating the synthesis of the plasmat-

ic proteins, among them, the ones that control the coagulation system and fibrinolysis. Initially, the estrogen present at the contraceptives was considered the sole responsible for the pro-thrombotic effects of hormonal contraception. However, with the continuous incidence of thrombosis induced by contraceptives containing low-concentrations of estrogen and different progestogens, it was possible observing that the hypercoagulant effect of the contraceptive was not only dose-dependent of estrogen, but also from the antiestrogenic activity of the progesterone used. Independent papers suggest that third and fourth generation progesterones have a smaller antiestrogenic activity in relation to the second generation one. In face of this, the purpose of this work was assessing the occurrence of hemostatic alterations on the Brazilian female population who is user of contraceptives from second and fourth generation. This study contemplated 70 participants from the Health Center School of Ribeirão Preto Medical School - USP and from the Faculty of Pharmaceutical Sciences of Ribeirão Preto - USP. These volunteers have given their consent, on written form, for participating on this study, and they were distributed into four groups: a control one (20 patients) and three oral combined contraceptive ones containing ethinylestradiol 20µg and 30µg combined with 3000 µg drospirenone (16 and 20 patients, respectively) and ethinylestradiol 30µg combined with 150µg Levonorgestrel (14 patients). From these were assessed the following parameters: TP, Factor VII, TTPA, Factor XII, Fibrinogen, Factor 1+2, Protein C, Protein S, Antithrombin, D-dimers and PAI - 1. The contraceptive containg ethinylestradiol 20µg and drospirenone has provoked favourable alterations to the hypercoagulability on the parameters TP, TTPA, Fibrinogen, Protein S and D-Dimers. For the formulation with ethinylestradiol 30µg and drospirenone we've found alterations on the parameters TP, Protein S and PAI - 1. And, for the formulation containing ethinylestradiol 30µg and levonorgestrel we've found alterations on TP and on the Protein C concentration. On this study, the alterations suggest that the fourth generation contraceptive (ethinylestradiol 20µg and drospirenone) is among the assessed formulations, the medicine with the larger number of alterations associated to the hypercoagulability, whereas, the second generation contraceptive (ethinylestradiol 30µg and levonorgestrel) continues being the safest option to avoid the development of thrombosis, since it has presented the least tendency to hypercoagulability, confirming the fact that the pro-thrombotic alterations generated by the contraceptives don't depend strictly from the estrogen dose, but are strongly related to the type of progestagen.

1688

TREATMENT WITH FIBRINOGEN CONCENTRATE IN A PATIENT WITH SEVERE POSTPARTUM HAEMORRAGHE

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Background. Significant blood loss is a common complication of childbirth. Fibrinogen deficiency often results, and is exacerbated by large-volume transfusions of blood products or colloids/crystalloids (dilutional coagulopathy). Substitution with fibrinogen concentrate can correct hypofibrinogenaemia and reverse dilutional coagulopathy, but there are few reports of its use in obstetric hemorrhage. Here, we describe the use of fibrinogen concentrate, along with other haemostatic agents, in the successful management of a severe case of postpartum hemorrhage. *Aims*. to describe the use of fibrinogen concentrate in severe postpartum hemorrhage. Methods: In July 2010, a 32 years old woman, 39 weeks into her first pregnancy was admitted to the obstetrics department due to decreased foetal movements (day 1) and was decided to induce labour on day 2. During labour was determined to perform a ceasarean section because of suspected acute foetal suffering and worsening of foetal bradycardia. In the post surgical unit she presented severe haemorrhage. Ongoing postpartum hemorrhage led to hypovolaemic shock and decrease of haemoglobin (8 g/dL, compared with 12 g/dL pre-admission) and also to hypotension and tachycardia. The patient presented uterin atony without response to conservative treatment, carried out with perfusion of oxytocin and sulprostone, as well as uterine compression. Was submitted to a second surgery, with subtotal hysterectomy and bladder reparation, during which several transfusions were administered: nine units of erythrocyte concentrate, 8 mg of recombinant factor VII, 7 g of fibrinogen concentrate, six units of fresh frozen plasma, one platelet concentrate obtained by aphaeresis and 1000 IU of antithrombin III. During surgery the patient remained hypotense and with conservative diuresis, resulting in transfer to ICU. Sedation was administered and mechanical ventilation was implemented. During the hospitalization coagulation was not compromised in the puncture locals, but there was blood present in gastric drainage and microscopic haematuria was diagnosed. Patient was transfused with two units of erythrocyte concentrate due to anemia, although there were not evidences of haematic loss. Sedation and ventilation were withdrawn and extubation was performed without complications. Diuresis decreased, with some retention of nitrogen, resolved by fluid administration. Results: She was transferred to the obstetrics department (day 3) and was asymptomatic, received no further transfusions, and responded well to iron-saccharate treatment, haemoglobin level increased to 12.6 g/dL. On day 7, patient was discharged in good overall health. Haemodynamic stability was confirmed: blood pressure 100/60 mmHg, pulse rate 80 bpm. Haemostatic tests (prothrombin time, fibrinogen level, platelet count, haemoglobin level) were normal, as were renal function and respiration. In the follow-up, dysfibrinogenemia was accessed and came out negative, the analytical parameters were also normal. Both mother and baby remained healthy upon follow-up at 3, 6, 10 and 15 weeks. Results of laboratory haemostatic tests performed during the patient's hospital stay and follow-up are presented in the table. Summary/Conclusions: The successful outcome of this case can be attributed to effective haemostatic intervention, in particular, administration of fibrinogen concentrate was key to re-establishing haemostasis. Rapid, effective haemostatic therapy is vital for women with severe postpartum haemorrhage.

1689

EVALUATION OF THE ADMINISTRATION EFFICACY OF PROTHROMBIN COMPLEX: RETROSPECTION OF 4 YEARS OF USE IN A PORTUGUESE DISTRICT HOSPITAL

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Background. The human plasma-derived prothrombin complex concentrate containing coagulation factors II, VII, IX, X, Protein C and Protein S is indicated when rapid correction of haemostasis is necessary. It is used in patients with acute haemorrhage, mainly hypocoagulated patients, and in the correction of coagulation parameters in patients with hepatopathy, substituting fresh frozen plasma, as prothrombin complex improves haemostasis in a more rapidly way, without causing circulatory overload. Aims: The aim of this work is to analyse the use of prothrombin complex in several pathologies during four years in a Portuguese district hospital. Methods: We performed a retrospective study of the use of the therapeutic in 172 patients, from 2007 to October 2010, in relation to gender, age, diagnostic, number of vials used, number of administrations, efficiency of the therapeutic and thrombosis occurrence three months after administration. Results: Average age was 67,4 years (15-91 years). Patients were treated in order to correct alterations of coagulation parameters. The first group was constituted by 135 (78,5%) hypocoagulated patients or patients with hepatopathy. From these, 39 (22,7%) were waiting for pre programmed surgery or pre invasive procedures, 39 (22,7%) had digestive bleeding, 23 (13,4%) had brain haemorrhage, 19 (11,0%) had the need of emergent abdominal surgery and 15 (8,7%) had thoracic and abdominal bleeding. The second group comprised 37 (21,5%) patients with coagulopathy: 16 (9,3%) were patients submitted to intestinal neoplasia surgery, 10 (5,8%) had bleeding caused by multiple trauma, 7 (4,1%) had bleeding caused by severe infection, 3 (1,7%) had post surgery uncontrollable hemathuria and 1 (0,6%) had post caesarean bleeding. The average number of vials used per patient was 2,5, each vial corresponding to 500 IU, and the average number of administrations was 1,2 times. The treatment was efficient in 152 (88,3%) patients. The patients that did not respond to the therapeutic died as a result of uncontrollable bleeding, multiorganic failure or fatal brain haemorrhage. Thrombotic events were not found in any patient treated with prothrombin complex after three months of treatment. Summary/Conclusions: Our experience revealed that prothrombin complex has an important role in the correction of coagulation parameters and control of haemorrhagic conditions in a rapid and efficient way, and without occurrence of thrombotic events.

1690

EPIDEMIOLOGY OF BLEEDING DISORDERS IN ZAGAZIG UNIVERSITY CHILDREN HOSPITAL, EGYPT: RETROSPECTIVE ANALYSIS

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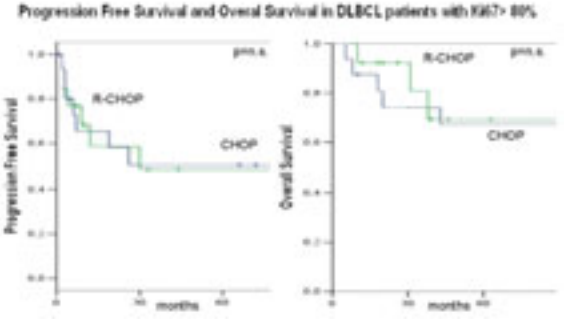
Background : Bleeding defects are of great interest in pediatrics since the prevalence of congenital forms and the early appearance of acquired ones. They have been identified with a heterogeneous group of clinical disease that differs from one another in etiology, pathogenesis, epidemi-

ology and incidence in population. Aims: to describe the epidemiological, laboratory, and clinical data of the various bleeding disorders observed in the Hematology Department of the Zagazig University Children Hospital, as based on retrospective analysis of clinical records between years 2006 and 2010. Patients and methods: The study included 279 children with bleeding disorders registered from 2006 to 2010 in Zagazig University Children Hospital. All cases with history of mucocutaneous bleeding or any other bleeding manifestations were subjected to full history and thorough clinical examination. Hematological profile (platelet count &function, bleeding time, PT, PTT and TT) and specific tests in congenital group (as coagulation factor assay and von willebrand factor assay) were done. Results: In this study bleeding disorders were significantly more common in males than females especially coagulation disorders. Platelets disorders were found in 71.3% of our patients while coagulation disorders in 28.7%. In this study hemophilia A was the commonest coagulation disorders (58.7%) followed by hemophilia B (20 %). Mild presentation represents the majority in 70% followed by moderate severity in 23.75 %. We reported 2 cases of mild hemophilia A in females (6.9%) in our study. The initial clinical presentation of coagulation disorders was not significantly affected by sex or severity. The commonest complication of coagulation disorders found was hemarthrosis. ITP was the commonest platelet disorders in our locality followed by aplastic anemia (45.9%, 22.6% respectively), 74.2% of patients with ITP were acute and 25.8% were chronic. The 5 years survival rate among patients with aplastic anemia was 69.3%, while the mortality rate was 31.7%. Conclusion: the most common bleeding disorders in our locality is ITP followed by aplastic anemia and hemophilia A. Attention must be given for early detection and accurate diagnosis of these disorders for appropriate management.



1691

THINK GLOBALLY, ACT LOCALLY - THE MEASURES OF LOCAL BLEEDING CONTROL IN PATIENTS WITH HEMOPHILIA AND INHIBITORS

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Background : By-passing agents made passible many invasive diagnostic or therapeutic procedures, in patients with hemophilia and high titer of inhibitors. But, these patients still have an increased risk of perioperative bleeding. In current literature, importance of the measures of local bleeding control are not enough emphasized. *Aims*: Analysis of our experiances in management of patients with hemophilia and inhibitors undergoing invasive diagnostic and therapeutic procedures. *Methods*: In our institution, since January 1st, 2008. until January 1st 2011, there were 7 invasive procedures in patients with hemophilia and high titer of inhibitors. Two of them were major surgeries (limb amputation due to compatment syndrome and phlegmona, nephrectomy due to renal tumor causing constant macrohematuria) and five minor interventions (3 surgical, mutiple tooth extractions, 1 diagnostic upper endoscopy and 1 upper endoscopy with hemoclipping and applicaton of fibrin glue for rare bleeding gastric Dieulafoy lesion). We used recombinant factor VIIa (Novoseven) or activated prothrombin complex (FEIBA). Former was used in dosis of 90-270 mikrogr/BM every 2 hours, with gradual prolongation of intervals between doses and later was applied in doses of 50 IU per kg/BM every 6, 8 or 12 hours. *Results*: Both by-passing agents showed excellent efficacy, but local measures of bleeding control (fibrin glue, hemocliping, compressive packing and suturae) were very important. Only after nephrectomy, not a single measure of local hemostasis was performed, due to large bleeding surface. As a result we had single significant bleeding. Bleeding was stopped when the renal lodge was filled with blood, and pressures were equalized. Later, that blood collection was transformed into the pseudotumor, still shrinking one year after intervention. All our patients had improved performance status after the invasive treatment. *Conclusions*: Both by-passing agents showed great efficacy, but mesures of local bleeding contol seems to be equaly important.

1692

INTRAMURAL HEMATOMA OF THE SMALL INTESTINE UNDER THE PROPHYLAXIS

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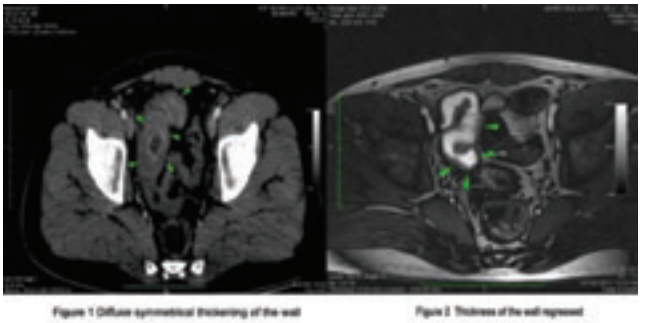
Intramural hematoma of the small intestine is a rare clinical condition. Spontaneous hematoma of the small intestine is seen in the warfarin overdose, and rarely in Hemophilia, ITP, vasculitis and malignancy. In this report, we will present a patient with Hemophilia developing small intestineal hematoma. A 25-year-old male patient who had been diagnosed since one year old as severe hemophilia was admitted to the emergency room with abdominal pain. He had a slight stomachache after meals for the last 2-3 days. A day before his admission, his abdominal pain got severed. After dinner on that day, he vomited. He was not suffering from fever, diarrhea or constipation. According to the laboratory results, The laboratory examination findings were as follows: white cell count: 10,500 (3,700-9,700), Hemoglobin: 15.9 (13.3-17,9) gr/dL, platelets: L, the level of Factor VIII: 1%, stool occult blood: positive,? 250,000 / abdominal X-ray and abdominal ultrasound were normal.Magnetic resonance imaging (MRI) of terminal ileum was also normal; there was a 1 cm diffuse symmetrical thickening of the wall in a segment of about 15 cm long (Figure 1). Intestinal hematoma was diagnosed. Factor VIII concentrate was started. Dose was calculated for to raise factor level to %40. After bolus dose of Factor, the level was checked as 55%. The patient's symptoms decreased within 2-3 days. A week later, MRI was taken again and in the ileum showed that the thickness of the wall regressed (Figure 2). Control stool occult blood was negative. The incidence of the intestinal hematoma in patients with hemophilia is not known. By the use of tomography, imaging the number of diagnosed cases has increased. Treatment is usually conservative. Surgery is not required. Bleeding can be stopped with a strong factor replacement. Hemophilia patients with developed intestinal hematoma have been reported in the literature. We have presented a case that developed intestinal hematoma under the prophylaxis. Unfortunately, the reason for health insurance system in Turkey, our prophylaxis doses are limited with 4500 Units in a week. This dose is not reaching to doses recommending, especially in adult patient. He was taking 1000 Units three times in a week. Nevertheless, the bleeding episodes in joints were controlled with this dose. He started to study in court as archive employees since last year. There was hard working such as carrying heavy files in his recent history, especially last week. This experience reminded us, prophylaxis doses should be reevaluated in hemophilia patients regularly and abdominal pain can be herald of the life threatening bleeding.



1693

INTEGRATED POSTURAL ANALYSIS IN CHILDREN WITH HEMOPHILIA: A COMPARATIVE STUDY

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Background. integrated postural analysis represents a new approach to assess the presence of musculoskeletal alterations in children with

haemophilia. *Aim*: To applicate the postural analysis in haemophilic children. *Methods*: children with severe haemophilia were evaluate for postural analysis methods and compared to healthy children: 1. Test of postural tone (PT); 2. antero-posterior equilibrium test (APE); 3. latero-lateral equilibrium test (LLE); 4. podalic support test. RESULTS: forty children, aged 5 to 17 years (median 12) with haemophilia A or B (36 and 4 respectively), of whom 21 severe, 8 moderate e 11 mild were evaluated. They were compared to 40 healthy, age-matched, male children (median 10 years, range: 5-17). Children with haemophilia showed a higher prevalence of multiple disharmonies compared to controls at PT test (60% vs 22%, respectively, p<0.002), and more frequent abnormalities at LLE test (p<0.006). No difference where found at APE test, while more frequent podalic support abnormalities in children with haemophilia did not achieve a statistical significance level (p=0.08). *Conclusions.* : integrated postural analysis can represent an useful instrument in the management of musculoskeletal health of children with haemophilia.

1694

ACQUIRED LOSS OF THE LARGEST VON WILLEBRAND FACTOR MULTIMERS IN SEVERE TETRALOGY OF FALLOT: A CASE REPORT

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Background. Congenital heart defects can be associated with loss of high-molecular-weight von Willebrand factor (VWF) multimers, potentially leading to severe bleeding complications.Case report. We describe a 3-month-old patient diagnosed with tetralogy of Fallot (TF) with atresia of the pulmonary and major aortopulmonary collateral arteries, and right aortic arch, an aberrant right subclavian artery, intrauterine growth retardation, congenital glaucoma and vertebral dysraphism. Before the first surgical correction, the patient underwent ventricular angiography complicated by right femoral artery thrombosis followed by renal hematoma and ischemic stroke, occurring during anticoagulant therapy. Laboratory findings showed prolonged PFA-100 closure times, reduced VWF collagen binding (VWF:CB) to VWF antigen (VWF:Ag) ratio (VWF:CB ratio) (0.53 vs normal >0.75), and the absence of large VWF multimers. All other hemostatic parameters, except for protein C, were normal. The parents revealed no VWF multimer abnormalities, ruling out an inherited VWF defect. First-stage cardiac repair involved systemic-pulmonary shunting with a homograft common iliac artery. On postoperative day 3, the patient had massive bilateral hemothorax with cardiac arrest requiring cardiopulmonary resuscitation. The VWF:CB ratio decreased (0.49) and the large VWF multimers remained absent. There was no laboratory evidence of intravascular coagulation or sepsis. After this first repair procedure, FVIII was 84.21%, the VWF:CB ratio increased (0.73) and the VWF multimer pattern improved to some degree, but PFA-100 closure time was yet prolonged (>300 sec). The patient underwent a new ventricular angiography and subsequent second-stage cardiac repair involving ventricular septal defect patch closure and right ventricular to pulmonary arteries connection. Plasma-derived FVIII concentrates containing VWF (75 UI R:Cof/kg bid - Haemate, CSL Behring Marburg - adjusting dose according to FVIII levels) was administered in peri-operative period; no haemorrhagic complications was recorded during and after surgical treatment. Conclusions. CHD could cause acquired Von-Willebrand syndrome, leading to high haemorrhagic risk. Administration of plasma-derived FVIII concentrates containing VWF prevent hemorrhagic complications.

1695

ORTOPEDIC SURGERY IN PATIENT WITH ACQUIRED INHIBITOR TO FACTOR XII

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Background: Acquired inhibitor to factor XII (FXII) is very rare and predominantly was describAim: The aim of this paper is to present a patient with inhibitor to F XII who should be underwent orthopedic surgery. Methods: A 68 year old woman presented with prolonged activated partial thromboplastin time (aPTT) during a presurgical screening for hip replacement. She had previous history of seronegative rheumatoid arthritis the last 20 years. She was treated with corticosteroids, sulphosalazine and methotrexate. Also, she had one delivery 43 years before, and did not receive any transfusion of blood products so far. Results: Her blood count and biohumoral status were without significant changes, prothrombin time (PT) was in normal range (91%) while aPTT was prolonged (57.5s) and failured to correct (44 s) with normal plasma in 1:1 mixing studies. Level of FXII level was 4% while levels of factors VIII, IX, XI were decreased 41%, 31%, and 31% respectively. Lupus anticoagulans (LA) was excluded with DRVVT test (LA1 44.3s, LA2 41.3 R=1.07). In mixing study with control plasma FXII insufficiently increased from 4% to 28% (control FXII 132%). Inhibitor to FXII detected with Bethesda like method shown level of 1.56 BU. This level of inhibitor correlates with ineffective increase of FXII with control plasma in mixing study. In next step clotting factors assayed at multiple dilutions. Factor XII appeared equally deficient no matter how much the patient plasma is diluted but other factors VIII, IX and XI increased over normal levels. This result strongly confirmed presence of FXII inhibitor. Also, we measured level of FXII in platelet reach plasma (PRP) before and after platelets lisys by freezen and heating. Levels were similar 4% and 6% respectively. All analysis indicated that patient have acquired deficience of FXII due to inhibitors against FXII. Conclusion: Patient received anticoagulant prophylactic therapy during surgery and postoperatively during 35 days with low molecular weight heparin (LMWH) in dosage range for anti-FXa between 0.2 to 0.5. Presence of inhibitor to FXII was not associated with bleeding complication during the surgery and with thromboembolic complications after surgery that was prevented with LMWH posteoperatively.

1696

CHILDHOOD STROKE - LOOKING FOR A CAUSE!

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Childhood stroke is a rare condition that requires etiological investigation. Among its causes we find Rendu-Osler-Weber syndrome. This is manifested as epistaxis, mucocutaneous and gastrointestinal telangiectases, arteriovenous malformations in the pulmonary, cerebral or hepatic circulation, which may trigger thromboembolic events. We report the case of a of 6 years old female child, admitted by change in level of consciousness, chewing movements, fixed eye deviations, focal motor weakness followed by generalized tonic-clonic seizure. Physical examination showed central cyanosis with persistent hypoxemia that was fairly well tolerated. She had a personal history of recurrent spontaneous epistaxis as her father. Her brother also had pulmonary arterio-venous malformations. The MRI showed focal signal change in bilateral frontal and parietal hemispheres, suggesting hypoxia / ischemia in the barrier territory and postictal changes. She was admitted with stroke diagnosis. On laboratory evaluation, iron deficiency anemia, protein C activity, protein S activity, antithrombin III, activated protein C resistance, and homocysteine level were unremarkable. We didn't found any functional heart defect or abnormal vasculature in transthoracic echocardiography. Chest X-ray films revealed an abnormal shadow in right lower lobe. CT scanning and pulmonary angiography showed an arteriovenous malformation of the right lower lobe. Rendu-Osler-Weber syndrome diagnosis was made based on positivity at family and personal history, clinical examination, laboratory and instrumental findings. Despite being one of the rare etiologies of childhood stroke, it should be considered in the differential diagnosis.

1697

TRANSIENT ACQUIRED FACTOR VII DEFICIENCY AFTER LOBECTOMY PERFORMED FOR LUNG METASTATIC ADENOCARCINOMA: A CASE REPORT

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A transient isolated deficiency of factor VII (FVII) is uncommon but has been described in association with infection, malignancy or inhibitor. Proposed mechanisms include proteases of neutrophils and macrophages and consumption associated with endothelial injury in sepsis; release of tissue factor (TF) from tumor; fixation of FVII to the tumor; increased concentration of tissue factor plasmatic inhibitor (TFPI) produced by tumor and complex binding FT-FVIIa-TFPI; specific inhibitor (autoimmunity). We report a case the taking in charge of a patient with acquired FVII deficiency in the context of lobectomy for lung metastatic adenocarcinoma. The possible mechanism of FVII deficiency are discussed.

This reports a case of a patient with acquired FVII deficiency in the context of lobectomy for lung metastatic adenocarcinoma. Preoperative coagulation studies were normal and there was no personal or family history of a bleeding diathesis. Post-operative pulmonary infection with systemic sepsis occurred (procalcitonin: 3.72 ng/mL - N < 0.05 ng/mL, detection of Enterobacter aerogenes in bronchial aspiration). This sepsis was controlled by antibiotics including amoxicillin/clavulanic acid and levofloxacin. Two days after surgery, haemostasis tests showed prolonged prothrombin time corrected by mixing test. Further investigations brought out isolated FVII deficiency. Bethesda test to assess inhibitor activity was negative. Plasma levels of liver enzymes were normal and vitamin K administration did not normalize the prothrombin time. To prevent bleeding during chest drains removal, the patient received recombinant activated FVII (eptacog-alpha, 1mg, 13.8 µg/Kg). Calibrated Automated Thrombinography (Diagnostica Stago) assay showed a longer lag time than plasma control and injection of recombinant activated FVII shortened the lag time without changing endogenous thrombin potential. Systematic biological control revealed a spontaneous normalization of coagulation tests in five days. This case report displays a conjunction of several possible aetiologies of transient acquired FVII deficiency, namely sepsis, malignancy and FVII inhibitor. It should be noted the presence of lung metastatic adenocarcinoma did not lead to FVII deficiency before surgery. With the Bethesda test, we could exclude the appearance of a FVII inhibitor. Although it was rapidly controlled by antibiotics, sepsis could have lead to a secretion of granulocyte-associated proteases that cleave FVII and to an overconsumption of FVII consecutive to damaged vascular endothelium. Finally, the transient acquired FVII deficiency could be linked to an increased FVII clearance by circulating tissue factor or tissue factor pathway inhibitor released during tumor resection surgery.

1698

FACTOR XI DEFICIENCY EPIDEMIOLOGY IN IRAN

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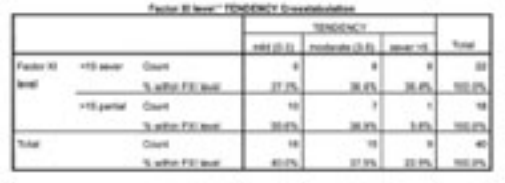
Background. Factor XI deficiency categorized as a rare bleeding disorders with several clinical manifestation in patients. *Aims*. This review gives an overview of the epidemiology, clinical manifestations, and bleeding severity in Iranian factor XI deficiency patients. The correlation between FXI levels and the bleeding tendency is much less clear than in the hemophilias, and consequently the bleeding risk can be difficult to predict. *Methods*. In this study 42 patients with factor XI deficiency were interviewed and a bleeding history questionnaire completed at Comprehensive Hemophilia clinic of Iran. Blood was taken for coagulation assays. The questionnaires contain personal information, clinical manifestation, bleeding tendency, family history, and factor XI level. Ordinal logistic regression with logit link function was used to predict bleeding tendency using FXI levels.



Results. 52.4% and 47.6% of patients were male and female respectively. Mean (±SD) age of patients was 26.38 ±16.51 years. 40.5% (n=17) patients have mild bleeding tendency. 38.1%(n= 16) patients have moderate tendency and the other have (n=9) severe bleeding tendency. 22 (52.4) of our patients have severe factor xi deficiency and 42.9 % have partial factor xi deficiency. There was negative correlation between bleeding tendency and factor XI level (r=-.37,P=0.018). In the patients with factor XI level less than 15u/dl, 27.3% were in mild tendency category versus it was 55.6% in patients with factor XI more than 15. Ordinal logistic regression with logit link function show that factor XI was a predictor variable for bleeding tendency (P=. 021). *Summary*. In contrast of our previous study and other research we find out a predictor variable of factor XI for bleeding tendency in mild and moderate factor XI deficiency patients with this method of data analysis. We need to do this research in a larger group to establish the correlation of factor XI level and bleeding severity.

1699

PFA100 TEST AND FUNCTIONAL VON WILLEBRAND FACTOR UTILITY AS A SCREENING METHODS FOR TYPE-1 VON WILLEBRAND DISEASE DIAGNOSIS

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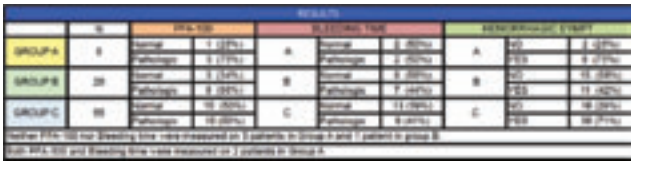
Background. Type 1 Von Willebrand disease (VWD) is the most frequently diagnosed congenital coagulopathy. It follows an autosomal-dominant pattern of inheritance. In Type 1 VWD, Von Willebrand Factor levels are quantitatively decreased (10-50 U/dL) making these patients more proned to hemorrhage. Nevertheless, the misknowledge of the association between bleeding probability and Von Willebrand Factor levels shows heterogeneous plasmatic concentration of FVW among population. The cut-off point between normal and low Von Willebrand Factor levels is not well defined and this difficults Type 1 VWD diagnosis. PFA-100 test is a convenient tool for measuring 'In Vitro' platelet aggregation with Collagen/Epinephrine (COL/EPI) or Collagen/ADP (COL/ADP). AIMS. The objective is to evaluate PFA-100 test sensitivity at different Functional-VWF (F-VWF) plasmatic concentrations and its association with hemorrhagic symptoms. *Methods.* Family history of hemorrhagic diathesis, hemorrhagic symptoms and plasmatic levels of Von Willebrand Antigen and F-VWF were studied in 89 patients with clinical suspicion of Type 1 VWD. 43 patients were evaluated with PFA100 test (COL/EPI and COL/ADP), 40 were evaluated using bleeding time and in 6 patients no test was performed. According to the F-VWF plasmatic concentration 3 patient groups were established: Group A: F-VWF<25% (8), B: F-VWF 25-40% (26), C: F-VWF 40-50% (55). PFA100 Closure Time (CT) and bleeding time (Simplate IIR) was evaluated in each group (CT COL-EPI normal≤155", CT COL-ADP normal≤110", Simplate IIR normal <8.5 min). *Results.* PFA-100 test was pathologIcal in 75% of patients showing F-VWF plasmatic concentration <25% whereas Bleeding time was prolonged in 50% of patients with F-VWF<25%. With higher plasmatic concentrations of F-VWF (25-40% and 40-50%) PFA100 test loses sensitivity. 75% of patients in Group A (6/8), 42% in Group B (11/26) and 71% in Group C (39/16) presented hemorrhagic symptoms non related with F-VWF plasmatic concentrations. *Conclusions.* PFA100 test sensitivity changes depending on F-VWF plasmatic concentrations, what does not make PFA100 useful as a single screening method for Type 1 VWD.



1700

FACTOR V LEIDEN IS THE MAIN ETIOLOGICAL FACTOR IN EGYPTIAN PATIENTS WITH BUDD-CHIARI SYNDROME

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Background. Budd-Chiari syndrome is a multifactorial disease where several prothrombotic disorders must concur for the development of thrombosis at this uncommon location. The prevalence and the interaction between genetic and acquired risk factors are variable in different parts of the world. Aims: To assess the prevalence and characteristics of inherited thrombophilia in Egyptian patients with Budd-Chiari syndrome. *Methods*. The study included 47 (20 children and 27 adults) patients with Budd-Chiari syndrome. Genotyping of factor V Leiden G1691A, Prothrombin G20210A and Methyltetrahydrofolate reductase C677T were performed by real time PCR using fluorescence melting curve detection analysis by means of the Light Cycler System. *Results*. Factor V Leiden was present in 28 patients (59.6%) which was signifi-

cantly higher compared to previous reports. It could be the sole factor causing Budd-Chiari syndrome in 19 patients and in 5 patients with inferior vena cava involvement. Myeloproliferative disease was present in only 10 (21.3%), antiphospholipid syndrome in 5 (10.6%) and Behcet disease in 3 patients (6.4%). Interestingly, 3 children with lipid storage disease had Budd-Chiari syndrome. *Conclusions*: Factor V Leiden could be considered as the main etiological factor for Budd-Chiari syndrome in Egyptian patients which could be attributed to the geographical distribution of this mutation in this area. Its sole presence could be considered as a strong thrombophilic factor and a leading cause of inferior vena cava thrombosis in these patients. Lipid storage disease should be included as a risk factor for this disease.

1701

STUDY OF URINARY 11-DEHYDRO-THROMBOXANE B2 AS A MARKER OF THROMBOEMBOLIC EVENTS IN BETA-THALASSEMIA PATIENTS

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Introduction: There is increasing evidence that chronic hemolytic anemia such as β thalassemia (βT) is characterized by a hypercoagulable state. In addition to increased thrombin and fibrin generation, increased tissue factor activity, and increased platelet activation, patients with hemolytic anemia manifest thrombotic complications, including venous thromboembolism and stroke. *Aims:* To evaluate the role of platelets activation by measuring the urinary level of 11-dehydro-thromboxane B2 as a marker of thromboembolic events in βT patients. *Subjects:* This study was carried out in at thalassemia outpatient clinic at Zagazig university hospitals. The study included a total number of 50 patients and 20 healthy controls, classified into: Group I: Included 20 healthy and haematologically normal subjects as a control group. Group II: Included 20 non-splenectomized β-thalassemia major (NSβTM) patients. Group III: Included 15 β-thalassemia intermedia (NSβTI) patients. Group IV: Included 15 splenectomized β-thalassemia major (SβTM) patients. *Methods:* All the patients and controls were subjected to the following: history taking and clinical examination, hemoglobin electrophoresis, prothrombin time (PT), Partial thromboplastin time (PTT), urine creatinine, D-dime, fibrinogen, brain MRI and Doppler ultrasound on the abdomen. The estimation of 11 dehydro-thromboxane B2 level was done by ELISA from DRG international company provided by clinilab and the results were reported as pg urinary 11dhydroTxB2 per ml urinary creatinine in order to normalize results for the concentration of the urine sample tested. *Results:* In this study Urinary 11 dehydroTXB2 pg/mg creatinine revealed very high significant increase in the all groups compared to that of the control group. In addition, there were significant difference between the patients group II and group III and IV ($P<0.05$), while there were no significant increase between group III & IV (βTI and SβTM), $P>0.05$.There was strong positive correlation in this study between 11 dehydroTXB2 and D-dimer level in groups II, III and IV. On the other hand there were strong negative correlation between 11 dehydroTXB2 and fibrinogen level in groups II, III and IV. In this results, no significant correlations were found between platelets counts and hypercoagulability markers (11 dehydroTXB2, D-dimer and fibrinogen) in all studied groups ($P>0.05$). A strong inverse correlation was found between HB F and both 11 dehydro TXB2 and D dimer in group II, III and IV. *Conclusion:* The elevated urinary 11-dehydro-TXB2 level can be used a marker of hypercoagulable state even in clinical asymptomatic thalassemic patients.

1702

RETROSPECTIVE ASSESSMENT OF RISK FACTORS FOR VTE IN ADOLESCENTS OF SOUTH MORAVIAN REGION, CZECH REPUBLIC WITHIN YEARS 2004-2010

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Background. Venous thromboembolism (VTE) is multifactorial disease. There are many known risk factors (RF) for VTE, both acquired and inherited. VTE is relatively infrequent in children and adolescents compared to adults. *Aims*. To analyse RF of VTE in adolescents. *Methods*. We retrospectively analysed RF for VTE in consecutive adolescent patients treated in regional referral centre for their first VTE episode in years 2004-2010. The patients were 14 to 18 years old. Analysed parameters were as follows: age; sex; thrombus location; pulmonary embolism (PE) and respective RF- both inherited (antithrombin (AT) deficiency; protein C (PC) deficiency; protein S (PS) deficiency; FV Leiden mutation (FVL); prothrombin gene mutation (FII G202010A); high FVIII levels; protein C global test (PCG) positivity and family history positive for VTE) and acquired (obesity, smoking, lupus anticoagulant, surgery, knee arthroscopy, immobilisation, pregnancy, delivery, abortion, autoimmune disease and oestrogen-containing oral contraception (COC)). We excluded central venous line (CVL) related VTEs as well as other non-VTE thrombotic events from our cohort. *Results:* Seventy-two consecutive patients were analysed for their first VTE (46 female (63,9%) and 26 male (36,1%)). Median of their age was 16 years, ranging from 14 to 18. Thrombus location in this cohort of patients was as follows: DVT of lower extremities and pelvis 56 (77,8%), DVT of upper extremities 8 (11,1%), one (1,4%) thrombosis of hepatic veins, one (1,4%) thrombosis of internal jugular vein and 2 (2,8%) thrombosis of cerebral veins. In 4 (5,6%) patients with PE we were not able to identify the location of primary thrombosis. The total number of patients with PE was 8 (11,1%). One patient died due to PE. Prevalence of respective RF was: positive family history 11 (15,3 %), FVL 20 (27,8%), FII G20210A 4 (5,6%), deficiency of AT 3 (4,2%), PC 3 (4,4%), PS 6 (8,8%); isolated positivity of PCG 11 (15,7%), FVIII high 20(43,5%), vessel pathology 3 (4,2%), obesity 2 (2,8%), smoking 6 (8,3%), knee arthroscopy 7 (9,7%), LA positivity 5 (7,2%), other acquired RF 36 (50%), COC 30 (47,6% from all, 65,2% of girls). In median, our patients had 2 RF (range 0-6 RF/patient). Only one patient (1,4%) was free of any known RF. Twelve patients (16,7%) hade only inherited RF, 23 (31,9%) had only acquired RF and 36 (50%) of patients in our cohort had combination of both inherited and acquired risks. We estimated the overall incidence of DVT (non CVL related) in age group of 14 to 17 years old adolescents in our region to be minimally 16/100000/year (2004-10); in females minimally 21/100000/year and in males 10/100000/year. *Conclusions*. Incidence of VTE in our cohort is relatively high (compared to published data), higher then that in children and closer to that in adults. VTE form thus a real threat for Czech adolescents. Less then 20% VTE episodes in our cohort were idiopathic/unprovoked. Prevention of VTE is necessary in this age group, and should be based mainly on recommendations for adults. Further prospective studies are needed to confirm our findings on a national-wide basis.

1703

POSSIBLE INFLUENCE OF ACQUIRED AND INHERITED THROMBOFILIC DISORDERS IN CHILDREN AND ADOLESCENTS WITH EXTRAHEPATIC PORTAL VEIN THROMBOSIS

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Aims. Aim of this study is to explore the prevalence of local and genetic thrombophilic disorders as risk factors for portal vein thrombosis (PVT) in our pediatric series. *Methods*: We conducted a case-control study enrolling 35 children with PVT and 26 age-matched controls. All were screened for thrombophilia, including genetic disorders, protein C, protein S and homocysteine deficiencies. All coagulation parameters were studied at least 3 mo after the diagnosis of portal vein obstruction. *Results.* In our study we showed that most pediatric patients with PVT have acquired prothrombotic risk factors, which are probably the most important factors leading to PVT. However, there is a clear association between the presence of inherited prothrombotic disorders and PVT, suggesting that these increase the risk of thrombosis in patients with local factors such as perinatal umbilical vein catheterization or sepsis. *Conclusions.* This series is the larger ever published so far. This study show that most patients with pediatric PVT have a history of a local prothrombotic factor, a figure much higher than that reported for adult portal vein thrombosis (around 30%). Patients with PVT should be screened for inherited prothrombotic disorders regardless of a history of an obvious local risk factor.

1704

SETTING A TARGET INTERNATIONAL NORMALIZED RATIO RANGE DOESN'T GUARANTEE GOOD ACHIEVEMENT OF THAT RANGE-A STUDY IN OMANI PATIENTS TAKING WARFARIN

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Background. Warfarin is a worldwide oral anticoagulant commonly used to prevent thrombosis, and international normalized ratio (INR) is used to monitor the dose of warfarin. INR is usually set either in the range of 2-3 or 2.5-3.5 dependent on the underlying disorders. Aims: To know the actual achievement of target INR in two groups of patients whose INR ranges were set either in 2-3 or 2.5-3.5. Methods: From September 2010 to January 2011, we consecutively enrolled 113 patients who were taking warfarin because of thrombosis, atrial fibrillation or mechanical heart valves (MHVs) and were regularly followed up in our specialist clinics. We reviewed their history, recorded the diagnosis, INR data and warfarin dosages. Two groups of patients were divided, Group A included patients with MHVs, the target INR range was set into 2.5-3.5; Group B included patients other than MHVs, and the target INR range was set into 2-3. We compared the percentage of time which was within the target INR ranges in these two groups. Results: Totally 113 patients, with 1976 results of INR were enrolled. The total follow-up period was 188.4 patient-years. Group A included 25 patients, their mean age was 43.4 ± 16.0 years, mean INR was 2.96±1.61, and mean warfarin dose was 4.69±2.03 mg. Group B included 88 patients with mean age 52.7±16.7 years which was significantly older than that in Group A (p=0.007). Their mean INR±SD was 2.67±2.16, which was significantly lower than that of Group A (p=0.001); their mean warfarin dose ±SD was 4.80±2.44 mg, which was not different from that of Group A (p=0.2155). 154 (32.8%) INRs were within the target range of 2.5-3.5 in Group A, whereas 533 (35.4%) INRs were within the target range of 2-3 in Group B. Interestingly, although the target INR in Group A was set in the range of 2.5 to 3.5, 187 (39.8%) INRs were found in the range of 2-3, which was more than those in the range of 2.5 to 3.5 (39.8% vs 32.8%) in the same group. Moreover, the percentage of INR range from 2-3 in Group A, which was originally set with INR range of 2.5-3.5, was more than the percentage found in Group B (39.8% vs 35.4%) whose INR range was initially set in 2-3. Thus, although the mean of INR in Group A was significantly higher than that in Group B, the percentage of INR ranging from 2-3 was higher in Group A than in Group B. i.e. the original setting of INR target with frequent monitoring could not result in a higher rate of that target. *Conclusions*. Simply setting the INR range and frequently monitoring INR by laboratory tests in a specialist clinic is not enough to achieve good INR targeting. Many other causes would influence the achievement of the target INR. As this might be a common phenomenon, attention to this problem with more studies to find out a way to solve this problem is urgently necessary.

1705

A COMPARATIVE EVALUATION OF SERUM FERRITIN LEVELS IN PATIENTS WITH DIABETES MELLITUS AND CHRONIC RENAL DISEASE WITH AND WITHOUT DEFECTS OF DIABETIC RETINOPATHY

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Background. It has been hypothesized that the diabetes mellitus type 2 related microangiopathy may be associated with elevated absortion and storage of dietery iron represented by increased serum ferritin levels. Aims: To comparatively evaluate the levels of serum ferritin in diabetic patients with and without diabetic retinopathy lesions and concomitant renal disease. *Methods*: 53 patients (34 male and 19 female) with a mean age of 70.45 years (SD 8.21) participated in the study. All suffered from type 2 diabetes mellitus and had chronic renal disease. 31 had no lesions (group I) and 22 had lesions of diabetic retinopathy (group II) during fundus examination. The mean duration of diabetes was 9 years (SD 4.67). 21 healthy controls (11 male and 10 female) with a mean age of 71.28 years (SD 3.37) also participated in the study (group III). The serum ferritin levels were measured in the three groups and were comparatively evaluated. *Results*: Mean serum ferritin values in group I were 146.69 ng/ml, in group II 131.9 ng/ml and in the group III 48.59 ng/ml. There was a significant difference in the values of serum ferritin between the diabetic patients with and without diabetic retinopathy lesions and the healthy controls (p=0.010 and p=0.006 respectively), while no significant difference in the values between the two groups of diabetic patients was found (p=0.704). Moreover, there was no significant correlation between ferritin values and the values of HbA1c in both groups of diabetic patients (p=0.365 and p=0.941 respectively). *Summary/conclusions*: The results show that the levels of iron in diabetic patients may play a role in the occurrence of microangiopathy or its evolution.

1706

WARFARIN LOADING & DOSING AT A DISTRICT GENERAL HOSPITAL

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Background. Warfarin is a Vitamin K antagonist. It is a synthetic derivative of dicoumarol, a fermentation product of coumarin. In 1940 dicoumoral was isolated at University of Wisconsin. It was the first drug in its class, patented in 1941. Warfarin is a combination of Wisconsin Alumni Research Foundation (W.A.R.F.) and -arin indicating the link with coumarin. Warfarin was first registered for use as a rodenticide in the US in 1948. In 1954 Warfarin was first approved for medical use for humans as an anticoagulent. International Normalized Ratio (INR), is a system established by the W.H.O. and the International Committee on Thrombosis and Haemostasis for reporting the results of blood coagulation tests. Objectives. The goal of this audit was to look at the Warfarin loading and dosing regimes of patients at our institution between January 2009 and January 2010. We wished to compare our findings to the National standards, and provide evidence for the development of better standards of INR monitoring especially during the loading phase. Our standards included :- 1) All patients loaded on Warfarin should reach their target INR with in 5 days of starting, as in keeping with Fennerty et al. findings. 2) INR checked daily during loading process. 3) Warfarin loaded according to hospital protocol. Methods. A Retrospective audit measuring against the BCSH and local guidelines was performed. The inclusion criteria for this audit required patients to be loaded on Warfarin during their inpatient stay in the year 2009. Based on this criterion, a number of elements were extrapolated, including: - Age/gender ratio, reason for admission, reason for Warfarin loading, Anticoagulation prior to admission, INR on admission, INR at first loading dose of Warfarin, INR checked daily, number of days to target INR, loading dose, INR checked daily, whether patient was on Heparin during Warfarin loading, are referral letters to the anticoagulation clinic present, and if the referral letters to the anticoagulation clinic complete.

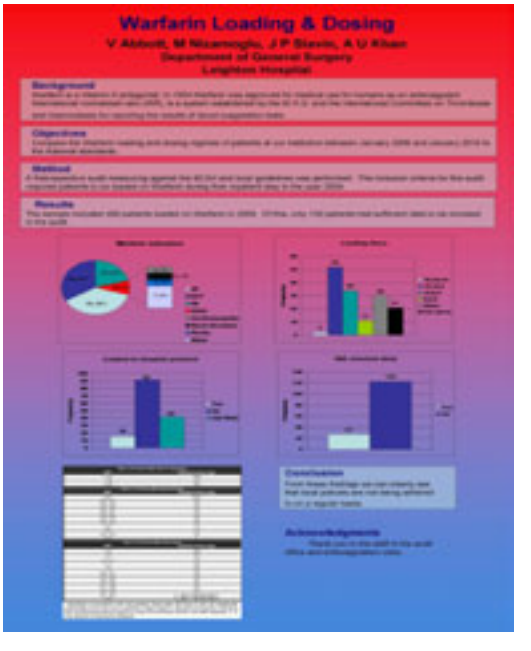

Figure 1. Warfarin loading and dosing poster.

Results. The sample included 400 patients loaded on Warfarin in 2009. Only 150 patients had sufficient data to be included in the audit. Of this

reduced sample, 41% reached their target INR with in 5 days. 18% of patients had an INR check on a daily basis. 11% of patients were loaded according to the hospital protocol that is based on the Fennerty et al study presented in the BMJ in 1984. *Conclusions*. Based on the findings of this audit, recommendations have been made including : (i) It is essential to implement loading protocols that all staff adhere to for general and vulnerable patient groups. (ii) INR should be checked daily, with all results and doses logged appropriately at the loading stage. (iii) Referral forms must be completed correctly; with dosing record should state clearly day 1 and (iv) Finger-prick INR to be introduced to enable daily easily implemented INR checks by nursing staff on a daily basis.

1707

PROLONGED CLINICAL OBSERVATION OF A GROUP OF PATIENTS WITH ASYMPTOMATIC ANTIPHOSPOLIPID ANTIBODIES

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Antiphospholipid antibodies (APA) are well known risk factor for thromboembolic events and/or obstetric complications. They may be found incidentally in patients without thrombotic complications (asymptomatic APA), and they often lead to an incorrect suspicion of hemorrhagic diathesis, as well as to an unnecessary disqualification from surgical procedures or withdrawal from a proper antithrombotic prophylaxis during these procedures. The aim of the study was to register venous and/or thrombotic events in a group of patients with asymptomatic APA, diagnosed according to the international guidelines (Myakis et all. 2006). The study group consisted of 25 patients (18 women and 7 men) of the mean age of 46 years (20 - 75 years). Concomitantly 9 of them had other autoimmune disorders and 4 had neoplasms. Among risk factors for arterial thrombosis 5 patients had hypercholesterolemia, 5 - hypertension, 4 were smokers and 4 were obese (BMI >30 kg/m2). None of the patients had hereditary thrombophilia (antithrombin, protein C or protein S deficiency, factor V Leiden, prothrombin G20210A mutation, increased activity of factor VIII). Family history of venous thromboembolic disease has been noticed in 4 patients and of arterial thrombosis in 6 patients. The observation lasted for 3 to 127 months (mean 35 months). The number and percent of patients (n = 25) with abnormal results of different laboratory diagnostic assays for APA: Assessment aPTT- n(%) dRVVT-n(%) ACA-n(%) anti- β2-GPI-n(%) initial 24 (96) 23 (92) 11 (44) 10 (40) after 12 weeks 24 (96) 22 (88) 9 (36) 11 (44) aPTT - activated partial thromboplastin time; dRVVT- diluted Russell viper venom time, ACA - anticardiolipin antibodies, anti-β2-GPI - anti- β2-glycoprotein I antibodies; Abnormal results of more than one assay in any combination were found in 16 patients, lupus anticoagulant alone in 9 patients. Only 4 out of 25 patients have taken aspirin - 75mg daily, in the other 4 a prophylactic dose of low molecular weight heparin was administered temporarily because of surgery. During observation time no venous or arterial thrombotic events occurred in the study group. In two patients asymptomatic APA disappeared. Conclusion: independently of the type and quantity of asymptomatic antiphospholipid antibodies, there were no venous or arterial thromboembolic events in the group of patients observed for meanly 35 months.

1708

THE INFLUENCE OF ELECTIVE PERCUTANEOUS CORONARY INTERVENTION ON THE PLASMA MICROPARTICLES CONCENTRATION IN PATIENTS WITH CORONARY ARTERY DISEASE

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Background. Mikroparticles (MP) are released into the circulation by cells including platelets, endothelial cells, leukocytes and erythrocytes. They play an important physiological roles (for exaple in coagulation) so are object of scientific interest. Aims. The aim of the study was to measure microparticles (MP) concentration in the plasma of patients (pts) with coronary artery disease (CAD) taking aspirin and clopidogrel, before as well as after elective percutaneous coronary intervention (PCI) with or without stent implantation. *Methods*. The study group consisted of 73 pts (44 men, 29 women) with CAD. During PCI stent implantation has been performed in 32 pts. MP concentration was measured using ZYMOPHEN MP-Activity test before, 24 h and 1 month after PCI. In the control group there were 26 healthy blood donors not taking antiplatelet drugs. Results. MP concentration (nM) in patients before and after PCI is listed in attached table. Result of the control group: 4,6 nM +/- 3,3; VC 72%.

	Patients with stent implantation					
	before stent implantation		24h after stent implantation		1 month after stent implantation	
	men	women	men	women	men	women
cMP[nM]	8,0	5,4	10,4	6,4	6,4	7,3
SD [nM]	4,1	4,2	4,2	3,5	3,2	5,6
VC [%]	51	78	40	54	50	77
	Patients without stent implantation					
	Before PCI		24h after PCI		1 month after PCI	
	men	women	men	women	men	women
cMP[nM]	6,1	5,7	7,7	9,4	7,6	7,9
SD [nM]	2,5	2,4	4,6	7,4	4,9	5,3
VC [%]	42	42	59	79	64	68

Conclusions. 1.Mean microparticles concentration in the plasma of patients with coronary disease taking aspirin and clopidogrel is higher than controls, but SD is high, therefore the difference is not statistically significant. 2.There is an increase of microparticles concentration 24h after percutaneous coronary intervention in women without stent implantation and in men 24h after stent placement.

1709

PREVALENCE, SPECTRUM AND CORRELATION OF LUPUS ANTICOAGULANT POSITIVITY WITH CLINICAL OUTCOME: EXPERIENCE FROM A SINGLE TERTIARY CENTRE IN OMAN

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Background. Lupus anticoagulants [LA] are immunoglobulins which prolong phospholipid-dependent coagulation tests. These inhibitors recognize anionic phospholipid epitopes and prevent or delay the generation phospholipid dependent activations complexes in vitro. However, in vivo, they are associated with endothelial injury that alters the fibrinolytic balance leading to vascular micro-infarcts which clinically manifest as thromboembolism, thrombocytopenia and recurrent pregnancy loss. *Aims*. To study the prevalence and spectrum of antiphospholipid antibody[LA] positivity diagnosed using the 2009 ISTH criteria and correlate it with one of the three clinical outcomes namely thrombosis, thrombocytopenia and recurrent pregnancy loss. 3. Methods: One hundred forty-seven consecutive patients (Males-47; Females-100) with mean age of 30.5 years [SD±17.1; Range 1-82 years] and an abnormal APTT that did not correct on mixing studies were retrospectively analyzed. Blood was collected for a complete blood count and serum and plasma samples were tested for IgG and IgM anticardiolipin[ACA] and anti-beta2-glycoprotein I(GPI) antibodies [by ELISA], along with a panel of Lupus coagulant test including a dilute APTT and a dRVVT followed by mixing and confirmation studies with normal plasma and hexagonal phospholipid reagent respectively. 4. *Results*: Out of 147 cases, 146 cases with a positive LA, 48 (32.9%) had SLE (primary-23,secondary-25); 26(17.8%) had thrombosis(DVT-12,Stroke-11, PE-1,MI-1,Portal vein thrombosis-1); 25(17.1%) had thrombocytopenia; 20(13.7%) had APLS (primary-8,secondary-12), and 13(8.9%) had pregnancy loss (Recurrent miscarraige-11, IUFD-2). Incidentally, one patient had an acquired factor V inhibitor with severe factor V deficiency (FV<1%). The overall prevalence of ACA and anti-beta2 GPI antibodies was 17.1% and 17.8%, whereas, in patients with SLE it was 2 fold higher at 31.3% and 29.2% , but in patients with APLS it was 4-fold higher at 70% each respectively[p<0.05; Chi Square test). Furthermore, there was a good correlation between the ACA and anti-beta 2 GPI antibodies (Pearson's correlation coefficient 'r'=0.55; p<0.05) 5. Conclusions: Antiphospholipid antibodies are heterogeneous, with LA positivity 5-fold more prevalent as compared to ACA or anti-beta 2 GPI antibodies. However, there was a good concordance between ACA and anti-beta 2 GPI antibodies positivity in the LA positive cohort. Furthermore both these antiphospholipid antibodies were equally prevalent in primary v/s secondary SLE and APLS patients.

1710

DETECTION OF MUTATIONS IN THE ATIII GENE IN PATIENTS WITH VTE AND ATIII DEFICIENCY

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Antithrombin III (ATIII) is a 58kd protein synthesized in the liver that belongs to the family of serpins. It inhibits thrombin by binding to it and forming a complex in a stoichiometric 1/1 relationship. The rate of inhibition is greatly accelerated in the presence of heparin (>1000 fold). ATI-II also inhibits the action of factors IXa, Xa, XIa, and XIIa and the TF/VIIa complex. Its deficiency predisposes to recurrent VTE episodes. Normal levels range from 70-120%. It is inherited in an autosomal dominant manner, and has been identified only in the heterozygous state, suggesting that its total absence is incompatible with life. Homozygosity has been described in mutations that involve the heparin binding site. It is found with a frequency of 1% to 2% of VTE patients. The gene, located on chromosome 1q25.1, is 13.5 kb long, consists of 7 exons and encodes for a 432kb amino acid protein. Various mutations have been described, including missense, nonsense, splice-site mutations, frameshifts, insertions and deletions. The functional (ATIII: C, mean 46%, range 19-62%) and antigenic (ATIII:Ag, mean 58%, range 38-113%) levels of ATIII were measured in VTE patients (n=30) with ATI-II deficiency. We present preliminary results of the detection of ATIII mutations. DNA was isolated from whole blood and exons and intron-exon boundaries were amplified. Multiplex PCR was also performed, where the 7 exons of the ATIII gene are simultaneously amplified, along with an internal quality control. All products were analyzed using dHPLC. PCR products were denatured and then gradually frozen to form heterodimers (detection of point mutations, small deletions and insertions) that are separated by the WAVE system. In cases where the chromatograms showed presence of heterodimers, samples were further analyzed by sequencing. The multiplex PCR products (detection of large deletions in heterozygous form that cannot be otherwise detected due to the presence of one normal allele) were also separated by the WAVE system and compared to the chromatograms of healthy subjects. The presence of lower peaks in the patient samples suggested the deletion of exons. Up to date, 10 patients have been examined. 3 were found to have the Budapest III mutation in exon 2 of the gene, leading to substitution of a cytosine by thymine (CTC to TTC), resulting in the substitution of a leucine by a phenylalanine (L131F). This is thought to change the isoelectric point of the protein that perturbs the geometry of the positively charged surface at the heparin binding site resulting in a low affinity for heparin. A frameshift mutation was also identified in exon 2 of one of the patients, as well as point mutations that result in a non-synonymous change of the amino acid and in premature stop codons (exons 1,4,5 and 6 and in particular W189R, Q334X, D342G, R359X). To our knowledge, this is the first documentation of mutations in the ATI-II gene in Greece.

1711

PLASMA COBALAMIN, FOLATE, PYRIDOXINE AND HOMOCYSTEINE LEVELS AND THROMBOSIS IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS AND HEMOLYTIC ANEMIAS

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Background. Hyperhomocysteinemia is a condition that may cause both arterial and venous thrombosis. In the etiology of hyperhomocysteinemia there are a lot of factors including folate, cobalamin and pyridoxine deficiencies. It has been shown that in chronic myeloproliferative neoplasms (MPNs) and chronic hemolytic anemias (CHA), the risk for thrombosis is higher than the normal population and plasma folate and cobalamin levels are significantly lower than the normal individuals. However there are small number of studies investigating the homocysteine (Hcy) levels in MPN and CHA patients. Aims: In this study, our aim was to investigate the thrombosis incidence, the effects of plasma total homocysteine (tHcy) levels on thrombosis formation and the correlations between folate, cobalamin, pyridoxine and homosisteine levels in MPNs and CHAs and to compare the results with the healthy control group. Methods:41 patients [MPN (n = 31) and CHA (n = 10)] and 40 age and sex matched healthy individuals representing the control group were included in the study. For all the objects plasma tHcy, folate, cobalamin and pyridoxine levels were measured, and bilateral lower extremity venous system B-Mode and colour Doppler compression ultrasound imagening was performed to investigate the deep venous thrombosis (DVT). All the patients and the objects in the control group were questioned for the past arterial or venous thrombosis and for the comorbities that may cause thrombosis. Also for each object smoking history was taken and recorded as package/year. The follow up durations were noted for each object as months. In addition we measured the D-dimer levels to exclude acute thrombosis and also investigated the thrombophilic disorders other than hyperhomocisteinemia in the objects with thrombosis. *Results*. 11 thrombotic events in 7 objects were detected among the patients whereas there were no thrombosis detected in the control group. Statistically significant and positive correlations were found between the follow up duration, comorbidity and thrombosis (r=0,455 and 0,248 respectively). There were no statistically significant correlations between the plasma tHcy and vitamin levels and thrombosis. In the MPN group, there were no statistically significant correlations between hematocrit, platelet counts and thrombosis. A statistically significant and negative correlation between plasma tHcy levels and folate (r=-0,312) also a statistically significant and positive correlation between tHcy and the amount of smoking (r=0,310) were detected. *Conclusions*. In conclusion, comorbidity may be an important factor in the etiology of the increased risk of thrombosis formation in MPN and CHA patients. Also we think that in these patients in order to keep the plasma tHcy levels low, folate replacement therapy and reducing smoking are the treatment modalities that may prevent thrombosis formation.

1712

THE EFFECT OF INFLAMMATORY CYTOKINES ON VON WILLEBRAND FACTOR SYNTHESIS

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Background: Upon vascular injury, during the early stage of systemic inflammation, endothelial cell stimulation lead to the secretion of a family of monocyte-derived peptides, which include the cytokines interleukin 6 (IL-6), IL-8 and tumour necrosis factor-α (TNF-α). Inflammatory cytokines stimulates the endothelial release of Ultra large Von Willebrand factor (ULVWF) multimers. Increasing VWF multimer levels may have inhibitory effects on the synthesis of the ULVWF cleaving enzyme, ADAMTS-13. This may ultimately lead to the deficiency of ADAMTS-13 during inflammation and the over expression of ULVWF multimers resulting in the initiation of thrombotic thrombocytopenic purpura (TTP). Furthermore, the role of the initiation of coagulation in TTP is not known.Aim: In this study, our aim was to examine the effects of inflammatory cytokines and coagulation factors such as tissue factor and thrombin on the release of ULVWF by cultured endothelial cells and the cleavage of these ULVWF by ADAMTS-13. This will allow us to evaluate potential links between inflammation and thrombosis and help us understand the mechanisms that lead to TTP in patients. Methods: Human umbilical vein endothelial cells (HUVEC) were treated with interleukin 6 (IL-6), IL-8 and tumour necrosis factor α (TNF-α) for 24 hours under static conditions. The cells were then assembled to form the bottom of the flow chamber and exposed to a shear stress of 2.5dyn/cm2 to expose VWF cleaving sites. The Von Willebrand factor secretion was then measured through an ELISA technique. Results: IL-8 and TNF-α stimulated the release of VWF after 24 hours of treatment while IL-6 did not induce the release of VWF due to the absence of the IL-6 receptor on HUVEC cells. *Conclusions*. These results suggest that inflammatory cytokines may stimulate VWF release. Further research will involve measuring ADAMTS-13 levels and activity after stimulation with the inflammatory cytokines as well as coagulation initiators, thrombin and tissue factor. The combined effect of specific cytokines with coagulation initiators will be tested to look at the possibility of enhanced secretion of VWF or inhibition of ADAMTS-13. The findings might describe a potential linkage between inflammation and thrombosis.

1713

ENDOTHELIAL MICROPARTICLES: A POSSIBLE ROLE IN INFLAMMATION AND THROMBOSIS

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Background Microparticles are vesicles formed from activation of cells. These particles have an effect on a number of disorders including inflammation, diabetes mellitus, atherothrombosis, thrombocytopenia,

cardiovascular and other cellular disorders. Elevations of microparticles might be an indication of cellular dysfunction and may thus be usefull as a diagnostic marker. Microparticles can also be used as therapeutic targets in treatment of certain diseases, since these particles transports proteins and signals across cell membranes. Inflammatory cytokines and thrombotic stimuli are responsible for activation of cells and mediate microparticle formation. Von Willebrand factor is expressed on endothelial microparticle membranes upon activation of endothelial cells. This may indicate an important role for microparticles in primary clot formation in circulation. Aim The aim of the study was to determine the effect of inflammatory cytokines (Tumour necrosis factor α, Interleukin 8 and Interleukin 6) on human umbilical vein endothelial cell composition and its effect on thrombosis and von Willebrand factor in vitro. Methods Human umbilical vein endothelial cells were cultured in sterile conditions. Specific concentrations (0μM and 100μM) of inflammatory cytokines (tumour necrosis factor α, Interleukin 8 and Interleukin 6) were used to stimulate endothelial cells (HUVEC) in static conditions for 24 hours. Treated cells and untreated controls attached on the flask were circulated in round petri dishes for half an hour on a horizontal shaker to create a shear stress of 2.5dyn/cm2. Microparticles were isolated by a Ceveron microparticle filtration unit. Thrombin generation assays were performed and von Willebrand factor antigen levels were measured using only the isolated microparticles. Results Thrombin was generated and von Willebrand factor levels were increased with both inflammatory and thrombotic stimuli. Conclusions Human umbilical vein endothelial derived microparticles have been illustrated to increase the von Willebrand factor levels from untreated to treated cells. Endothelial microparticles also generated thrombin after inflammatory stimulation of cells. This suggests that microparticles might have an effect on thrombin generated in locations other than the static endothelial cell environment, where inflammation or thrombosis may occur. Continued studies on von Willebrand factor activity and the metalloprotease of von Willebrand factor, ADAMTS-13 will be conducted to determine the effect of endothelial microparticles on the breaking down of ultra large von Willebrand factor mulimers. This will give more insight on the effect of endothelial microparticles in inflammation and thrombosis

1714

ACQUIRED ACTIVATED PROTEIN C RESISTANCE IN PATIENTS WITH SARCOMA

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Background: Although the relationship between cancer and thrombosis has been known for long years and the pathogenesis has partially been understood, the relationship between sarcoma and thrombosis has been reported recently and detailed information about its pathogenesis is lacking Aims: We studied the frequency of clinically detectable thrombosis and presence of acquired activated protein C resistance (aAPCR) in patients with newly diagnosed sarcoma. Methods: We detected the cases who developed aAPCR prospectively in 52 patients (in the period after the diagnosis and treatment) and 52 healthy controls. We also determined the Factor (F) V and FVIII levels. Results: While thrombosis was present in one patient (2.17%), we did not observe thrombosis in the control group after the cases with Factor V Leiden (FVL) mutations were excluded. Although the normalized activated protein C sensitivity ratio (nAPCSR) values which were minimum (median 87.25%) at the time of diagnosis increased following treatment (median 94.35%), it was found to be significantly lower compared to that of the control group (median 106%, $p<0.05$, $p<0.001$ and $p<0.001$, respectively). aAPCR was found as 13%, 0% and 4.2%, respectively, according to the groups. Post-treatment FV levels were found to be higher compared to the values at the time of diagnosis ($p<0.001$). An opposite relationship was found between the FV levels and the nAPCSR values of the patients in the post-treatment period ($r= -0.38$, $p<0.02$). *Conclusions*. We found out an increased frequency of venous thromboembolism (2.17%) in sarcoma patients. As an original finding, we also found out a decrease in the nAPCSR, persisting even after treatment. Thirdly, we found out that the significantly higher rate of aAPCR at the time of diagnosis (13% versus 4.2%), totally disappeared after treatment (0%).

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1715

CHANGES IN PLASMA HEMOSTATIC VARIABLES IN AMBULATORY CANCER PATIENTS ENROLLED IN A TRIAL OF THROMBOPROPHYLAXIS WITH LOW-MOLECULAR-WEIGHT HEPARIN (LMWH) NADROPARIN DURING CHEMOTHERAPY (PROTECHT)

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Background. The results of the PROTECHT clinical trial (Agnelli et al., Lancet Oncology 2009) demonstrated the efficacy of thromboprophylaxis with the LMWH nadroparin in reducing the rate of thromboembolic events in ambulatory patients receiving chemotherapy for metastatic or locally advanced solid tumors. *Aims*. In this placebo-controlled randomized clinical trial, a study of plasma thrombotic markers [i.e. thrombin-antithrombin (TAT) complex, plasminogen activator inhibitor-1 (PAI-1) and von Willebrand factor (vWF)] was planned to evaluate: 1. the biomarkers' predictive value for thrombosis, and 2. the modulation of the biomarkers by thromboprophylaxis during chemotherapy. Methods Plasma samples from 141 patients were available for analyses at baseline (T0), i.e. before starting treatments (96 LMWH/45 placebo). Additional plasma samples from 31 study subjects (17 LMWH/14 placebo) were also obtained: before the 3rd chemotherapy cycle ± LMWH (T1), and at the end of treatments (T2). One hundred healthy subjects acted as a control group. *Results*. At T0, the levels of TAT, PAI-1 and vWF were significantly increased in patients compared to healthy controls ($p<0.05$). In the 31 patients analyzed over time, a significant reduction of TAT levels, from T0 to T1, occurred in subjects given LMWH, but not in those receiving placebo ($p<0.05$). After discontinuation of the study drug (T2), TAT levels returned to pre-treatment values. PAI-1 levels remained steadily elevated from T0 to T1 to T2 in both LMWH and placebo groups, while vWF levels were even significantly increased ($p<0.05$) over the chemotherapy period, with a similar profile in both study arms. Three out of the 141 patients experienced thrombosis during treatment. The small number of events did not allow to calculate the predictive value of plasma markers, but, interestingly, patients with thrombosis had significantly higher baseline levels of vWF than those without (247±5 vs 155±5%; $p<0.05$). *Summary/Conclusions*. The data show that LMWH nadroparin improved the patients' hypercoagulable state, as demonstrated by the reduction of plasma TAT levels, but did not influence the endothelial perturbance during chemotherapy, as measured by the levels of endothelial activation markers (i.e. PAI-1 and vWF). The alterations of endothelial markers may be possible candidates to identify ambulatory cancer patients at high thrombotic risk during chemotherapy.

1716

ALTERATION IN HUMAN ENDOTHELIUM CELL FUNCTION INDUCED BY HYPERGLYCEMIA AND HYPERLIPIDEMIA

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Excess vascular permeability due to secondary factors could promote more aggressive development of hematologic malignancies. We studied a development of endothelium dysfunction by hyperglycemia or hyperglycemia employing cultivating primary human endothelium cells (HUVEC) in different conditions. In order to study the effects of hyperglycemia we assessed cell response to normal glucose (5.6 mM) or high glucose (25.0 mM) content and used high sorbitol content (glucose 5.6 mM + sorbitol 19.4 mM) as osmolarity control. For hyperlipidemia, we used postprandial plasma collected from healthy volunteers 2.5 hrs following a fatty meal (300 g bread, 60 g butter); fasting plasma samples isolated from the same healthy volunteers served control. We than cultured HUVEC in a presence of 10% of plasma with high lipid content (HL) or fasting plasma with normal lipid level (NL) for 6 days. Lipid analysis revealed higher amount of triglycerides in postprandial plasma and similar level of high density and low density lipoproteins and cholesterol probably due to presence of excess amount of chilomicrons and remnant lipid-containing particles. Staining cells with lipid-soluble dye (Oil-red) reveal accumulation in cellular cytoplasm lipid-containing granules after two days of incubation. Growth rate analysis reveal that incubation with HL strongly suppresses cell proliferation and decreases percentage of viable cells, even more than high glucose level. We have observed that

when cultured in high glucose or high fat conditions for 2 days, HUVEC cells presented considerable signs of senescence, as detected with ? -galactosidase activity assay. We further found that a combined exposure to high lipid and high glucose has a substantial synergistic effect on induction of endothelial cells senescence. That could cause inability of the cells to sustain proper vascular homeostasis. Constant activation of pro-proliferative signaling pathways could result in decrease in cell viability by compensatory mechanisms, inhibiting excessive signal. We revealed that protein kinase Akt1 is strongly activated by HL in shot (30min) or long (6 days) incubation. Cells could be unable to proper react to growth factors stimulation and therefore decrease viability and become unable to sustain a barrier function. We also analyzed cellular contacts by staining for intercellular junctions (VE-cadherin and PECAM) and found that cells incubated in presence of high glucose and high lipid conditions in confluent monolayer were unable to sustain tight cell-cell contacts, possibly due to decreased level of particular proteins. In summary, we found that chronic exposure of endothelial cells to high glucose and high lipid conditions could alter cell viability and decreases barrier function.

1717

D-DIMERS: A SIMPLE, SENSITIVE MARKER OF PROCOAGULANT ACTIVITY

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Background. It is established that occurrence of thrombosis requires the coexistence of two or more, acquired or congenital, thrombophilic factors. Acquired thrombophilic factorscan be easily recognized. Congenital thrombophilic factors are difficult to be detected, because of bioethical and practical issues; a thorough investigation is very expensive, while only half of all congenital defects are known. *Aims*. The aim of the current study was to evaluate the measurement of D-Dimers as a simple and sensitive screening test, which could reveal early activation of coagulation, thus leading to investigation of congenital thrombophilic factors and/or adoption of preventive measures. *Subjects-Methods*. Our study group consisted of 32 individuals, with no personal history of thrombosis, who were tested for thrombophilia. We used ACL Advance coagulation analyzer in order to measure plasma antithrombin III (ATI-II), protein C (PC), protein S (PS), factor VIII (FVIII) and factor FXII (FXII) activities. We used the same analyzer in order to measure plasma total homocysteine and to investigate the existence of V-Leiden mutation (rdVAPC-resistance). Testing for prothrombin G20210A mutation was performed by use of an in house PCR protocol. Finally, ACL Advance coagulation analyzer was used for a latex-based determination of plasma fibrin D-Dimers. Demographic data and medical history of participants were collected through a structured questionnaire. Results: Study participants were divided into three groups, A,B or C, according to their positivity for the tested blood thrombophilic factors and into two groups, according to their positivity for plasma D-Dimers, as they are shown on the table below. All participants with positive D-Dimers had slightly increased levels (265-466 ng/ml, median: 290 ng/ml). According to the questionnaires, the three subjects with positive D-Dimers of group A had a family history of thrombosis at a relatively young age. Two of them also reported heavy consumption of cigarettes and coffee and the third had protein C activity at the lower normal level (77%). Two out of the three Group B subjects with positive D-Dimers had sickle/beta-thalassemia or sickle cell trait, respectively, while the third subject reported heavy cigarette and coffee consumption.

Groups	Thrombophilic factors	n	DDimers>255ng/ml (positive)	DDimers<255ng/ml (negative)
A	-	11	3	8
B	1	16	3	13
C	2	5	5	-

Conclusions: D-Dimers' test may be a simple and sensitive marker of early coagulation activity. The slightly elevated D-Dimers' levels in all subjects with positive D-Dimers, suggest that laboratories must use D-Dimers' tests with high sensitivity, not only to exclude a thromboembolic episode but also to indicate the presence of hypercoagulability.

1718

STUDY OF URINARY 11-DEHYDROTHROMBOXANE B2 AS A MARKER OF HYPERCOAGULABLE STATE IN CHILDHOOD HEMATOLOGICAL MALIGNANCIES

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Background. CXancer can confer a hypercoagulable state through an altered balance between the coagulation and fibrinolytic systems, which can be related to long term treatment. Association of prothrombotic markers with thrombosis in pediatric oncology patients is conflicting. Patients who exhibit elevated 11 dTXB2 levels; as a marker of hypercoagulablity; may be at increased risk for thrombembolic events. Aims : The aim of the work is to predict thromboembolic state in childhood hematological malignancies by estimation of 11-dehydrothromboxane B2 in urine. *Patients and methods*. This study was conducted on twenty-five patients with newly diagnosed hematological malignancies. They were followed up and treated in Oncology unit, Pediatric department, Zagazig University Hospitals. Patients were subjected to full history taking and thorough clinical examination with special attention to thromboembolic manifestations. Laboratory investigations were done before and after four months from starting chemotherapy: CBC, PT, PTT, urine creatinine, D-dimer and estimation of urinary 11 dehydro-thromboxane B2(TXB2) level. *Results*. Our data showed that there was no significant clinical presentations suggesting presence of thrombosis either before or after chemotherapy in both leukemic and lymphoma patients. There was a significant increase in D dimer and TXB2 / creatinine ratio levels in leukemic and lymphoma patients before the start of chemotherapy when compared to the controls. Also, there was a highly significant decrease in these levels after 4 months from starting chemotherapy in leukemic and lymphoma patients. Our data showed that there was significant correlation between TXB2 / Creatinin ratio values and D- dimer values, while, there was no statistical correlation between TXB2 / Creatinin ratio values neither with platelets count, PT and PTT values before and after 4 months from chemotherapy in both leukemic and lymphoma patients. *Conclusions*. children with hematological malignancy are at high risk of thrombosis; even if asymptomatic. So, those patients should be screened for additional prothrombotic risk factors and appropriate measures should be taken to prevent the development of thrombosis.

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IMPLEMENTATION VALIDATION OF THE REAL-TIME PCR FOR FACTOR V LEIDEN AND PROTHROMBIN G20210A MUTATION ON THE GENEXPERT DX SYSTEM

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Background Venous thromboembolism (VTE), including deep venous thrombosis and pulmonary embolism, has an estimated annual incidence of approximately 100 per 100.000 in the general population and is a major source of morbidity and mortality (White, 2003). Factor V Leiden and the prothrombin mutation are the most common inherited risk factors for VTE. After diagnosis of VTE, these trombophilic risk factors are commonly performed for etiological purpose and risk assessment of recurrent thrombosis. Aim To validate the Xpert HemosIL FII & FV kit (Instrumentational Laboratory, IL), a commercially available (CE-IVD and FDA approved) kit on the GeneXpert Dx System (Cepheid), for detection of factor V Leiden and prothrombin G20210A mutation. Methods All validation experiments were performed with 50 µl of EDTA whole blood. Molecular testing was performed on the GeneXpert Dx System according to the manufacturers instructions. This system combines extraction, real-time PCR, detection and result interpretation. The assay was checked for analytical sensitivity, specificity, precision and accuracy following international publications on molecular validation methods (e.g. ACMG, 2006). Twenty two patient samples were collected in Imelda Hospital over a 4-month period. To evaluate the clinical performance, these samples were tested with the HemosIL FII & FV kit as well as with the Light Cycler FRET system (Roche), performed in the University Hospitals Leuven. Results Sensitivity: The cycle

threshold (Ct) values for the prothrombin and factor V Leiden mutation ranged from 21.2 to 25.6 Ct and 21.8 to 28.1 Ct respectively. In this Ct-area errors related to limited or excess sample are rare. According to the manufacturer a volume of 50 µl of EDTA whole blood is sufficient to obtain a correct genotype. Specificity: Silent mutations (SNPs) in the probe binding area can occur. The manufacturer demonstrated that known rare factor V SNP's like A1696G, G1689A and A1692C can lead to an invalid result. However, valid results always revealed a correct genotype. Precision: Two samples (FII homozygous normal - FV homozygous normal patient sample and FII homozygous normal - FV heterozygous mutant control sample), were analyzed in triplicate on 3 different days. The standard deviation (SD) (Ct) was between 0.5 and 0.8 for both samples, meeting our validation criterion of SD < 1 (Ct). Accuracy: The 2010 INSTAND controls (whole blood), the NIBSC WHO reference panels and a 1 CE-IVD / FDA FII & FV control (IL) were used to check the accuracy of the HemosIL kit. A 100 percent agreement was found. Clinical performance: In 3 of the 22 patients APC resistance was detected and subsequently a heterozygous Factor V Leiden mutation was genetically confirmed. Nineteen patients were tested for the prothrombin mutation and only one heterozygous mutation was found. The other patients were homozygous normal for both factors. A 100 percent agreement with the Light Cycler FRET method was observed. Conclusion: The HemosIL FII & FV assay on GeneXpert Dx System met all our analytical and clinical validation criteria and was implemented in the daily routine.

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THROMBOPHILIA AND CRYPTOGENIC FOCAL EPILEPSY AMONG CHILDREN AND YOUNG ADULTS

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Background. Epilepsy has frequently a multifactorial aetiology and recognize a single cause is often impossible. Advanced neurodiagnostic investigations resulted in decreased rate of epilepsy of unknown etiology. Magnetic Resonance Imaging (MRI) brain allowed to identify the cause of focal epilepsy and generalized epilepsy, respectively in 70 and 30% of cases. To determine the pathogenesis of idiopathic focal epilepsy in MRI negative cases in children and young adults, this study would investigate a possible role of prothrombotic risk factors. *Methods*. We studied 48 patients (32M, 16F), aged between 1 and 32 years (median age 11.5 years). All were affected by epilepsy in which it was possible to exclude any form of focal idiopathic epilepsy. The diagnostic procedure included a first phase includings history, physical and neuroradiological examinations, waking and/or sleeping EEG. In a second phase patients diagnosed with cryptogenic partial epilepsy were subjected to coagulation tests (plasma fibrinogen level, plasma D-dimer level, PT, PTT, antithrombin III) and thrombophilia screening (Factor VII, Factor VIII, Protein C anticoagulant, protein S anticoagulant, APC resistance, polymerase chain reaction-based analysis for the factor V Leiden, prothrombin gene G20210A mutations, total plasma homocysteine. Results: 15 patients (12M, 3F) showed increased levels of factor VIII (> 120%) (range 123-226, median 159), 10 patients (8M, 2F) had hyperhomocysteinemia (> 15 mmol / L) (range 15.9 - 47.3, median 24.5), 1 patient (F) had protein S level of <50% (37%). One patient presented a heterozygous Factor V Leiden mutation and another patient an heterozygous mutation of Factor II prothrombin. The study did not reveal more significant correlations for the other parameters. *Conclusions*. We observed a lower incidence of Factor V Leiden and an higher incidence of increased levels of factor VIII and homocysteine in our cohort compared to general population (11% and 5% respectively). In addition we reported an increased frequency of thrombophilic disorders in children and young adults studied. Further studies are necessary to confirm these data.

1721

VENOUS THROMBOSIS IN PEDIATRICS: THE ROLE OF METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) MUTATION

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Background. Although the incidence of venous thrombosis is lower in children than in adults, it represents a significant source of morbidity and mortality in pediatric population. Both congenital and acquired factors contribute to the development of thrombosis. Hyperhomocysteinemia is a risk marker for venous thrombosis and it is consistently associated with methylenetetrahydrofolate reductase enzyme (MTHFR) gene mutation. Not always this association has been found and presence of MTHFR gene mutation with normal homocysteine level in vascular event suggests its role as independent risk factor. Our aim is to estimate the impact of MTHFR mutations alone as prothrombotic risk factor childhood. Methods: From 2002 through 2010 one hundred and twenty children aged between 1-192 months, affected by deep venous thrombosis were admitted into Bambino Gesù Children Hospital of Rome. They were submitted to thrombophilic screening and vascular imaging. Thrombophilic screening included the following tests: measurement of APC resistence, protein C, protein S, antithrombin III, polymerase chain reaction-based analysis for the factor V Leiden, C667T MTHFR and prothrombin gene G20210A mutations and assays for lupus anticoagulants, anti-cardiolipin/antiphospholipid antibodies and homocysteine. Results: The MTHFR polymorphism C677T was found in sixty patients (fifteen in homozygosis and forty-five in heterozygosis) with no other inherited thrombophilic risk factors.Eight heterozygous and eight homozygous patients presented increased plasmatic and urinary levels of homocysteine. Forty-four of these sixty (73%) patients presented normal homocysteinemia, thirthy-seven (84%) with MTHFR heterozygous genotype and seven (16%) with MTHFR homozygous genotype. Sixteen of sixty (27%) patients presented elevated levels of homocysteine, eight (13%) with MTHFR heterozygous genotype and eight (13%) with MTHFR homozygous genotype. Twenty-six were treated with low molecular weight heparin (enoxaparin 100 U/kg s.c. x 2 daily), then they all continued the treatment with oral anticoagulant for 6-12 months. Five patients were treated with antiaggregation therapy and all of them performed long term profilaxis with folic acid and B group vitamins. About remaining patients, since venous thrombosis was subsequent of thromboembolic event, three patients did not received treatments. *Conclusions*. Our data raise the question whether MTHFR gene polymorphism alone, with or without hyperhomocysteinemia, may somehow contribute to thrombophilia and suggest to perform anticoagulant prophylaxis in all patients with MTHFR mutation with previous thrombotic events in case of other thrombotic risk factors (pregnancy, oral contraceptives, sepsis and immobilization). Not always the MTHFR polymorphism leads hyperomocysteinemia. This may be due to the effect of homocysteine levels on thrombotic disease and to the variations of homocysteinemia that may depend on others conditions. The impact of the MTHFR mutation as independent thrombophilic marker on outcome and recurrence risk of venous thrombosis needs to be further investigated.

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HEMOSTATIC AND RHEOLOGIC CHANGES OF ACUTE MYOCARDIAL INFARCTIONS IN NIGERIANS

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Background. Myocardial infarction (MI) is defined as necrosis of a portion of cardiac muscle caused by obstruction in coronary artery through either artheriosclerosis, a thrombus or spasm. The causative factors has been well documented and its risk has been reduced to the barest minimum in advanced countries of the world while in developing countries such as Nigeria, the advent of MI as a major cardiovascular problem is moderately recent. Therefore, researches into the responses of of rheologic and fibrinolytic parameters are modestly new and ongoing. *Aims*. We therefore aimed to highlight basic information on haemorheologic and fibrinolytic parameters with a view to indicate their possible use as diagnostic and prognostic indices in MI. Methods: We investigated longitudinally, 10 acute myocardial infarction (AMI) patients together with 20 age and sex -matched apparently healthy subjects as controls. Blood samples were taken at the point of admission (Day 0), on the 4th and 7th day respectively after treatment has commenced. Rheologic and fibrinolytic indices such as complete blood count (CBC), erythrocye sedimentation rate (ESR), Plasma Fibrinogen concentration (PFC), D-dimer concentration (DDC), Euglobulin lysis time test (ELT) and Plasma viscosity (PV) as well as lipids were measured using standard laboratory methods. Results: We recorded a significantly reduced values of haematocrit and fibrinolytic activity coupled with significantly increased D-dimer levels, PFC, ESR and PV in AMI patients on admission compared with controls (P<0.05, respectively). However, PFC, DDC and PV became significantly lowered from the 4th day of admission while all the parame-

ters became significantly reduced from the 7th day of admission and treatment (P<0.05, respectively). On the other hand, lipids such as Cholesterol, Triglycerides, VLDL and HDL remained relatively unchanged from admission and throughout the duration of the study. *Conclusions*. We conclude therefore that hyperfibrinogenaemia coupled with hypofibrinolytic activity and high plasma viscosities could be likely associated risk factors of thrombosis in Nigerians with AMI and their reduction during treatment are positive indicators as factorials in its pathogenesis. The role of lipids in AMI pathogenesis in Nigerians seem to be unpronounced.

1723

D-DIMER ASSAY IN EGYPTIAN PATIENTS WITH GAUCHER DISEASE: CORRELATION WITH BONE AND LUNG INVOLVEMENT

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Background. Gaucher disease (GD) is the most frequent lysosomal storage disorder. Bone and lung involvement are two major causes of morbidity in this disease. D-dimer is a reliable indicator of active microvascular thrombosis, even in patients without overt hypercoagulation. Aim: This study aimed to assess D-dimer levels in GD correlating this marker to clinical characteristics and radiological parameters to investigate its role as a potential predictor for the occurrence and severity of skeletal and pulmonary manifestations. *Methods*. The study population consisted of 56 Egyptian patients with GD; 36 had type 1 (64.3%) and 20 had type 3 (35.7%). Thirty healthy individuals were enrolled as a controls. All patients were receiving regular ERT (the recombinant imiglucerase enzyme). Laboratory investigations were done including complete blood count, liver function and quantitative plasma D-dimer assay. Radiological; investigations X-rays long bones, abdominal ultrasound for assessment of liver and splenic volume, high resolution C.T chest (HRCT) as well as magnetic resonance imaging for pelvic spines and femur bones. *Results*. All Gaucher patients had shown remarkable improvement of growth and hematological parameters, as well as reduction of hepatic and splenic volumes after 6 months of the ERT. D-dimers in all Gaucher patients were significantly higher compared to controls with a mean of (2011±499 and 396±94 ng/ml respectively., p=0.001). D-dimers were significantly higher in type III compared to type I patients with a mean of (1063.5±414 and 830±275.2 ng/ml respectively., P=0.03). Pulmonary involvement and HRCT findings (ground glass appearance, interlobular thickening) were present in a higher percentage among type III compared to type I (71%, 37.5% respectively) (P=0.03). While bony involvement and MRI findings (bone marrow expansion, cortical thinning) were present in higher percentage of type I compared to type III patients (25%, 21% respectively) (p=0.62) .D-dimers were significantly higher in patients with ground glass appearance on HRCT chest (1163.61±457.1 ng/ml) compared to those with normal study (810.77±250 ng/ml; p=0.05). Platelets count were significantly higher in patients with ground glass appearance (218±95x10^9/L) compared to patients with normal study (117±49x10^9/L; p=0.03). *Conclusions*. D-dimer is significantly elevated in Gaucher patients particularly in type III. Higher D-dimers levels in Gaucher patients with clinical and radiological manifestations of bony and pulmonary involvement were found. Thus it's assay may be potentially predictive of bone and lung involvement in Gaucher disease and suggesting that the occurrence of these complications is induced initially as microthrombi.

1724

INFLAMMATORY BIOMARKERS IN ACUTE CORONARY SYNDROMES

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Background. Acute coronary syndromes occur in response to inflammation, plaque rupture and subsequent thrombosis. Inflammation is associated with the progress and stability of atherosclerotic vascular lesions and much interest has focused in some inflammatory parameters, such as white blood cell (WBC) count, interleukin-6 (IL-6) and matrix metalloproteinases (MMPs). Traditionally elevated WBC count considered as an indicator of systemic inflammation, but it has also been accepted as part of the healing response following acute myocardial infarction. IL-6 is an inflammatory cytokine which appears to play an important role in atherogenesis. Many studies have demonstrated that MMP-mediated degradation of extracellular matrix is critical for atherosclerotic plaque rupture, responsible for acute atherothrombotic syndromes. *Aims*. The present study was designed to investigate the association between these inflammatory biomarkers (WBC count, MMPs and IL-6), with acute coronary syndromes. Methods: We studied 45 patients (33 males - 12 females, mean age 64.4±12.9 years), either with ST-segment elevation (STEMI) or without ST-segment elevation (non-STEMI) myocardial infarction. From blood samples, which were taken from a peripheral vein, in the first post-infarction day, human pro-MMP-10 and IL-6 plasma levels were measured, using an enzyme immunoassay method. WBC count was also measured in the same samples. Results: WBC (and neutrophils), MMPs and IL-6 levels were found elevated to our study population. No statistically significant differences between STEMI and non-STEMI could be demonstrated. Results are summarized in the table. *Summary/Conclusions*. All inflammatory parameters were found elevated in patients with acute coronary syndromes, possibly because of the existing inflammatory process. Whether elevated WBC count, MMPs or IL-6, are only a marker of the inflammatory process or a direct risk factor for acute coronary events remains unclear.

Variable	All	STEMI	NSTEMI	p-value
Number of patients	45	20	25	-
WBC (x10^3/μL)	10.65±3.5	11.05±3.4	10.32±3.5	0.48
NEUT (x10^3/μL)	8±3.9	8.2±3.8	7.9±4.3	0.8
MMPs (pg/mL)	566.2±257.7	615.8±308.6	524.8±203.8	0.24
IL- 6 (pg/mL)	22.1±27.4	19.1±15.4	24.8±35.2	0.5

1725

DETECTION OF THROMBOPHILIC MUTATIONS IN WOMEN WITH ADVERSE PREGNANCY OUTCOMES

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Introduction. Inherited thrombophilia is associated with a high risk of pregnancy complications including early recurrent miscarriage, intrauterine fetal death, intrauterine growth restriction (IUGR) and preeclampsia. Factor V Leiden (FVL) G1691A, methylenetetrahydrofolate reductase (MTHFR) C677T/ A1298C, and factor II (FII) G20210A mutations are important causes of thrombophilia. The aim of this study is to evaluate the prevalence of these mutations in a group of obstetric patients. *Material and Methods*. The study enrolled 58 women with obstetric complications divided in: maternal complications - 3 patients with post C-section venous thromboembolism, and fetal complications - 31 women with 1-2 early miscarriages, 15 with >3 early miscarriages, 6 with IUGR, 2 with preeclampsia and 4 with intrauterine fetal loss. DNA was isolated from peripheral blood samples and then analyzed by Real-Time PCR and melting curve analysis (LightCycler 480 platform) for Factor V Leiden G1691A and FII G20210A mutation. The presence of C677T and A1298C mutations was investigated using Polymerase Chain Reaction - Restriction Fragment Length Polymorphism method based on Hinf I and Mbo II endonuclease digestion. *Results*. FV Leiden was detected in 17 women (29.3 %), 3 were homozygous and developed VTE. Five women (8.6%) had heterozygous prothrombin mutation and three of them presented intrauterine fetal death with severe IUGR. A high frequency of MTHFR mutations was obtained: 41 women (70.6%) with C677T mutation (9 homozygous, 32 heterozygous) and 28 women (48.2%) with A1298C mutation (6 homozygous, 22 heterozygous). Three patients had 3 simultaneous mutations, 29 had 2 mutations and one patient was found without any mutations. *Conclusions.* The investigated thrombophilic mutations were frequent among our patients. FV Leiden and prothrombin mutations generated the thrombotic events

with the highest morbidity. Thrombophilic molecular evaluation should be performed in selected women with adverse pregnancy outcomes. *Funding. This work was supported by the grant PN 42-099 from the Romanian Ministry of Research and Technology.*

1726

RENAL INFARCTION: CASE REPORTS IN PATIENTS WITH THROMBOEMBOLIC RISK FACTORS

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Background. The renal infarction is a rare, but serious and often misdiagnosed, condition. There is not a therapeutic consensus to treat this disease, because of the unfrequent episodes reported in different series. We can found different treatment choices: surgery, fibrinolysis and anticoagulation. *Aims*. Review of the cases of renal infarction diagnosed in our hospital: clinical diagnostic, therapeutic management and evolution. Case 1: 52-year-old male, with history of hypertension, active smoker, pulmonary emphysema, generalized atheromatous, chronic vascular ischemia, angor haemodynamic, cocaine use in treatment with methadone, derived from another hospital due to acute and refractary to analgesic pain in right hypochondrium, radiated to epigastric and neck associated with nausea and vomiting. Abdominal exam: painful to the palpation in flank and right iliac fossa, without signs of peritoneal irritation. Ultrasound: normal size and structure kidneys. Angio-CT: atheromatous stenosis of the principal branch of the right renal arterial. Reevaluation CT: right kidney with decrease of size and poor parenchymal opacification, suggesting chronic renal infarction. Treatment: conservative with oral anticoagulation developing good response. Angio-CT: resolution of the injury with arterial permeabilization. Case 2: 53-year-old male with history of hypertension, presented right hypochondrium pain, persistent and refractary to analgesic treatment. Abdominal exam: painful to the palpation in right flank, without signs of peritoneal irritation. Angio-CT: extensive renal infarction involving the middle third of the right kidney, with hypoperfusion in this area. Treatment: Fibrinolysis and non fractionated heparin. Hipercoagulability study reveled positive lupus anticoagulant suggesting an antiphospholipid syndrome. Genetic study showed heterozygote mutation of the MTHFR with high levels of homocistein. Case 3: 84-year-old male with history of hypertension, cardiac insuficiency, auricular fibrillation and chronic pulmonary disease, came to emergency from another hospital due to abdominal pain for 11 days, with nausea and vomiting, suggestive of cholecystitis. Ultrasound: biliar bladder with inflamatory signs, cortical cysts in right kidney. CT: severe aortoiliac atheromatous, hypoperfusion renal area, 35 % right and 15% left. Patient was in surgery because of perforated cholecystitis, then continued with anticoagulantion. Echocardiogram: Normal.

	Case 1	Case 2	Case 3	Case 4
Abdominal pain	+	+	+	+
Nauseas	+	+	+	-
Vomiting	+	-	+	-
Fever	-	-	+	-
Hematuria	-	-	+	-
Arterial Hypertension	+	+	+	+
Leucocyte count	18200	11600	13900	8600
Hemoglobin	9.5	13.3	12.2	15.2
Platelet count	588	176	233	234
Haemostasis	Act. Prot: 19% Tto: oral anticoagulation	Normal	Normal	Normal
Creatinine	1.4	1.3	1.3	0.99
LDH	-	484	355	335

Case 4: 56-year-old man with history of myorcardial infarction, prostate cancer and hypertension, presented abdominal pain and constipation for 15 days, associated to weight lost. Abdominal exam: reduced bowel sounds, painful to the palpation in rigth flank, without signs of peritoneal irritation. Angio-CT: stenosis of transverse colon because of tumor presence, splenic and bilateral renal infarction. At the same time it is detected positive for lupus anticoagulant. During hospitalization patient developed a small cerebral infarction, kidney failure, and worsening of previous clinic described. He was under surgery because of intestinal obstruction, subsequently worsening of kidney failure, multiorgan failure and death. *Comments*. Although acute renal infarction is a rare entity, it is necessary to suspect it in patients with abdominal pain and risk factors for thromboembolism. Early diagnosis is critical in order to diminish the morbimortality of this disease. Physicians must extend the ethiology study, because there are many entities that can cause this patology in patients without known risk factors like use of cocaine, sepsis, neoplasm and thrombophilia.

1727

EVALUATION OF THE EFFECT OF SUBCLINICAL HYPOTHYROIDISM ON BLOOD COAGULATION PARAMETERS

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Background. Subclinical hypothyroidism (SCH) is defined as a status of mildly elevated thyrotropin (TSH) levels associated with normal total or free T3 and T4 values. In general, it is an asymptomatic entity, usually diagnosed after routine screening or while exploring nonspecific clinical presentations, which has been associated with enhanced cardiovascular risk. There has been considerable controversy regarding the alterations of blood coagulation parameters in patients with SCH as both hypercoagulable and hypocoagulable states have been referred. *Aims*. To evaluate platelet activation and coagulation disorders in patients with SCH and the influence of TSH, peripheral thyroid hormones (FT3, FT4) and thyroid autoantibodies such as thyroid antithyroglobulin (anti-TG) and antithyroperoxidase (anti-TPO) on them. *Methods*. A total of 24 patients with SCH (males/females: 10/14) and 24 euthyroid control subjects matched for age and gender were enrolled to the study. Platelet indices [platelet count (PLT), mean platelet volume (MPV) and platelet distribution width (PDW)] were measured in whole blood samples by flow cytometry method with the use of XE-5000 Sysmex (ROCHE). Coagulation parameters [prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), protein C and protein S] were measured in plasma samples using a fully automated analyser (ACL Top, ALAPIS). Serum TSH, FT3, FT4, anti-TG and anti-TPO levels were determined by electrochemiluminescence immunoassay with Elecsys Modular E170 analyser (ROCHE).

Table 1. Platelet and coagulation parameters in patients with subclinical hypothyroidism (SCH) and normal controls.

Laboratory parameter	Patients with SCH (n=24)	Normal controls (n=24)	p-value
PLT (K/μL)	277 ± 108.60	278 ± 57	0.960
MPV (fL)	10.80 ± 0.81	10.25 ± 0.42	0.009*
PDW (%)	12.95 ± 1.67	11.37 ± 2.49	0.016*
PT (sec)	12.04 ± 2.05	11.52 ± 0.89	0.406
INR	0.99 ±0.18	0.94 ±0.08	0.408
aPTT (sec)	29.61 ± 8.72	27.55 ± 2.46	0.405
Protein C (%)	96.25 ± 24.87	97.21 ± 21.29	0.916
Protein S (%)	90.25 ± 31.31	99.92 ± 22.08	0.366

Results: The results are presented in Table 1.The levels of MPV and PDW were significantly higher in the SCH group than in the euthyroidic one ($p<0,05$). No differences were observed in other coagulation parameters between SCH patients and control subjects. The above indices were not found to be correlated neither with the thyroid hormone concentrations nor with the measured autoantibodies levels. Summary/conclusions: Subclinical hypothyroidic patients present alterations in some blood coagulation parameters, which are indicative of platelet activation. The speculation that elevated MPV values may be involved in the increased risk of atherothrombotic complications in subclinical hypothyroidism should be further investigated.

1728

ANTICARDIOLIPID ANTIBODIES IN THE REAL WORLD: WHAT IS THE IMPACT OF A CONFIRMATORY TEST AT 12 WEEKS?

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Background. Because of the possible transient nature of antiphospholipid antibodies, Sydney classification criteria for antiphospholipid syndrome recommend a confirmatory test, which was postponed at least 12 weeks apart from the first measurement. *Aims.* The aim is to evaluate the impact of this confirmatory test in patients referred to our hospital and having a first positive anticardiolipin antibody (aCL) ELISA. *Methods.* Home made aCL ELISA (IgG and IgM) was performed according to the requirements of the European Forum on antiphospholipid antibodies using cardiolipin for coating and with locally established cut-off values (>99th percentile 15UGPL -7UMPL). CV was 10%. *Results.* Among the patients prospectively included in our database (2005-2010), we identified 91 patients with a positive aCL ELISA either IgG (37) or IgM (41) or both (13). Median and range values were 60.9 (15-576) UGPL and 17 (7-288) UMPL for IgG and IgM respectively. Among them, 53 had a positive second result. Patients persistently positive had a higher level of antibodies at the first test: median and range were 63.8 (15-576) and 20.7 (15-26) UGPL respectively for persistent and non persistent aCL IgG and were 17.9 (7-288) and 8.3 (7 14) UMPL for persistent and non persistent aCL IgM (borderline statistical. *Conclusions.* At or beyond 12 weeks 58% were found to remain positive, but a subset of patients with further testing could become negative. Thus a second, confirmatory test, even when performed at 12 weeks rather than at 6 weeks as previously recommended, could be insufficient. The clinical relevance of the differing patterns of persistency remains to be elucidated.

1729

PROTHROMBIN MUTATION G20210A AND MUTATION MTHFR - RISK FACTOR FOR RECURRENT FETAL LOSS

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Background . In the recent years, thrombophilia as a risk factor for pregnancy complications and fetal loss gained much attention in the scientific community. However, data on this topic in the literature are conflicting. Prothrombin mutation G20210A and mutation MTHFR result in an increased susceptibility to develop venous thrombosis. The associated clinical manifestations can be heterogeneous as regards severity as well as type of event (VTE or obstetric complication). *Aims*. This study estimates the risk factor of the presence of inherited thrombophilia genetic defects in women with recurrent fetal loss. *Methods*. 95 women with a history of recurrent fetal loss were referred to our laboratory for thrombophilia genetic testing (prothrombin G20210A, MTHFR 677T) by PCR methodology during one year (2009). RESULTS. 88 (92.63%) women had a positive genetic testing: 24 (27.27%) had prothrombin G20210A mutation, 4 (4.54%) had homozygous for MTHFR, 29 (32.95%) had heterozygous and 31% (35.22%) had double heterozygosity. *Conclusions*. Considering the higher percentage of these genetic mutations in women with multiple fetal losses, there are many voices in the scientific community who suggest that maternal screening before pregnancy is a must, in spite of the high cost of this investigation.

1730

INHERITED THROMBOPHILIA IN WOMEN WITH RECURRENT PREGNANCY LOSS

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Background. Recurrent pregnancy loss (RPL) is a common clinical problem. Several groups have reported an association between inherited thrombophilia and RPL. *Aims*. The aim of this study was to investigate the prevalence of inherited thrombophilia in women between the ages of 18 and 45 referred to our clinic for evaluation of thrombophilia as the etiology of RPL. *Methods*. We retrospectively analyzed the records of women who had complete thrombophilia testing for RPL, defined as 2 or more otherwise unexplained pregnancy losses, between the period of January 2008 and December 2010. The following thrombophilic disorders were included: protein C deficiency (PC), protein S deficiency (PS), anti-thrombin deficiency (AT), activated protein C resistance (APCR), factor V G1691A (FVL), prothrombin G20210A (PTG) and Methyl tetrahydrofolate reductase C677T (MTHRF) done outside of pregnancy or the immediate post-partum period. Results: A total of 43 patients with complete data were identified. Median age was 33 years (range: 20-45). Median number of pregnancy losses was 3 (range: 2-8). 15 women (35%) were found to have a thrombophilic condition. PS deficiency was identified in 1 woman (2%), AT deficiency in 1 (2%) and APCR in 2 (4%), while no cases of PC deficiency were identified. In addition, 2 (4%) had FVL (same women identified as having APCR, both heterozygous), 1 (2%) had PTG and 11 (25%) had MTHFR (10 heterozygous and 1 homozygous). *Summary/conclusions*. Inherited thrombophilias are not uncommon in women with RPL. These results suggest that testing for thrombophilia may be warranted in women with RPL. A prospective study with a control group and more detailed thrombophilia testing, including confirmatory DNA analysis, to assess the true prevalence and significance of such defects in women with RPL is highly warranted.

1731

EVALUATION OF IMMATURE PLATELET FRACTION, MEAN PLATELET VOLUME AND ENDOTHELIAL DAMAGE IN PATIENTS WITH CORONARY ARTERY DISEASE

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Background: The development of vaso-occlusion is believed to be multifactorial. The presence of activated platelets, subclinical inflammation and consequent activation of the vascular endothelium are important contributors that lead to coagulation activation. Large platelets are more active and more thrombogenic than smaller ones. Immature platelet fraction (IPF) is an index of reticulated platelets which are newly released platelets, larger and more reactive than mature ones. Increased mean platelet volume (MPV) and platelet distribution width (PDW) have been connected to morbidity and cardiovascular mortality in patients with coronary artery disease. Evidence of vascular endothelial damage, includes levels of plasma von Willebrand (vWf) factor. However, relationships between these factors in cardiovascular disease are unknown. Aims: The purpose of our study was the evaluation of the levels of IPF, MPV, PDW and vWf in patients with coronary artery disease . Methods: Our material consisted of 80 patients, (47 males and 33 females, aged 18-74 years old) suffering of coronary artery disease. A second group consisted of 40 normal controls (20 males and 20 females matched for age and ethnicity). Platelet count (PLT), IPF, MPV and PDW were measured by flow cytometry with the SYSMEX XE2100, when vWf was measured using the automated analyser ACL Top (ALAPIS). Exclusion criteria for entry into the study was the presence of co-existing thrombotic or hematological disease . Results: Mean values of PLT, IPF and MPV of patients presented statistically significant difference (all $p<0,05$) with that of the normal controls while mean value of PDW was not significantly different. Positive correlation was found between the values of IPF and MPV ($r= 0,638$, $p<0,01$). Von Willebrand factor was significantly higher in patients compared to the normal controls ($p<0,001$) and was also positively correlated with IPF ($r= 0,648$, $p<0,01$). Conclusions: Patients with coronary artery disease present abnormalities in their platelets. These patients present more numerous platelets, which are bigger and activated. In this disease activated platelets and activation of the vascular endothelium, may lead to coagulation activation.

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EVALUATION OF IMMATURE PLATELET FRACTION, MEAN PLATELET VOLUME AND HOMOCYSTEINE LEVELS IN PATIENTS WITH CARDIOVASCULAR DISEASE

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Background. Immature platelet fraction (IPF) is an index of reticulated platelets which are newly released platelets, larger and more reactive than mature ones. Mean platelet volume (MPV) and platelet distribution width (PDW) are indicators of platelet function and activation. Increased mean platelet volume and platelet distribution width have been connected to morbidity and cardiovascular mortality. Increased mean platelet volume has also been recognized as a risk factor for myocardial infarction in patients with cardiovascular disease. Hyperhomocysteinaimia has

been identified as an independent risk factor of cardiovascular disease and thromboembolic incidents. *Aims*. The purpose of our study was the evaluation of IPF, MPV, PDW and levels of homocysteine (Hcy) in patients with cardiovascular disease. *Methods*. Our material consisted of 70 patients, (37 males and 33 females, aged 18-74 years old) suffering of coronary artery disease. A second group consisted of 40 healthy persons (20 males and 20 females matched for age and ethnicity). IPF, MPV and PDW were measured by flow cytometry with the SYSMEX XE2100 analyser. The levels of Hcy were determined with immunofluropolarimetry on the AxSYM-plus analyser. Exclusion criteria for entry into the study was the presence of co-existing thrombotic or hematological disease. *Results*. Patients with cardiovascular disease presented significantly higher IPF, MPV, PDW and Hcy values than normal controls (all p<0,05). A positive correlation was found between the values of IPF and Hcy (r=0,648, p<0,01) while no positive correlation was found between the value of Hcy and the value of MPV or PDW. *Conclusions*. IPF, MPV, PDW and Hcy are elevated in patients with cardiovascular disease. From our study is concluded that the combination of measurement of IPF, MPV, PDW and homocysteine is useful in patients suffering with cardiovascular disease as indices of risk of new cardiovascular incidents.

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HEMATURIA AND GINGIVAL BLEEDING IN A PREGNANT WOMAN

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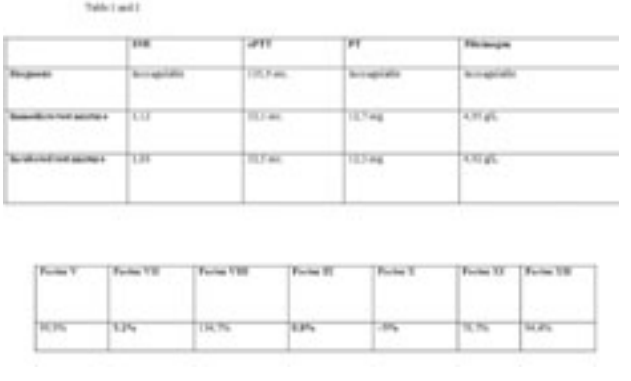
Background. Antagonists of vitamin K (Vit K) are currently the most used rodenticides. Ingestion causes nausea, vomiting and bleeding within 36-48 hours. Serious intoxication is caused by repeated doses or by a single massive dose (>15 g). Diagnosis is based on clinical findings and the determination of the specific toxic is interesting from a forensic point of view. Bleeding risk may last for several weeks for compounds of long average life and Vit K treatment may be necessary for weeks or months until total recovery of coagulation parameters. Case report: 17 weeks pregnant woman without pathological history, was admitted for mild bleeding (hematuria and gingival bleeding) for 1 week. Laboratory findings presented global changes in coagulation parameters. Previous studies are normal. Amniocentesis had been performed 3 weeks before without complications. Analitical findings ares shown in Table 1. A moderate to severe acquired Vit K dependent clotting factors deficiency was confirmed: FII 3.3%, FV 99.9%, FVII 3.1%, FVIII 134.7%, FIX 8.8%, FX <5%, FXI 78.7%, FXII 94.4% . After the initial intravenous Vit K treatment bleeding improved and clotting parameters normalized. 24-48 hours oral Vit K was iniciated developing recurrence of coagulation disorders, suspecting the possible action of some long life substance. Differential diagnosis: bowel disease, malabsorptive, drugs ingestion, oral anticoagulants and/or rodenticides were discarded. The patient reported the use of rodenticide (brodifacoum) last year due to a rat invasion at her home, but denies current contact. Given the high suspicion of possible intoxication by *superwarfarin*, determination of levels in blood and urine samples were studied resulting positive in both samples. Hemostasis study was conducted in cohabitants to discard multiple intoxication resulting normal.



The patient was discharged from hospital with treatment of oral Vit K (initial dose 40 mg/day) lasting for 2 months and pregnancy came to term without complications. Conclusions: The 'superwarfarins' displays exert its toxic effect by inhibiting the reduction of Vit K, preventing its activation and resulting in a lengthening of TP, and in more serious cases lengthening of aPTT. Intoxication should be suspected when sustained and high doses of intravenous Vit K are needed to correct coagulation parameters, and this treatment may be sustained for one or two months. The most common cause is the accidental ingestion, especially in children, although the manifold intake in familiar surroundings must be discarded. Other causes are important due to legal involvement.

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IS PSYCHOLOGICAL ADJUSTMENT TO A DIAGNOSIS OF ACUTE LEUKEMIA INFLUENCED BY ACCEPTANCE OF DIAGNOSIS AND AMOUNT OF TIME ELAPSED TO ACCEPTANCE?

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Background: Coping with cancer is a subject intensely analyzed by psycho-oncologists. It was proven that there are several factors that help patients to develop an effective coping: personality traits, efficient coping with other problems, internal and external psychological resources. *Aims:* We intend to analyze how acceptance of the diagnosis and the amount of time elapsed to acceptance influence coping with acute leukemia. *Methods:* From November 2007 to April 2009 we conducted 100 interviews with patients admitted at the Cluj Hematology Clinic, all with a diagnosis of acute leukemia, and treated with standard protocol chemotherapy. Patients were asked how long did it take to accept their diagnosis and to rate the degree of acceptance on a scale from 1 to 10 (1 = does not accept diagnosis, 10 = total acceptance). At the end of the interview (which focused on more aspects than those addressed by this study), the author (a psycho-social counselor and clinical hematologist) determined patients' coping strategy to leukemia as either efficient or inefficient. Data was analyzed with SPSS and Excel. Correlations between time/degree of acceptance of diagnosis and coping were made using Cramer contingency coefficients. Patients signed informed consent, and the study was approved by the Ethics Committee of Medicine and Pharmacy University Cluj-Napoca, Romania. *Results:* Patterns of efficient coping indentified in this group of patients were: fighting spirit, realistic approach toward disease, direct confrontation of disease, overcoming denial, and considering every possible disease outcome (favorable or unfavourable). In this group, 93% of the patients developed an efficient coping to acute leukemia Patterns of inefficient coping were: long-term denial of disease or prognosis, passive attitude, blaming others, anger, depression, and concealment of truth. The time period to accept the diagnosis ranged from immediate to four months. During the interviews patients were asked to describe and analyze the reason for their immediate or delayed acceptance of diagnosis. The reasons for the immediate acceptance were: presence of severe or neoplastic disease in patient's or his family's medical history, severe symptoms at initial meeting, hearing of suspicion of neoplastic disease from a different hospital, belief in a cure, hearing the diagnosis after a prolonged investigations. The degrees of acceptance varied from 4 to 10 on the scale described above, where about 2/3 of the patients ranked at ≥ 8. The time period to acceptance of diagnosis was not statistically relevant associated with efficient coping (F=3.73, p=0.508>0.05). A high degree of acceptance of the diagnosis was significantly associated with efficient coping (F=7.13, p=0.019<0.05). *Conclusions:* A high degree of acceptance of diagnosis influences the development of an efficient coping strategy. The time period to acceptance of diagnosis does not appear to influence coping. Patients with a low degree of diagnosis acceptance (4-6 on the 1-10 scale above) developed an inefficient coping and these patients would benefit from targeted and individualized psychological support.

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QUALITY OF LIFE IN NON-HODGKIN'S LYMPHOMA PATIENTS TREATED WITH 21 R-CHOP IN ASSOCIATION WITH PROFILACTIC GRANULOCYTE GROWTH FACTORS

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During the last few years 21 R-CHOP has become the standard choice of treatment for patients with B cell non-Hodgkin`s lymphoma. It has been demonstrated that this combination is effective and safe. But we observed a relatively high frecquency of severe toxicity, infections, anemia and malnutrition in many patients, responsible for increased morbidity during treatment. The aim of this study was to evaluate whether the quality of life was improved after the use of profilactic granulocyte growth factors. QOL was assessed using the validated EORTC QOL-30 questionaire, which is a 30-item instrument developed specifically for

use in clinical cancer research. Methods: 80 patients were included between January 2008 and January 2011. 54 out of them were stage I-II and 26 stage III-IV, 42 were male and 38 female. Profilactic use of granulocyte growth factors began after the first cycle for 40 patients and in the other group of 40, after the first neutropenia. All patients completed the QOL-C30 questionaire at least 3 times: pre-treatment, after four cycles and post-treatment. In the second group, patients completed the questionaire after the first neutropenia and before every cycle until the end of treatment. Pre-treatment global health status and role functioning were almost identical. The first group followed the treatment at exact time and the neutropenia developed did not interfere with the course of treatment. They did not develop febrile neutropenia or grade III or IV neutropenia. Therefore, global health status and role functioning was not significantly decreased compared with the pre-treatment value. In contrast, in the second group, 32 patients developed neutropenia after approximately 3 cycles and global health status, physical and role functioning were significantly decreased and fatigue, dyspnoea and appetite loss significantly increased. After the introduction of granulocyte growth factors the incidence of neutropenia decreased and the patients continued with the treatment at regular times. The six parameters improved, especially the last 3 that were associated with respiratory infections that disappeared after the remission of neutropenia. After three months of post-treatment the patients generally scored equal.Conclusion: The use of profilactic granulocyte growth factors improves QOL in NHL patients treated with 21 R-CHOP and should be a standard in the course of the treatment because of the benefic effect over the patient`s state of well being during the treatment and also because of the costs that a febrile neutropenia involves.

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THE SOUTHERN TRANSYLVANIA PATIENTS OPINION ABOUT THE IMPORTANCE AND MANAGEMENT OF LIFE QUALITY OF PATIENTS WITH MALIGNANT HEMOPATHIES

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Background. Clinicians are both interested in the therapeutic results and quality of life for patients. Therefore, regular questioning of patients on quality of care and quality of their life may contribute to increased efficiency of medical act. *Aims*. Our goal was to find out the opinion from the southern Transylvania patients about the importance and management of life quality of hematological patients. Methods: We had realized a transversal study on a group of 252 consecutive hospitalized patients who had agreed to answer at a questionnaire regarding their opinion about the importance and management of life quality of patients with malignant hemopathies. We have analyzed the rates of the answers, their significations and we have drawn useful conclusions for medical practice. *Results*. The medium age of the studied group was 55.42+/-15.02 years. The gender repartition: 59.52% women and 40.48% men. 61.9% of the interviewed patients considered that quality of life is more important than its duration. On a scale of 1 to 10, 64.29% of patients gave the importance of quality of life a 10. Despite the side effects, most patients (85.71%) would recommend chemotherapy/radiotherapy and only 14.24% a natural treatment. 99.21% agreed upon an adjuvant treatment during polychemotherapy sessions. 96.83% considered that anemia decreases quality of life. Despite the side effects of erythropoietin, 76.98% of respondents would recommend it for treating anemia. If they had to choose between erythropoietin and herbal treatments, however, 51.59% would indicate the last. If an iron deficiency is present in these patients, 63.49% would recommend medical treatment and only 36.51% - iron rich foods. 80.16% of questioned patients would indicate stem cells transplantation, if the patients had indication for this. If the patient did not respond to chemo/radiotherapy, to maintain a quality of life as little altered as possible, 53.97% would further indicate palliative chemo/radiotherapy, 21.43% - only the treatment of anemia and potential hemorrhage, 19.84% - only natural treatment and 4.76% - no treatment. 90.48% considered that patient's socioeconomic status can affect quality of life. 94.44% would be willing to donate blood and only 76.19% would be willing to donate bone marrow to increase the quality of life. Almost all (97.62%) felt that an adequate information made by the medical staff can enhance their quality of life. 92.86% found the questionnaire for assessing quality of life useful. Only 68.25% felt that mass media dissemination of information on patient's pathology may affect quality of life, and 29.07% of them felt that in a negative way. Summary : Most interviewed patients are concerned about quality of life, they support therapies with curative intent in parallel with the adjuvant ones, are willing to donate blood and bone marrow and wish to be adequately informed by the medical staff. Not everyone appreciates the proper risk/benefit ratio on erythropoietin therapy. Not all of them want that information on the pathology of the patient to be broadcast by the media. Most of the patients are aware of our present-day reality: socioeconomic status can affect quality of life for patients.

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RELIGIOUS COPING WITH MALIGNANT DISEASE

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Background: Malignant patients develop a psychological adaptation (coping) process which relies on patients' internal or external psychological resources (family, hobby, personality, culture, friends). An important resource is faith and religion. Aims: Analyze the efficiency of religious coping of acute leukemia patients, and identify patterns of religious coping. Methods: We have performed 89 semi-structured interviews with acute leukemia patients who were admitted to the Cluj Hematology Clinic during 2009 and were treated with chemotherapy. Study admission criterion was patient's acknowledgement of religion as a psychological resource. Interviews were conducted by a psycho-social counselor who is also a clinical hematologist, and were analyzed by a bioethics committee (theologian, physicians, philosopher, law specialist).Patients signed informed consent, and the study was approved by the local Ethics Committee. The interviews were analyzed qualitatively using thematic analysis. This study is a part of POSDRU/89/1.5/S/61879 Project co-financed from European Social Fund through Human Resources Development Sectorial Operational Program 2007-2013. Results: Out of the 89 patients, 37% were Christian Orthodox, 5.6% Catholics, and 6.7% Protestants. These percentages are also reflected in the general population. All patients reached an efficient coping. 30% of them experienced inefficient coping periods such as frantically praying for a miracle, questioning God's love, hopelessness, blaming God for the disease, resignation (the disease was God's will), or interpreting cancer as a punishment. Patterns of efficient religious coping were: keeping the faith, and following Christ's example by combining suffering with trusting God. Some patients became more religious after the diagnosis, thus entering a process of spiritual awakening. They reinterpreted the disease as an opportunity for spiritual growth and study of religion. There was only one atheist patient, but his illness brought him to faith. The patients described bargaining with God as an efficient coping pattern. This is one of the phases in Elisabeth Kubler Ross' coping model. In *bargaining* the patient asks God to prolong his life or to allow him to take part in an important event in exchange for a Christian life. In our group, 38% of patients bargained for family-related matters (marrying, having children or grandchildren, seeing their children graduate), 7% bargained for profession-related matters, 2% committed to go on religious pilgrimages, and 17% did not reveal the subject of their bargaining. Some patients said that their religious coping was initiated by their priests (many priests advise patients to keep hoping and to maintain a positive atitude). However, other patients displayed a passive behaviour and accepted the disease as a fatality. Conclusion: It is important for the multidisciplinary team (oncologist, nurse, psychologist, theologian, social worker, family) that provide medical care to malignant patients to realize that religious coping is not always efficient, and to recognize the most common patterns of religious coping. It is recommended that when a member of the team identifies patients with inefficient religious coping, to refer those patients for specialized psychological or spiritual counseling.

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STUDY OF EFFECTIVENESS RECOMBINANT HUMAN ERYTHROPOIETIN AND QUALITY OF LIFE IN LYMPHOPROLIFERATIVE DISORDERS PATIENTS WITH ANEMIA

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Background. Anemia in patients with lymphoproliferative disorders (LPD) is a frequent symptom and can influence the efficacy of antitumor 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), reduce the number of RBC trans-

fusions and improve QoL in patients with chemotherapy induced anemia. Aims. To study the efficacy of rHuEPO and improving QoL in LPD patients with anemia. Methods. There were done this prospective study to investigate the effectiveness of rHuEPO in reducing RBC transfusion-dependency, increasing hemoglobin concentration and QoL in patients (n=83) with low-grade non-Hodgkin's lymphoma (n=18), chronic lymphocytic leukemia (n=21) and multiple myeloma (n=44). The median age of patients was 65.0 years (range 24-82). Recombinant human erythropoietin 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 and planned duration of rHuEPO treatment 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 8.73±1.53 g/dl (37-100 g/dl). Before rHuEPO-therapy 24 patients had received RBC transfusion (2-17 units) during last 3-6 months because of low Hb (3.7-8.0 g/dl). The period of rHuEPO-therapy was from 6 to 16 weeks (mean 9.0±3.4 weeks and median follow-up of 8.5 weeks). During the study period 7 patients (29.2%) followed RBC transfusions also after finishing of rHuEPO treatment and 17 ones of them (70.3%) showed RBC transfusion-independency. In whole group normal Hb concentration (<12.0 g/dl) showed 39 patients (47%). Whole we observed positive response in 53 patients (63.9%), Hb concentration increased from baseline to 12.4±1.19 g/dl (10.5-15.4 g/dl; p<0.01). However 4 patients who didn't receive transfusions before rHuEPO-therapy showed RBC transfusion-dependency because of their Hb fell down less 8.0 g/dl. The reason appearing of transfusion-dependency in these patients was progression of their diseases and so poor effectiveness antitumor therapy. FACT-An demonstrated that rHuEPO-therapy reduced symptoms such as: fatigue, force and physical efficiency, depression, drowsiness, giddiness, headaches, pain in thorax and dyspnea. On a scale from 0 to 4 points, the symptoms reduced from 1.79 to 1.28 in positive response patients indicating an improvement in QoL in the study patients (p<0.05; n=53). However we didn't observe any difference of the symptoms between before beginning and after finishing rHuEPO-therapy in non response patients (from 1.92 to 1.93; p>0.5; n=30); their Hb concentration wasn't changed significantly: from baseline to 9.03±1.99 g/dl (6.9-11.4 g/dl; p>0.1). Conclusions. The study has shown that rHuEPO is effective treatment of reducing RBC transfusion-dependency, increasing Hb and improving QoL in a group of anemic patients with lymphoproliferative disorders.

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THE EVALUATION OF EORTC QLQ-C30 AND ITS ASSOCIATION WITH ANXIETY AND DEPRESSION IN TURKISH MULTIPLE MYELOMA PATIENTS

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Background. Multiple myeloma (MM) patients have depression, anxiety and alterations in quality of life which could be explained by disease itself and by side effects of treatment modalities. AIMS: We evaluated quality of life, depression, anxiety, and their associations with parameters like patients' personal and demographic features and stage of disease. *Methods*. We included 50 (15 females, 35 males) MM patients (mean age, 61.8±9.1; median disease duration, 20.7 months) diagnosed according to IMWG criteria. Patients' age, sex, disease duration, disease type and stage, treatment modalities, and other medical data were recorded from hospital files. For staging purposes, ISS was used. The performance status of the patients were evaluated by means of the ECOG performance scale. Patients were administered EORTC QLQ-C30, EORTC QLQ-MY20, General Health Questionnaire (GHQ) and Hospital Anxiety and Depression Scale (HADS). In order to compare categoric variables, Chi-square test and to compare continuous variables, unpaired-t-test were used. Pearson correlation test was utilized for correlation analysis. Multiple linear regression analysis was used to determine independent factors which affected EORTC QLQ-C30. *Results*. The monoclonal protein was IgG in 22 (44%) of MM patients, IgA in 17 (34%), light chain kappa in 6 (12%) and light chain lambda in 4 (8%). Patients in early (stages I, II) and late (stage III) ISS stages were similar in functional scales, symptom scales, global QoL, subscales of EORTC QLQ-MY20, and HADS-A and HADS-D scores. When MM patients with good (ECOG 0, 1) and poor (ECOG 2 and higher) performance status were compared, it was observed that the functional and symptom scales of EORTC QLQ-C30, global QoL, subscales of EORTC QLQ-MY20, and HADS-A and HADS-D scores were significantly different (p values <0.05). Patients with HADS-D scores >7 were accepted to have depression. The functional and symptom scales of EORTC QLQ-C30, global QoL, subscales of EORTC QLQ-MY20, and HADS-A were significantly different between patients with HADS-D scores >7 and ≤7 (p values <0.001). The physical functioning (OR, 2.53; 95%CI, 1.7-5.8; p=0,001) and role functioning (OR, 4.09; 95%CI, 2.3- 45.5; p=0.03) scores of EORTC QLQ-C30 were independent factors which had positive effect on general quality of life. The treatment-related side effect score of EORTC QLQ-MY20 (OR, -3.2; 95%CI, -4.9 to -1.4; p=0.001) and the presence of depression as assessed by HADS-D (OR, -4.4; 95%CI, -2.6 to -0.4; p=0,007) were factors which had negative effect on general quality of life. *Conclusions*. The performance status as assessed by ECOG, presence of anxiety and depression were associated with quality of life and severity of symptoms in Turkish MM patients. The presence of depression in HADS-D was an independent prognostic parameter which had negative impact on general quality of life score. The factor which had the greatest negative influence on quality of life in MM was observed to be the treatment-related side effect score of EORTC QLQ-MY20.

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ERYTHROPOIETIN USE IN PATIENTS WITH AML OR UNDERGOING ALLOGENEIC HSCT SIGNIFICANTLY IMPROVES QUALITY OF LIFE AND REDUCES RED BLOOD CELLS AND PLATELETS TRANSFUSIONS WITHOUT ANY SURVIVAL EFFECT

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Background: Despite frequent anemia and multiple transfusions during allo-HSCT, recommendations and marketing authorization for EPO use are still missing.Aims: In this prospective study, as primary objective, we evaluated the effect of EPO on patient's quality of life (QOL). Secondary objective was hemoglobin (Hb) recovery. In addition, a paired matched analysis was conducted to compare platelets (Pt) and red blood cells (RBC) transfusion number. Material and methods: We included adult patients with Hb level ≤11g/dl induced after allo-HSCT for any hematological disease. EPO (NEORECORMON® 30000IU) was administered Sc. once/week during a maximum period of 6 months; Hb level was monitored every week. Injections were stopped once Hb level reached 12g/dl without any transfusion. If after 4 injections, no improvement was observed, doses were doubled, and if after 8 injections, no improvement was observed, patient was taken off-study for EPO inefficiency. The QOL was measured at baseline, at 1, 2, 3 and 6 months by the Functional Assessment of Cancer Therapy-Anemia (FACT-An). EPO responders patients were defined as having Hb level ≥12g/dl (EPO CR) or a ≥ 2g/dl increase [EPO partial response (EPO PR)] compared with baseline value without any transfusion requirement. The matching analysis took into account: diagnosis, conditioning, HSC source, number of previous transplants and GVHD. Results: Between April 2006 and December 2009, 61 patients were included, patient characteristics are summarized in Table1. The median number of EPO injections/patient was 8 (2 - 28). We have noticed a trend for improvement of QOL during the 6 months follow-up according to FACT-An anemia (p=0.07). There were 71% of EPO CR after a median time of 39 days (14 - 180). After the pair-matched analysis, 44 patients were matched with at least one case-control patient. When comparing RBC and Pt transfusions, there were 355 units and 555 units in the matched population versus 227 and 574 and in the EPO population p=0.004 and p=0.6 respectively. The multivariate analysis on EPO CR showed the positive impact of Pt levels at baseline, the negative impact of female recipient and major ABO incompatibility. We did not find any significant difference in terms of overall (OS) and event free survival (EFS) between EPO and control group. Conclusion: We showed a positive trend of EPO administration on QOL, an achievement of a normal Hb level and a significant spare of RBC transfusions. A cost-effectiveness study is ongoing and results will be communicated.

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COGNITION AND QUALITY OF LIFE IN ADULT *DE NOVO* ACUTE LEUKEMIA PATIENTS

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Background. A significant percentage of cancer patients will develop cognitive impairment, during or after completion of aggressive treatment. Cognitive deficits are usually subtle and mild, but can occur in various cognitive domains. In AML/MDS patients, impaired cognitive functions have been described, even prior to initiation of chemotherapy. In addition, classical side-effects of aggressive chemotherapy in patients with acute leukemia can have a significant impact on quality of life and on emotional aspects of well-being. Aims: To measure baseline cognitive functions and short-term cognitive evolution, and to assess depression, emotional well-being, and quality of life in de novo acute leukemia patients before and after induction treatment. Methods: Longitudinal-prospective study of adult de novo acute leukemia patients, treated with chemotherapy. This study was approved by the local Ethical Committee and informed consent for each patient was obtained. Eligible patients were enrolled and investigated with a comprehensive cognitive test battery, within five days after admission (pre-induction) and after completion of induction (pre-consolidation). Cognitive functions assessed are attention, executive functions, motor dexterity, and also verbal memory. Depression, emotional aspects of well-being, and quality of life were assessed with self-report questionnaires. *Results*. Twenty adult patients were enrolled between 01/2009 and 06/2010. The median age was 43 years, 50% were male, 80% had AML (20% ALL), and median duration of education was 12 years. Baseline hematological values were assessed, with WBC count (10,4 10E3/uL), RBC count (3,1 10E6/uL) and HgB (9,8 g/dL). Adult patients had normal cognitive functions (range: 1 SD below normative mean) in different cognitive domains, and mainly in attention and executive functions (COWAT, and SCWT), except for mild cognitive deficits in verbal learning (AVLT A1-5), and also especially in motor dexterity (PPT). These functions were heterogeneous at baseline, ranging from severely impaired to good within a cognitive domain. In particular, at baseline low quality of life and role functioning, and a high level of fatigue were found. Global health status related with social functioning, and role functioning related with fatigue. At follow-up, attention, executive functions, motor dexterity, and verbal learning had all improved significantly ($p \leq 0.02$). Changes during chemotherapy treatment were found in depression ($p<0.005$), global health status ($p<0.001$), emotional functioning ($p<0.001$), and symptom scales fatigue ($p \leq 0.05$) and pain ($p<0.01$), and all five scales of well-being ($p<0.05$) (CES-D, EORTC QLQ-C30, and POMS). Global health status related with four of the five functional scales, except for cognitive functioning, and with symptom scale fatigue. Global health status related with depression and vigor. Conclusions: Adult de novo acute leukemia patients exhibit at baseline normal cognitive functions, except for verbal learning and motor dexterity. Cognitive deficits in attention, executive functions, and verbal learning were found similar to previous reported research. However, our data showed less impaired motor dexterity. Moreover, cognitive functions improved at follow-up. At baseline, adult de novo acute leukemia patients have low quality of life, high levels of depression and negative status of well-being. Changes over time, were observed across global health status, dimensions of quality of life, depression, and emotional aspects of well-being.

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PROSPECTIVE EVALUATION OF ORAL MUCOSITIS BY DAY-BY-DAY ASSESSMENT IN HEMATOLOGICAL PATIENTS UNDERGOING STEM CELL TRANSPLANTATION

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Background. Oral mucositis (OM) is a major issue in the setting of hematopoietic stem cell transplantation (HSCT), with serious effects on quality of life. Aim. A prospective observational study was conducted in our BMT Unit to assess the pattern and severity of OM and GIM in patients (pts)

Methods. Assessment of OM in patients (pts) undergoing autologous (auto) or allogeneic (allo) HSCT was conducted daily from conditioning regimen to day +20 or discharge. OM was assessed according to WHO scale. Results. 60 HSCT (10 alloHSCT, 50 autoHSCT) were performed from January 2008 to Agoust 2010 (high dose chemotherapy conditioning regimen), in adult patients with lymphoma (n=21), multiple myeloma (n=29), acute leukemia (n=8) or other diagnosis (n=2). All patients received high dose chemotherapy as conditioning regimen, according to the international guidelines for lymphoma or myeloma. OM prevention measures were: in allogeneic HSCT, mouth rinses alone (clorexidine based); in autologous HSCT, mouth rinses (clorexidine based) alone or associated with probiotics (Lattobacilus brevis CD2) in 43 and 7 pts, respectively. Oral criotherapy was associated in 42/50 pts undergoing intermediate-high dose melphalan-based conditioning regimen and autoHSCT. 57/60 HSCT were eligible for analysis. Data about OM are presented in table. Prevention of OM with oral criotherapy in autoHSCT (high dose melphalan conditioning in myeloma pts only) resulted on reduction of severe OM (grade 3-4). Data about OM prevention with probiotics are encouraging, although preliminary. *Conclusions*. Although preliminary, these data may be helpful to define the impact of OM on patients undergoing high-dose chemotherapy and evaluate preemptive and treatment approach of this complication, with the primary aim to improve patients quality of life.

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ACUTE AND CHRONIC STRENUOUS EXERCISES ALTER ANTIOXIDANT STATUS IN HEMOGLOBIN E TRAIT CARRIERS

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The question of whether Hb E trait carriers are exposed to exercise intolerance at biochemical (antioxidant and oxidative damage) and physiological (physical fitness) levels is addressed. First, 10 Hb E trait(21.2±0.6 yrs) and 10-paired normal Hb (21.4±0.5 yrs) participants performed a maximal oxygen uptake test (VO2max) on a treadmill. Second, 16 Hb E trait (15.6±1.8 yrs) and 16-paired normal Hb E (15.6±1.9 yrs) athletes participated in a 10-week training camp. In Hb E trait, the activity of erythrocyte glutathione peroxidase (GPx) failed to recover after 45 minutes of rest (p=0.049). In both groups of athletes, the training camp allowed to improve anaerobic capacity and maximal muscular strength but depression score was increased and VO2max was decreased. Activities of plasma and erythrocyte GPx and erythrocyte superoxide dismutase were higher at the end of the training camp compared to before (p<0.001). Plasma GPx increase was higher in Hb E (p=0.002) compared to normal Hb athletes. We conclude that Hb E trait could explain inter-individual variability in blood antioxidant markers in response to exercise. Proper training programs (suitable training load and recover) should be of importance concerning Hb E carriers.

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FUNCTIONAL HEALTH STATUS ASSESSMENT IN HAEMOPHILIA PATIENTS WITH THE INTERNATIONAL CLASSIFICATION OF FUNCTIONING, DISABILITY AND HEALTH (ICF, ICF-CY)

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Background. People with haemophilia experience a progressive deterioration of their functional health status. Its rRegular clinical assessment of functional health status provides insight into their process of disablement. As such, the development of a core-set of measurement tools is warranted. AIM: The aim of this the study was to gather data to identify clinically feasible items from existing haemophilia-specific instruments assessing the functional health status and Quality of Life (QoL) of haemophilia patients using the ICF for adults and children, (ICF-CY) frame. *Patients and methods*: Five haemophilia-specific instruments were linked to the ICF separately by 3 trained health professionals according to linking rules developed specifically for this purpose. The degree of agreement between health professionals was calculated by means of the kappa statistics. Bootstrapped confidence intervals were estimated. *Results*: Within the 5 selected instruments 365 concepts were identified, of which 283 concepts were linked to the ICF and ICF CY and mapped into 70 different categories. The estimated kappa coefficients ranged between 0.85 and 0.88. 19 Nineteen categories were included for body function (27%), 4 for body structure (6%), 35 for activities and participation (50%) and 12 for environmental factors (17%). High prevalence in health areas corresponding to ICF and ICF-CY categories for activities and participation were reported for mobility, work, school, sport and social activities. Environmental factors included in the instruments were health economic aspects such as treatment, assistive products for mobility and transportation, and restriction/facilitation in accessing health resources. *Discussion*: The results of This work may contribute to the formation of a preliminary core set of instruments to assess the functional health status of patients with haemophilia (children and adults) giving providing a new insights tool not only for clinical studies but also for epidemiological and health economics studies.

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QUALITY OF LIFE IN ACUTE LEUKEMIA PATIENTS

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Background. Quality of life is now considered an important outcome measuring in patients with malignancies. *Aims*. The aim of our study was to examine the relationship between self-reported quality of life and some clinical, socio-demographic and psychological factors in acute leukemia patients. *Methods*. 63 patients of 15-69 years of age (Median 42 years) with newly diagnosed Acute Myeloid (n=42) or Acute Lymphoid Leukemia (n=21) admitted to a Central Regional Hospital for treatment were eligible. The Linear Analog Self-Assessment was used as an instrument for quality of life examination. Infections, hemorrhage/bleeding, severe anemia (HBG<7.0g/dl), central nervous system leukemia, age, income, education, family status, social support and depression were investigated in their relationship to a self-reported quality of life. The Hospital Anxiety and Depression Scale (HADS) was used for the depression screening. The data were collected shortly after the diagnoses and after induction of remission therapy. The inform consent was obtained from every patient. *Results*. 33 patients were eligible early after the diagnosis. Their Mean Quality of life score was 53.5. The self-reported quality or life did not depend of the clinical course of the disease (such as severe anemia, bleeding, central nervous system leukemia, infections. But quality of life was significantly worse in patients older then 50 years old (t-test, p=0.03) and in depressed patients (p=0.0003). 60.6% of patients (n=20) met the depression criteria early after the diagnosis. After induction of remission therapy 30 patients were eligible. Most of them (n=23) achieved complete remission. Mean Quality of life score was 69.7 at this period of treatment. 46.7% of patients (n=14) reported depression. The self-reported quality of life measuring after induction of remission therapy did not depend of such complications of the treatment as bleeding, pneumonia, mucositis, hepatitis, enteropathy, nausea. This complications were successfully treated (antibiotics, anti-emetics, transfusions, etc.) Quality of life was significantly poorer in patients who had febrile neutropenia (p=0.01), age older then 50 years(p=0.006), low social support score (p=0.045), singe family status (living alone) (p=0.01) and depression (p=0.001). Quality of life of patients with different education level and different income level were not significantly different (p=0.18 and p=0.7 respectively). The income level did not differ much between patients. *Conclusions*. Self-reported quality of life of newly diagnosed acute leukemia patients was significantly poorer in patients with depression or low social support level, and patients older then 50 years old. Depressive symptoms are prevalent.

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IMPACT ON QUALITY OF LIFE OF OUTPATIENT TREATMENT IN HIGH RISK MYELODYSPLASTIC SYNDROMES: RESULTS OF A PILOT STUDY

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Background. quality of life (QoL) in myelodysplastic syndromes (MDS) depends on various factors: global health status, social setting, disease characteristics (anaemia). Moreover uncertain evolution of the disease induces emotional distress that impacts on QoL and patients experienced a high level of fatigue. Treatment of anemia with erythropoiesis stimulating agents (ESA) or hypomethylating agents, depending on the status of the disease, will improve patient functional status and quality of life. Purpose: few data are available to know if home treatment improves patient QoL: 102 patients with advanced colon cancer were treated with chemotherapy either in outpatient or inpatient care(1). QoL was improved in outpatient group as well as social function score. In hematological issue one study including 41 patients in bone marrow transplantation2 showed benefit of home treatment in term of QoL. No data are available concerning outpatient MDS treatment. *Patients and methods*: 40 patients were included in a single center, 20 patients receiving chemotherapy at home and 20 patients at hospital, matched on gender and age. Quality of life (QoL) assessment was performed using EORTC QLQ-C30. In addition locus of control, stress and mental adjustment strategies were evaluated (using MAC 44, CLCS and stress scale). Results: mean age was 70 years. No difference was found between the 2 groups excepting stress which was higher in female than in male. 50% of patients who received treatment at hospital would like to change. At the opposite 80% of patients treated at home prefer to continue A negative correlation was found at home between age and stress and a positive correlation between number of person at home and stress. Conclusion and perspectives: better knowledge of relevant factors which determine QoL in this population as family, caregivers, distress, and strategy of adjustment will be necessary. Larger number of patients is needed to better understand underlying conditions that could affect QoL.

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FREQUENCY AND SIGNIFICANCE OF PSYCHIATRIC DISORDERS IN HEMATOLOGICAL AND NONHEMATOLOGICAL MALIGNANCIES

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We investigated the frequency of anxious-depressive psychiatric disorders (anxiety and depression) in subjects with hematological and non-hematological malignancies; we evaluated 1031 subjects hospitalized in the past 30 months, among them 615 (59.65%) were diagnosed with non-hematologic malignancies, 341 (39%) were diagnosed with malignant hematologic disease and 75 (7.35%) were diagnosed with cancer and hematologic manifestations; frequency of mental disorders in subjects with hematological malignacies was 39%, statistically significantly different ($p < 0.0001$) of frequency of mental disorders in subjects with depressive anxious and non hematological malignancies: 26.5%; In the subjects with hematological malignant diseases, we identified a 25% frequency of psychiatric disorders in subjects with acute myeloproliferative disorders, 40% in subjects with acute lymphoproliferative disorders, 23.5% in subjects with MDS, 33.75% in subjects with chronic myeloproliferative disorders, 44.96% in subjects with chronic lymphoproliferative disorders, 46.6% in subjects with Multiple Myeloma ; Note a frequency of only 21.33% of anxious depressive psychiatric disorders in subjects with para neoplastic disease (39% in hematologic malignancies, 23.33% in para neoplastic hematologic manifestations:$p < 0.01$). In the non malignant hematologic diseases lower incidence of mental disorders has been identified in autoimmune hemolytic anemia (10.3%) and highest incidence was found in Anti Phospholipid syndrome (49.5%) ($p < 0.01$). Impact of psychiatric disorders in non-hematological and hematological malignancies was significant in terms of quality of life, therapeutic compliance. Impact of the prognosis will be estimated by expanding the lot and monitored the dynamics analysis; in terms of APL syndrome, high frequency of mental disorders is an argument for APL antibodies involvement in the pathogenesis of neuro psychiatric disorders; Phase of study conclusions indicate depressive mental disorders associated with hematological malignancies anxious to represent a significant factor influencing quality of life, therapeutic compliance and possibly prognosis of these diseases, requiring strong multidisciplinary approach.

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USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINE IN CHILDREN WITH CANCER: REPORT OF A BELGIAN CENTER FOR PEDIATRIC HEMATOLOGY ONCOLOGY

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Background: Complementary and alternative medicine (CAM) is a heterogeneous group of products, practices, medical and health care systems defined as not presently part of the conventional medicine. For the last decades, the use of CAM has become increasingly frequent, particularly in children with chronic illness and cancer in whom CAM is commonly reported along with conventional care. Aims: To define better the actual place of CAM in children with cancer in Belgium and to understand better the circumstances of their use and the expectations of patients and families using CAM, we have realized an observational study in children treated at the pediatric hematology oncology department of the Saint-Luc University Hospital in Brussels. Methods: A questionnaire was distributed to parents of children diagnosed with cancer between January 1st 2003 and December 31st 2009, who were not deceased or in palliative care. The participation was volunteer and the study was approved by the hospital's ethical committee. Results: The survey was sent to 301 families of patients aged from 0 to 21 years at the time of diagnosis. 153 questionnaires (50,8%) were returned and analyzed. In these, the prevalence of CAM use was 63 % (55% after excluding prayer and psychotherapy). For 21,5% of users, CAM therapy was initiated prior to cancer but maintained or expanded during or after conventional treatments. 58,1% of patients used CAM for the first time during conventional treatments and 20,4% started CAM after conventional treatments. The most frequently used CAM were homeopathy (24%), massage therapy (16,2%), dietary supplements (15,6%). The most common expectations of users were an improvement of the child's wellness (22%), a decrease of the side effects of conventional treatments (13,9%) and a reinforcement of immunity (12,4%). Parents reported benefits in 90,5% of cases although negative effect was described in 10% of cases. The duration of use was 1-3 months in 13,9%, 3-6 months in 11,3%, 6 months-1 year in 18,3% and > 1 year in 46,1% of users. The frequency of use depends on the type of CAM: alternative medicines such as homeopathy were mainly used daily while manipulative and body-based methods such as massage were commonly administrated on demand. Despite a full agreement on conventional treatment, 55% of families did not disclose their CAM use to their physician. Factors as age, sex, type of tumor, place of residence or prior CAM use were not predictive for use of CAM. However, we find a significant correlation with the level of education of the mother and/or the main person in charge of the child. Conclusions: CAM is part of the treatment in a large number of children with cancer. It is commonly perceived as safe by patients and families but its undisclosed combination with conventional therapies increases the risk of drug interaction and could interfere with the analysis of clinical trials. This work underlines the necessity for physicians, CAM users and practitioners to improve their communication. Such attitude could also allow a better study of CAM efficacy.

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A WAY TO REDUCE PAIN IN BONE MARROW DIAGNOSTIC PROCEDURES

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Background. Bone Marrow core biopsy and aspiration represents a standard procedure in diagnosis and staging of hematological malignancies. Attempts to reduce pain during aspiration and biopsy procedure have led to the development of new techniques such as electrically powered devices.

The use of these devices may reduce pain in bone marrow aspiration. Nevertheless there is no protocolized clinical studies comparing different pain scores using this new technique. versus manual techniques. *Aims*: The objective of this randomized, protocolized, single blind study is to measure the seconds it takes for the needle to penetrate the skin and reach the bone marrow just before aspiration and measure the pain produced by this technique compared to manual bone marrow aspiration. *Methods*. 30 patients 18 men and 12 women were selected, and randomized into 2 groups. In the group 0 (n=15) powered aspiration (Vidacare battery powered device) was applied whereas in group 1 or control (n=15), aspiration was performed with a manual device (Cardinal Health Illinois Bone Marrow Needle). Every patient received subcutaneous 2% Mepivacaine in the punction area. Time from the cortical contact to aspiration was measured in seconds. Previous pain and pain during insertion were registered according to the Patient Pain scale in which 0 is absence of pain and 10 is the most extreme pain. In order to evaluate the pain reduction a new parameter called Pain Reduction was obtained by subtracting previous pain minus pain during insertion. Statistical analysis was performed throug non parametrical tests for unpaired data. *Results*. Time to aspiration was lower and pain reduction was higher on the electronically powered device ($p=0.025$ and $p=0.001$ respectively) compared to control group. *Conclusions*. The use of ellectrically powered device has shown in our series a significative reduction in operational biopsy time, as well as a pain reduction compared to traditional manual devices.

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CHLORPROMAZINE PLUS METHOCLOPRAMIDE AND PREDNISONE IS COST-EFFECTIVE IN CONTROL OF LATE HEMESIS IN PATIENTS PRETREATED WITH PALONOSETRON THAN IN THOSE TREATED WITH TROPISETRON

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Background. Late hemesis is often difficult to resolve, especially when patient received highly hemetogenic chemotherapy regimens. Data regarding the most useful drug to employ in late hemesis on the base of the type of anti HT3 drug previously used in acute hemesis (palonosetron or tropisetron) are lacking. *Aim.*Aim of this study is to define which is the best cost-effective antihemetic therapy in late hemesis when palonosetron or tropisetron are used in acute hemesis in the same type of patient. *Methods.* This study is a prospective monocentric study. We considered 95 patients in period June 2007- January 2011, receiving highly and moderate hemetogenic chemotherapeutic regimens for haematologic malignancies. 50 patients (5 HD, 25 NHL, 6 ABMT, 14 AML) received tropisetron 2 mg tid i.v. until 48 hours after the end of chemotherapy. Of these 15 received DHAP-like regimen, 15 received IGEV, 6 BEAM and 14 "3+7"regimen. M/F was 28/22 and median age was 65 years (R30-75). 45 patients (4HD, 26 NHL, 5 ABMT, 10 AML) received palonosetron 250 mcg i.v. day 1 of chemotherapy. Of these 20 received DHAP-like regimen, 10 received IGEV, 5 BEAM and 10 "3+7"regimen. M/F was 26/19 and median age was 67 years (R28-76). All chemotherapy regimens were of 5 or 7 days of duration. If patients presented nausea and vomiting 48 hours after the end of chemotherapy, they received dexametason 4 mg + methoclopramide 20 mg i.v. tid. If nausea and vomiting persisted, they added largactil 12.5 mg i.v. tid. Results were evaluated by Fisher exact test. A cost analysis was performed considering the median of global direct and indirect antiemetic expense for each patient. *Results.* Out of 45 patients receiving palonosetron, 5 had late hemesis, 4 requiring chlorpromazine and all responded to treatment. Out of 50 patients receiving tropisetron, 8 had late hemesis, 6 requiring chlorpromazine and only one responded to treatment.No difference were noted in late hemesis between palonosetron and tropisetron (two tailed Fisher text p 0.56). Chlorpromazine was effective in control of late hemesis mainly in palonosetron group (two tailed Fisher text p 0.046) Only observed side effect was a slight drowsiness in all patients receiving chlorpromazine. In palonosetron group, median global antiemetic direct expense for each patient was 107.25euro (R107.25-834.53), while in tropisetron group was 410.5euro (R30-515). Median antiemetic indirect expense was 20 euros in palonosetron group, while in tropisetron group was 280 euros .*Conclusions.* In this study chlorpromazine in association with methoclopramide and dexametason is cost effective in management of late hemesis of patients treated with palonosetron and receiving moderate and highly hemetogenic chemotherapy regimens for 5-7 days.

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CONTRIBUTION OF AGE, GENDER, BODY WEIGHT, CYP2C9, CYP4F2 AND VKORC1 GENOTYPE TO THE WARFARIN ANTICOAGULANT RESPONSE IN OMANI PATIENTS

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Objectives. The objective of this study is to assess the contribution of age, gender, body weight, CYP2C9, CYP4F2 and VKORC1 genetic variants to the warfarin dose requirement in the Omani patients. Methods: Blood was collected from 240 Omani patients taking warfarin daily and on stable anticoagulation with INR values between 2 to 3 at 3 consecutive readings, after an informed consent. The genetic polymorphisms of warfarin dose influencing loci (CYP2C9, CYP4F2 and VKORC1) were studied and the demographic factors (age, sex, weight) were recorded. Results: CPY2C9 genotyping (mutant alleles - *2, *3 & *8) showed that 73.2%, 23.5% and 3.3% of these patients were homozygous for wild type allele, heterozygous and homozygous or compound heterozygous mutant allele respectively. VKORC1 (g.-1639G>A) genotyping showed that 46.6%, 44.5% and 8.9% of these patient had GG, GA or AA genotype respectively. Patients with VKORC1 (g.-1639GG+GA)and CYP2C9 *1/*1 genotypes respectively required 5.5+3.8 mg/day and 5.42+4.2 mg/day ,which was significantly higher than in those with -1639 AA (2.5+1.3 mg/day; p <0.05) or CYP2C9 homozygous or double heterozygous *2 or *3 (2.5 + 1.6 mg/day; p<0.05) genotype. Univariate analysis revealed that age, weight, CYP2C9, and VKORC1 g.1639AA were significantly associated with warfarin dose. However, the multiple regression model for warfarin dose indicated that a significant contribution to the warfarin dose was seen only from age (r2=0.0208; p=0.02), CYP2C9 (r2=0.0144; p=0.046) and VKORC1AA genotype (r2=0.154; p=000).Conclusions: The study showed that although age, weight, CYP2C9, and VKORC1 polymorphisms affect the warfarin dose requirements in univariate analysis, stepwise regression analysis model showed that only VKORC1 was the dominant predictor of warfarin dose requirement overshadowing all other variables. Therefore, VKORC1 AA is the most important factor to consider while using dosing algorithms to improve safety of warfarin therapy in Omani patients.

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IMPROVING STANDARDS OF CARE FOR HAEMOGLOBINOPATHIES IN A LOW PREVALENCE REGION

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Births of patients with major Haemoglobin disorders tend to be concentrated in a few urban areas throughout the UK. This has led to the development of some highly specialised centres for the care of patients in these areas. However in areas of lower prevalence, services may be poorly developed, coordinated and funded, leading to inequalities in access to care for affected patients. Progress in the roll out of antenatal and neonatal screening programmes, publication of peer reviewed national standards of care, information on systems failures from the NCEPOD report on deaths of patients with haemoglobin disorders and Dept of Health funded review of services, have all contributed to a drive to commission effective services in low as well as high prevalence areas.We report an initiative by the East Midlands Commissioning Team, to support a multidisciplinary hub and spoke network of services for patients throughout the East Midlands, with outreach services from two linked centres to surrounding areas. The £250k package supported the appointment of a data manager, two WTE hospital based nurse specialists and support for roll out of the regional transcranial Doppler (TCD) and psychology support services. Within a year a regional database has been established, >50% patients have been recruited to the newly established National Haemoglobinopathy Register, the EMidlands TCD service has achieved 100% coverage of the main centres, with outreach service for small centres available from 2011, development and access to agreed regional guidelines for acute management of sickle crises, positive report of nationally run peer review of children's services, impact of nurses on outpatient non-attendance, as well as an annual report to commissioners. Novel approaches to the use of scarce resources are being developed (eg screening assessement tools for referral to psychology services, assisted regional audit, and education material). The process has contributed to the concept of developing a national commissioning tool for haemoglobinopathy services, which will outline standards of care and services that must be provided in all areas. These will be available for use by any commissioning structure that emerges from NHS reforms in the UK.

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REVIEW OF OUT OF HOUR COAGULATION SCREEN REQUESTS IN TWO UNIVERSITY TEACHING HOSPITALS

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Background. Coagulation screens are invaluable tests for diagnosis and investigation of congenital or acquired bleeding disorders. They designed to provide rapid non-specific information, which allows an initial broad categorization of haemostatic problems. Unfortunately false positive or negative results are common. Haematologists are frequently contacted by other teams for abnormal coagulation screens and authorisation of such results is part of their daily duty. It is very important that these tests to be requested properly and results explained in the context of clinical condition. Anecdotal studies indicated that irrelevant requests and lack of clinical details on request forms are more common in out of hour requests. *Methods*. We retrospectively analysed out of hour coagulation screens requested between Wednesday 13.05.2010 and Monday 18.05.2010 in Royal Victoria Infirmary and Newcastle General Hospital- both teaching university hospitals in Newcastle, UK. Relevant information obtained

from request forms, patients notes and laboratory electronic data. *Results*. There were total of 321 requests 139 (43%) were form A&E, 52 (17%) from ICU, 51 (16%) were from medical, 40 (12%) were form surgical, 24 (7%) were from paediatrics and 15 (5%) were from obstetric and gynaecology. 163 (51%) had irrelevant clinical details and 96 (30%) had relevant information. There were no clinical details available on 62 (19%) requests, most commonly from A&E (46%). Only 49 (15%) screens yielded abnormal results. *Conclusions*. These results indicate a large number of irrelevant requests. They also show a considerable amount of forms either with no clinical details or irrelevant information. Such unnecessary or poorly informed requests waste a significant laboratory and clinical time and could potentially raise the need for further detailed investigations. Eventually this can result in more costs, delay in patients' discharges and further unnecessary stress to patients and workload to staff. Moreover lack of proper clinical details and irrelevant requests would affect the outcome of sample authorization reports by haematologists.

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AN AUDIT OF COAGULATION SPECIMENS REQUIRING DIRECT HUMAN INVOLVEMENT AT AN ACADEMIC LABORATORY

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Background. Currently there is an increasing trend towards automation in the laboratory testing of coagulation. Some coagulation specimens cannot be summarily tested in currently utilized fully automated systems because of suboptimal pre-analytical influences, such as underfilled tubes, that would yield spurious results. Other specimens require additional sample preparation, such as double centrifugation, before testing. *Aims*. To determine what proportion of specimens received by the coagulation section of the Universitas Academic Laboratory required direct human involvement for screening and/or additional sample preparation prior to testing and to analyze the contributing factors. Methods: All specimens received for coagulation testing from 1 December 2008 to 30 September 2009 were audited. Specimens inspected by technologists and found to be under-filled, clotted, collected in incorrect specimen tubes, lipaemic, icteric, haemolysed, from patients with haematocrits >0.55 L/L, old (>4 hours since sampling), unlabelled or incorrectly labelled, and specimens for lupus anticoagulant or activated protein-C resistance (APCR) testing (both requiring double centrifugation), were recorded in a logbook on a daily basis. *Results*. Of the 8614 specimens received, 1091 (12.7%) fell into one or more of the categories. Underfilled: 246 (2.9%); clotted: 148 (1.7%); collected in incorrect specimen tubes: 127 (1.5%); lipemic: 8 (0.1%); icteric: 43 (0.5%); haemolysed: 75 (0.9%); from patients with haematocrits >0.55: 2 (0.02%); old (>4 hours since sampling): 160 (1.9%); unlabelled or incorrectly labelled: 20 (0.2%); lupus anticoagulant: 347 (4.0%); activated protein-C resistance (APCR) testing: 6 (0.1%). In total, 353 (4.1%) required additional sample preparation (double centrifugation) and 829 (9.6%) required screening to avoid spurious results. *Conclusions*. A substantial proportion (12.7%) of specimens received for coagulation testing required direct human involvement prior to testing and would not have been suitable for testing in currently utilized fully automated systems. Of these specimens, 9.6% were subject to suboptimal pre-analytical influences and would have yielded spurious results had they been summarily tested in a fully automated system. Strategies must be developed to prevent this phenomenon

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MICROSCOPIC EXAMINATION OF BONE MARROW ASPIRATION SMEARS: DIAGNOSTIC AGREEMENT OF HEMATOLOGISTS AND HEMATOPATHOLOGISTS ON COMMON HEMATOLOGICAL DIAGNOSES

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Background. Morphological examination of bone marrow aspiration slides is one of the routine and the most valuable diagnostic tools to evaluate hematological disorders for the hematologists. Hematopathologists evaluate those in combination with more complicated techniques such as immunohistochemistry. However, there are no data about the agreement levels on microscopic evaluation of bone marrow aspiration slides between hematologists and hematopathologists. *Aims*. To determine the agreement levels between hematologists and hematopathologists on common hematological diagnoses. *Methods*. A random sample of 569 patients who underwent bone marrow aspiration procedure with or without trephine biopsy for clinical purposes between January 2008 and December 2009 were chosen. Archived Wright-Giemsa-stained bone marrow aspiration smears and corresponding pathology reports belonging to those patients were reviewed. Patients who had an adequate bone marrow aspiration smear and corresponding hematopathology report were included into the study (n=325). Two-hematology fellow and a consultant hematologist with a 12, 24, and 120-month experience on microscopic evaluation, respectively, were assigned to evaluate smears. Hematologists evaluated 15 bone marrow smears per week during their training period. In order to determine inter-observer agreement, hematologists reviewed 10 smears. The diagnoses of smears were obtained from hematopathology reports. For each slide, brief clinical and laboratory information was provided. Hematologists gave a diagnostic code for every slide according to predefined diagnoses based on hematopathology reports. A good inter-observer agreement was achieved between the hematologists. Then a pilot test was performed in order to test the feasibility of coding system and to accommodate hematologists to coding system. Hematologists reviewed 30 smears and coded every slide for one diagnosis. An acceptable agreement was observed between hematologists and hematopathologist. At last, hematologists evaluated all 325 slides. Agreement between hematologists and hematopathology reports was assessed (Figure 1). Agreement analyses were performed by Cohen's kappa, Fleiss kappa, and AC1 statistics. Kappa and AC1 values were interpreted as follows: less than 0.20, poor; 0.20 to 0.40, fair; 0.40 to 0.60, moderate; 0.60 to 0.80, good; and 0.80 to 1.00, very good agreement. *Results*. Overall agreement between hematologists and hematopathologist was good (κ: 0.76, AC1: 0.78). Hematologists were especially successful for the diagnoses of acute leukemia, multiple myeloma, and chronic myeloproliferative neoplasias with excellent agreement levels (Kappa: 0.91, AC1: 0.90; kappa: 0.92, AC1: 0.91; and kappa: 0.91, AC1: 0.91, respectively). Diagnosis of iron deficiency anemia by bone marrow aspiration smear was a challenge. (κ: 0.31, AC1: 0.22). Agreement for the other hematological diagnoses was acceptable (Figure 2).

Figure 1.

Figure 2.

Conclusions. Microscopic evaluation of the bone marrow smears performed by hematologists gives mostly reliable and valuable results. This is especially important for the diagnosis of acute and life-threatening conditions such as acute leukemia. Assessment of bone marrow smears as well as basic history, physical examination, laboratory data, and flow cytometry may prevent treatment delays. It may allow verification of diagnosis made by hematopathologists. Routine use of iron staining in patients with anemia may increase the diagnostic power of bone marrow smear when used by hematologists.

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