Acute myeloid leukemia - Biology

B1244

PROLIFERATION OF ACUTE MYELOGENOUS BLASTS AS EFFECT OF DIFFERENT CYTOKINES CAN BE USED TO IDENTIFIED PATIENT AND CYTOKINE SUBSETS ALSO BY GENE EXPRESSION PROFILES

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Background: Acute myeloid leukemia (AML) is a heterogeneous group of hematopoietic disorders characterized by bone marrow accumulation of immature leukemia cells. Uncontrolled proliferation of blasts is a hallmark of AML. Several cytokines can function as growth factors for AML blasts, and abnormalities of cytokine and growth factor signalling pathways are characteristic for AML.

Aims: We investigated proliferation features of two different groups of AML patients, to further use the findings for classification of patients and linked them to distinct gene expression profiles.

Methods: AML cells were cultured in standard in vitro condition in the absence or presence of seven given cytokines; stem cell factor (SCF), granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), FLT3-ligand (FLT3-lg) and IL3 (IL-3) and interferon γ (IFNγ). Gene expression patterns were obtained for 84 samples belonging to the two groups containing 40 (group I cohort) and 54 (group II cohort) patients respectively. Proliferation rates were detected after seven days by thymidine incorporation assay. Results were used to classify patients by clustering models.

Results: Microarray experiments performed using the Illumina iScan Reader. Resulted-based proliferation rates of AML blasts compared to blasts cultured in the absence of cytokines (P<0.001). An unsupervised hierarchical cluster model identified three main patient clusters based on cytokine dependent proliferation (i) high proliferation rates both in the presence and absence of cytokines, (ii) high proliferation rates in the presence of cytokines, but low proliferation without cytokines, (iii) lower proliferation rates with or without cytokines. The high proliferation clusters were characterized by high expression of CD34 and low frequency of the 

B1225

DIFFERENTIAL EXPRESSION OF NEW METNASE (SETMAR) TRANSCRIPT VARIANTS IN HEMATOLOGICAL CANCERS - INCREASE IN AML PATIENTS

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Background: Evolutionary, the Metnase gene, also known as SETMAR and present in several transcript variants, is regarded as a unique fusion protein leading factor (G-CSF), macrophage colony stimulating factor (M-CSF), FLT3-ligand and IL3 (IL-3) and interferon γ (IFNγ). Gene expression patterns were obtained for 84 samples belonging to the two groups containing 40 (group I cohort) and 54 (group II cohort) patients respectively. Proliferation rates were detected after seven days by thymidine incorporation assay. Results were used to classify patients by clustering models.

Results: Microarray experiments performed using the Illumina iScan Reader. Resulted-based proliferation rates of AML blasts compared to blasts cultured in the absence of cytokines (P<0.001). An unsupervised hierarchical cluster model identified three main patient clusters based on cytokine dependent proliferation (i) high proliferation rates both in the presence and absence of cytokines, (ii) high proliferation rates in the presence of cytokines, but low proliferation without cytokines, (iii) lower proliferation rates with or without cytokines. The high proliferation clusters were characterized by high expression of CD34 and low frequency of the

B1226

NEWLY DIAGNOSED ADULT AML AND MPAL PATIENTS FREQUENTLY SHOW CLONAL RESIDUAL HEMATOPOIESIS

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Background: The current WHO classification of myeloid neoplasms identifies three major subgroups of heterogeneous diseases, -acute myeloid leukemia (AML), myelodysplastic syndromes (MDS) and myeloproliferative neoplasms

Methods: PCR using primers located in exon 1 and exon 3 of Metnase target-
(MPN) - with a significant degree of overlap among them. Within AML, disease heterogeneity translates into six major subgroups each of which still contains several specific diagnostic entities, being of utmost clinical relevance because of their distinct prognosis. Among cases classified as de novo AML, a significant percentage of patients show AML with myelodysplasia related changes, representing a unique poor-prognosis WHO category of the disease, independency of the lineage and cytogenetic alterations of myeloid blasts. Overall, these criteria confirm the clinical relevance of the presence of MDS-associated features in AML, for unequivocal differential diagnosis among de novo AML, secondary AML and AML with myelodysplasia related changes, that reflects the presence of an underlying clonal disorder of residual hematopoiesis, fact that at present, neither cytomorphology nor cytogenetics alone is sensitive enough to assess in every de novo AML patient.

Aims: To investigated the existence of an underlying clonal disorder of residual mature/maturing neutrophils, monocytes and NRC in the BM of de novo AML and MDS. The potential correlations between the molecular findings and both dysplastic features by cytomorphology and altered immunophenotypes by multiparameter flow cytometry.

Methods: iFISH studies, immunophenotypic studies, HUMARA assay (in female cases) and presence of Kit mutation was performed on FACS-purified cell populations from newly-diagnosed AML and MPM patients.

Results: The clonal nature of blast cells was demonstrated in all (59/59; 100%) newly-diagnosed AML and MPAL patients analyzed, meanwhile clonality of residual BM compartments of mature/maturing neutrophils and monocytes and/or NRC was confirmed for at least one of these three cell compartments in most newly-diagnosed AML and MPAL cases (49/59; 83%).

No statistically significant differences were observed as regards the distribution of cases with clonal vs. non-clonal residual hematopoiesis among the different diagnostic subgroups of newly-diagnosed AML.

Residual BM mature/maturing granulomonocytic and NRBC showed aberrant phenotypic expression of mature neutrophils in 52/59 cases (90%). Accordingly, immunophenotypically altered neutrophils, monocytes and NRBC were found in 51/58 (88%), 38/52 (73%) and 27/45 (60%) patients respectively.

Cases with clonal residual hematopoiesis showed a significantly higher frequency of aberrant phenotypic expression of mature neutrophils (98% vs. 50%; P<0.001), monocytes (83% vs. 30%; P=0.003) and NRBC (71% vs 13%; P=0.01) and a higher mean number of phenotypic alterations/case (6.9±3.0 vs. 3.7±2.5, P<0.001) as well as greater number of altered cell populations/case (2±0.8 vs. 1±1.2, P=0.03), than the other cases.

Summary / Conclusion: The vast majority of adults with newly-diagnosed AML and MPAL displays an underlying clonal hematopoiesis, residual mature/maturing granulomonocytic and/or erythroid cells displaying chromosomal alterations, which are frequently shared by the blast cells, in addition to multiple aberrant phenotypes that appears to involve most WHO 2008 diagnostic subtypes of AML and also MPAL. Whether the presence versus absence of clonal residual hematopoiesis contributes to a better prognostic stratification of newly-diagnosed adult AML and MPAL patients deserves further investigations.

B1228 FLOW CYTOMETRIC ASSESSMENT OF HUMAN EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1 (hENT1) EXPRESSION IN ACUTE MYELOID LEUKEMIA (AML) AND MYELODYSPLASIA (MD) AND ITS RELATIONSHIP TO THE ASX1 Mutant

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Background: Assessment of hENT1 expression by multiparameter flow cytometry (MFC) in AML and MDS. To correlate results to morphology, cytogenetics (CG) and molecular genetics (MG).

Methods: We studied 130 AML and 94 MDS at diagnosis, 125/99 male/female, median age 66.5 (AML) and 73.3 years (MDS). CG was done in 115 AML and 84 MDS. hENT1 expression was quantified by a new multicolor flow cytometry assay with monoclonal antibodies against hENT1, CD45, CD64 and myeloperoxidase. Median fluorescence intensities (MFI) of hENT1 were determined in myeloid progenitors (MP), granulocytes (Gra) and mononuclear cells (Mo) and correlated to hENT1 expression in AML and MDS.

Results: hENT1 expression by MFC was feasible. In AML patients, significantly lower hENT1 expression was found in myeloblasts (M) compared to mature (M1, M2, M3, M4, M5, M6) leukemic populations (M1, M2, M3, M4, M5, M6) and granulocytes (Gra) as well as in intermediate and high risk pts (3.07 vs. 1.65, P<0.001) as compared to low risk pts (3.07 vs. 1.65, P<0.001). hENT1 expression was significantly higher in M and Gra as compared to very low risk pts (4.07 vs. 1.77, P=0.001, 4.07 vs. 1.77, P=0.001), respectively. In intermediate risk pts, hENT1 expression was significantly lower in Mo as compared to very low risk pts (4.07 vs. 1.77, P=0.001, 4.07 vs. 1.77, P=0.001) and Gra as compared to very low risk pts (4.07 vs. 1.77, P=0.001, 4.07 vs. 1.77, P=0.001), respectively. In high risk pts, hENT1 expression was significantly lower in Mo as compared to very low risk pts (4.07 vs. 1.77, P=0.001, 4.07 vs. 1.77, P=0.001) and Gra as compared to very low risk pts (4.07 vs. 1.77, P=0.001, 4.07 vs. 1.77, P=0.001), respectively. In low risk pts, hENT1 expression was significantly higher in Mo as compared to very low risk pts (4.07 vs. 1.77, P=0.001, 4.07 vs. 1.77, P=0.001). hENT1 expression was significantly lower in Mo as compared to very low risk pts (4.07 vs. 1.77, P=0.001, 4.07 vs. 1.77, P=0.001) and Gra as compared to very low risk pts (4.07 vs. 1.77, P=0.001, 4.07 vs. 1.77, P=0.001), respectively.

Summary / Conclusion: At present, neither cytomorphology nor cytogenetics alone is sensitive enough to assess in every de novo AML patient.

B1227 MINIMAL RESIDUAL DISEASE AND CLEARANCE OF LEUKEMIC BLASTS IN ACUTE MYELOID LEUKEMIA: TIMING AND CUT-OFF VALUES BETWEEN MFC AND WT1

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Background: The detection of leukemia–associated immunophenotypes (LAIPs) by multiparameter flow cytometry (MFC) and the quantification of WT1 RNA levels were widely used to monitor minimal residual disease (MRD) in AML and MDS. To correlate results to morphology, cytogenetics (CG) and molecular genetics (MG).

Methods: Fresh bone marrow samples from 88 AML patients were obtained at diagnosis and during post-induction and pretransplant times. Cut-off values needed to lower the AML compared to previous times and the clearance of LAIPs to monitor disease progression.

Results: The assessment of MRD by MFC and WT1 at T4 predicted the recurrence better than the other time points. The more reliable cut-off values resulted 0.1% for MFC at T1, T2 and T3 and 0.05% at T4. The cut-off values by WT1 were 90.0 at T1 and T3, 71.0 at T2 and 54.0 at T4. When the clearance was considered, the best prediction of relapse was evidenced at T4 with cut-off values of 3.07 and 1.65 for MFC and WT1, respectively. Patients with values of MRD above 0.05% at T1 equaled or fell below 2.81 (P=0.02, 62%) had a significantly poorer expected disease survival compared to those with higher levels (n=18 pts, 40%; P<0.01; crude HR: 5.0; 95%CI: 1.4-17.5). This difference was preserved after adjusting for age, gender, Hb levels and stem cell transplant (P=0.01; adjusted HR: 7.7; 95%CI: 1.8-29.6). Results from MFC clearance showed that values of MRD above 0.1% at T1 equaled or fell below 2.81 (P=0.02, 62%) had a significantly poorer expected disease survival compared to those with higher levels (17 pts, 38%; P<0.01; crude HR: 9.7; 95%CI: 2.2-43.2). This difference was preserved after adjusting for age, gender, Hb levels and stem cell transplant (P=0.01; adjusted HR: 6.0; 95%CI: 0.1-0.7). At T1 no statistically significant correlations were observed between WT1 values, WT1 Log clearance values and DFS. No significant correlations between MRD and DFS were obtained at T2.

Summary / Conclusion: From our study, the most predictive evaluation of MRD was performed after the transplant while the post-induction evaluation stratified better high risk patients among pre-transplant times. Cut-off values needed to be lower than the transplant compared to previous times and the clearance of LAIPs to monitor disease progression.
es to induction therapy while overall worse outcome is mostly due to early relapses. Higher index in AML than MDS may be considered causal for better response to nucleoside-based chemotherapies in AML. Data within MDS may be interpreted accordingly, low risk CG (IPSS-R1) showing higher index in MP and pt with poor risk MG presenting lower index in Gra, though the latter results have to be judged cautiously due to low pt numbers. Further analyses are warranted to explore hENT1 expression in AML and MDS more comprehensively and to correlate with outcome.

B1220

CELL CYCLE MODELING OFFERS A PLATFORM TO OPTIMIZE COMBINATION OF CYTARABINE AND FLT3 INHIBITORS IN ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) patients with FLT3 mutations have poor response to cytarabine-based therapy. FLT3 inhibitors may over come cytarabine resistance and improve treatment outcome. Depending on the sequence of administration, combining FLT3 inhibitors and cytarabine can achieve synergistic or antagonistic effects.

Aims: The goal of our work was to develop a pharmacodynamic model that mechanistically describes the effects of cytarabine and FLT3 inhibitors on cell proliferation, cell cycle distribution, and optimize combination regimens.

Methods: Three AML cell lines were exposed to varying concentrations of cytarabine and a panel of FLT3 inhibitors (AC220, PKC-412, sorafenib, and JP116) over 96 hours. The cell lines were: HEL (negligible expression of wild-type FLT3), EOL1 (wild-type FLT3 which depends on FLT ligand for activation), MV4-11 (FLT3 with internal tandem duplication resulting in constitutively active kinase). Cell proliferation kinetics and cell cycle analysis were assessed using trypan blue and propidium iodide staining. Model fitting was performed using Phansisght Modeling Language in Phoenix. Plasma concentration profiles for cytarabine and AC220 in humans were digitized using computer digitizing program (Digitizer Version 1.9). One and two compartment models were fit to cytarabine and AC220, respectively. Estimated cell cycle, drug sensitivity, and pharmacokinetic parameters were used to simulate different combination regimens to predict synergism.

Results: Experimental data and model selection criteria showed that cytarab ine induced apoptosis in S-phase. FLT3 inhibitors induced apoptosis and cell cycle arrest at G1 phase (Figure 1). MV411 was most resistant to cytarabine followed by EOL-1, with HEL cells being most sensitive reflecting the role of FLT3 status in conferring resistance to cytarabine. AC220 showed highest sensitivity among tested FLT3 inhibitors (K50 = 0.4 nM in MV411 cells). HEL cells lacking FLT3, were resistant to all FLT3 inhibitors (K50 HEL >40 fold higher than K50-MV411). Simulations of candidate clinical regimens predict better cell kill upon adding FLT3 inhibitors simultaneously with or immediately after cytarabine exposure. In vitro combination experiments to validate the effects of administration sequence on cell kill are ongoing.

Summary / Conclusion: Patients with FLT3 perturbations are likely to benefit from combining FLT3 inhibitors to cytarabine. Simultaneous administration of cytarabine and FLT3 inhibitors is predicted to achieve highest cell kill. Our model presents a mechanistic interpretation for cytarabine and FLT3 inhibitors effects in AML cell lines and provides a useful tool to optimize combination regimens.

References
2. James et al. (2008) (ASH Abstract/Poster)

B1223

EXPRESSION OF PIM-2 GENE, BAD AND 4E-BP1 PROTEIN IS INCREASED IN PATIENTS WITH AML AND ALL AND CORRELATES WITH COMPLETE REMISSION RATE, OVERALL SURVIVAL AND APOTOPSIS 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 kinase suppresses apoptosis and promotes cell survival. These events are a consequence of phosphorylation of pro-apoptotic factors: 4E-BP1 translation inhibitor and BAD protein belonging to BCL-2 family. PIM-2 is highly expressed in neoplastic tissues and in lymphoma and leukemia cell lines which is consistent with its role during oncogenic transformation. In particular, PIM-2 plays an important role in bone marrow cell growth, differentiation and survival.

Aims: The aim of this study was to investigate whether the PIM-2 (both mRNA and protein level), BAD, 4E-BP1, p-BAD p-4E-BP1 expression is altered in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Patients and methods. One hundred twenty-six patients were included: 74 with AML and 52 with ALL aged 18-85 (median=48.1). Seventy-one patients reached complete remission (CR): 39 with AML and 32 with ALL. Bone marrow samples were collected at the time of diagnosis and in CR phase. Control samples were obtained from healthy donors. Median expression level of PIM-2 and 4E-BP1 mRNA was determined by semi-quantitative RT-PCR. PIM-2, BAD, 4E-BP1, and ATRA expression was assessed by Western blot. Moreover siRNA targeting human PIM-2 in leukemia cell line HL-60 was used to examined influence on apoptosis rate.

Methods: Reverse transcriptase-polymerase chain reaction and analysis of the expression of PIM-2, BAD, 4E-BP1 mRNA and protein level, followed by Western blotting. Moreover siRNA targeted human PIM-2 expression in both AML and ALL patients. In leukaemic cell line HL-60, the siRNA-mediated decrease of PIM-2 expression induced the increase of cell apoptosis rate.

Results: Our data indicate that PIM-2, BAD, 4E-BP1 expression is increased in patients with AML and ALL. In AML patients PIM-2 expression correlates with CR, OS and apoptosis rate.

Summary / Conclusion: Our data indicate that PIM-2, BAD, 4E-BP1 expression is increased in patients with AML and ALL. In AML patients PIM-2 expression is increased in patients with AML and ALL. In AML patients PIM-2 expression correlates with CR, OS and apoptosis rate.

B1231

FLUVASTATIN CAN INCREASE APOPTOSIS INDUCED BY VALPROIC ACID AND ALL-TRANS-RETINOIC ACID IN FLT3-ITD-POSITIVE AML CELLS

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Background: FLT3 internal tandem duplications (ITDs) represent the most frequent molecular aberration in acute myeloid leukemia (AML) associated with an impaired prognosis. The pattern of downstream activation by this constitutively activated receptor tyrosine kinase is influenced by the localization of mutated FLT3 depending on its glycosylation status. The important process of post-translational maturation can be inhibited by fluvastatin. Furthermore, overexpression of resistance tyrosine kinase inhibitors (TKIs) reflects a current challenge in the treatment of FLT3-ITD-positive AML.

Aims: The objective of this study was to investigate whether fluvastatin has an impact on the induction of apoptosis or on differentiation of FLT3-ITD-positive AML cells in the presence of the histone deacetylase inhibitor valproic acid (VPA) and all-trans-retinoic acid (ARA). Methods: The murine Ba/F3 leukemia cell line was stably transfected with a FLT3-ITD variant resulting in IL-3-independent growth. Signal transduction after exposing cells to fluvastatin, VPA, and/or ATRA was analysed by Western blotting. Apoptosis, cell cycle analyses, and differentiation were detected by flow cytometry.

Results: In FLT3-ITD expressing Ba/F3 cells, VPA or ATRA or the combination of both compounds were not able to induce apoptosis while fluvastatin alone resulted in a slight increase of apoptotic cells compared to the DMSO control. Co-treatment with fluvastatin, VPA, and ATRA, however, demonstrates additive effects and is associated with a significant increase of apoptosis in this cell model. Interestingly, acetylation of histone H3 is much more pronounced in the presence of fluvastatin as compared with VPA alone. Besides, phosphorylation of the anti-apoptotic protein Akt (Ser473) is strongly decreased in the triple combination while VPA plus ATRA results in the highest phosphorylation level of Akt. Surprisingly, these observations do not correlate with the phosphorylation status of the p70S6 kinase.

Summary / Conclusion: Co-treatment with fluvastatin can increase the sus-
capability to VPA and ATRA. We suggest that compartmentalization of FLT3-ITD by statins might improve the effect of such a therapeutic approach and could represent a promising strategy to overcome TKI resistance in FLT3-ITD-positive AML.

**B1232**

**OVEREXPRESSION OF WILMS TUMOR GENE 1 (WT1) IN ACUTE PROMYELOCYTIC LEUKEMIA.**

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**Background:** WT1 gene is known to be highly expressed in the majority of acute myeloid leukemia (AML), but remains unclear its role in the development of myelodysplastic and leukemia cells. WT1 gene is not to be expressed in highly proliferative cells (1) and recently, an anti-apoptotic effect of WT1 has been demonstrated in acute promyelocytic leukemia (APL)-cell lines (2). However, there are only a few data regarding expression levels of WT1 in APL.

**Aims:** Between January 2007 and June 2011 we evaluated the WT1 expression in 169 AML patients at diagnosis. To assess the pattern of expression in different subtypes of AMLs, we performed a quantitative polymerase chain reaction (RQ-PCR) assay on bone marrow (BM) samples using the ELN Pro-fileQuant Kit (Ipsogen, Marseille, France) following the European Leukaemia Net protocol. As normal expression cut-off was established 250 WT1 copies/10⁶ BM, we observed: 49 (29%) AMLs with myelodysplasia-related changes, 27 (16%) AMLs with minimal differentiation, 15 (9%) AMLs with maturation, 22 (13%) Acute Myelomonocytic leukemia, 35 (21%) Acute Monocytic leukemia and 21 (12%) AML. All the APL were PML-RARApha and (1;17)(q22;q12) positives.

**Results:** There were only 9 WT1 normal expressing patients (6%), whereas 160 (95%) AML cases presented levels of WT1 expression higher than the established cut-off, with a median value of 6776 copies WT1/10⁶ copies ABL (range 235-62567). Inside each FAB subtypes, the median level of WT1 in the over-expressing group at diagnosis was: 8669 WT1 copies/10⁶ copies ABL (range 135900-26292) in M0-M1 group, 4965 copies/10⁶ copies ABL (range 10025-255829) in M3 group, 8111 copies/10⁶ copies ABL (range 415-11484) in M4 group, 6226 copies/10⁶ copies ABL (range 15661-4530) in M5 group, 4530 copies/10⁶ copies ABL (353-24204) in secondary AML (Msec) group. The level of WT1 expression in AML was significantly higher (P<0.01) than in all other subtypes (figure 1). All APL patients presented an over-expression of WT1, differently from M4, M5 and secondary AML groups. Moreover, over the majority of APL patients (51%, 17/21 pts) had more than 20000 WT1 copies at onset. Conversely, only 9 out of 139 (6%) Non APL cases demonstrated a number of WT1 copies higher than 20000.

**Summary / Conclusion:** In our cohort of AML patients, Acute Promyelocytic Leukemia group showed highest levels of WT1 expression. In particular, we observed that all APL patients overexpressed WT1 gene at diagnosis and the transcript levels were significantly higher in APL cases than in other FAB subtypes. It is still unclear the role of WT1 gene expression to promote or sustain proliferation of leukemic cells, but this observation could reinforce a possible relationship between the pathway stimulated by PML-RARA transcript and the WT1 anti-apoptotic and pro-proliferative mechanisms(1;2).

**References:**


**B1233**

**CLINICAL AND BIOLOGICAL CHARACTERISTICS OF ACUTE MYELOID LEUKEMIA WITH ABDERRANT EXPRESSION OF CD56+CD11b+**

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**Background:** Immunophenotyping is a widely used diagnostic tool which, in combination with cytology and cytogenetics, allows for a more accurate diagnosis of acute myeloid leukemia (AML). The literature describes a group of AML with a peculiar clinical and biological characteristics, such as monocytic/monoblastic AML, whose blasts coexpressed CD56 and CD11b (CD56+CD11b+). This subtype of leukemia has been associated most often with aberrant karyotypes (mainly 11q23/MLL gene rearrangement), as well as more aggressive disease and refractoriness to treatment and, ultimately, with a worse prognosis.

**Aims:** In our study we analyzed all cases of AML diagnosed at our institution in the years 2002-2012, and in particular those whose blasts coexpressed CD56+CD11b+ and its possible association with extramedullary involvement, extramedullary disease and overall survival.

**Methods:** We analyzed a total of 133 cases divided into three different groups according to WHO classification: Group 1 (n = 60), not otherwise specified AML; Group 2 (n = 44), AML with myelodysplasia-related changes; and Group 3 (n = 29), AML with recurrent genetic abnormalities. Among others, analyzed variables included age, sex, cytogenetics (karyotype and FISH), immunophenotyping, especially the coexpression CD56+CD11b+, blood count at diagnosis and LDH, number of blasts in the blood and bone marrow, extramedullary involvement at diagnosis (skin, liver, spleen, lymph nodes and other locations), treatment features (complete remission after induction, number of treatments to achieve complete remission), and data on survival (disease-free survival, overall survival and the mortality rate). Statistical analysis was performed using SPSS (Chicago, IL) and significance level (p value) was established at 0.05.

**Results:** Ten of the 133 studied cases (7.5%) had this phenotype. Extramedullary involvement at diagnosis was detected in 50% of patients with CD56+CD11b+ coexpression, whereas only in 18% of AML with CD56-CD11b-. Concerning overall survival, we did not find differences in these patients when compared to double CD11b and CD56 negative (P=0.105). Cases of AML CD56+CD11b+ more frequently correlated to those with monocytic component, while AML with monocytic and myelomonocytic, clearly different to the remaining AML subtypes (p <0.001). The frequency of this phenotype was similar in monocytic/monoblastic (62.5%) and myelomonocytic (66.7%) AML (P=0.898). Extramedullary involvement was also more common in those AML with monocytic component (52.4%) than not (18.5%) (P=0.003).

**Summary / Conclusion:** In our experience, AML with the CD56+CD11b+ coexpression present more frequently a monocytic component (monoblastic/monocytic and myelomonocytic subtypes of AML), and also show a clear tendency to present with extramedullary involvement. By contrast, in our study it was not possible to establish this phenotype with 11q23/MLL gene disorders (which has been reported by other authors), and CD56+CD11b+ coexpression did not seem to correlate with overall survival in AML patients.
Background: The 90 kDa Heat Shock Protein (HSP90) is a dimeric molecular chaperone required for conformational maturation and stabilization of numerous oncoprotein targets involved in cell cycling, receptor function, signal transduc-
tion, and apoptosis. A high expression of HSP90 has been reported in leukemic cell lines and in patients with acute myeloid leukemia (AML) which has been associated with poor prognosis. In acute promyelocytic leukemia (APL), the disease-specific PML-RARA oncoprotein deregulates the expression of various genes, including the immortalized and apoptosis-resistant target gene PMLRARA which by p53 and p21 is a critical mechanism for the pathogenesis in APL. To date, no studies have investigated the potential role of HSP90 in APL pathogenesis.

Aims: Objective of this study was to comprehensively analyze the involve-
mant of HSP90 in pathogenesis of APL.

Methods: HSP90 expression levels were analyzed using Western Blot and Real Time PCR in APL patient blasts and in NB4 cells both at baseline and after treatment with ATRA and ATO, as well as in the zinc inducible, PML/RARA transfected PR9 cells. ChIP was used to analyze the regulatory sequence of HSP90 in dependence on PR9. Using ChIP, we found in-vivo binding of Pu. 1 and PML-RARA to the HSP90 alpha and beta promoter regions. Pu. 1 motifs coexisted with one or more rare sites in HSP90 promoter regions.

Summary / Conclusion: The DNA binding domain, conserved in the PML-
RARA fusion protein, displaces the gene without dysplastic features. In the promoter sequence a Pu.1 containing complex is necessary for the activation of HSP90 transcription, thus suppress-
ing the expression of the protein. We demonstrate here a new peculiar feature of APL which differentiates this subset from other tumors and leukemias, usually showing increased HSP90 level.

Further studies are needed to define the significance of this finding.

B1237

ARE MUTATIONS IN THE IDH2 GENE SUITABLE MOLECULAR MARKERS FOR MRD MONITORING IN AML PATIENTS?

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Background: Heterozygous missense mutations in isocitrate dehydrogenase 2 gene (IDH2) have been recently reported in patients with acute myeloid leukemia (AML), notably in cytogenetically normal AML (incidence 12,1 %). These mutations affect mainly arginine codons p.R140 and/or p.R172, both involved in the IDH2 enzymatic domain.

Aim: to verify the use of most frequently detected mutation c.G419A (p.R140Q) in AML patients, and ii) to compare these results with those obtained from routine monitoring of NPM1 mutations in AML patients, who harbor IDH2 and NPM1 mutations simultaneously.

Methods: Method of DNA-based RQ-PCR with a specific set of primers and Locked Nucleic Acid (LNA) probe were used for quantification of IDH2 c.G419A (p.R140Q) mutation in a total of 73 samples (45 bone marrow and 28 peripheral blood samples) of AML patients. These samples were obtained during AML treatment at the different time points. All patients signed an informed consent before the samples were collected. Six of nine (6/9) analyzed patients harbored parallel mutation in NPM1 gene. Quantification of NPM1 mutation was performed according to previously published work. The results were normalized as the normalized copy numbers (NCN) defined as the number of mutated IDH2 or NPM1 gene copies for every 10^6 cell equivalents (CE).

Results: In our cohort, 7/9 AML patients revealed concordant results for the NCN of the IDH2 c.G419A mutation and disease status. Moreover, in 5/6 analyzed patients, the kinetics of IDH2 mutations was nearly identical to the kinetics of NPM1 mutations. However, in the remaining two patients, IDH2 mutation status did not correspond to clinical course of AML. Both patients revealed persistent IDH2 c.G419A positivity (10^-2 – 10^-6 NCN) although they were in hematological remission. Moreover, one of them was also in molecular remission according to NPM1 response. In this case further analyses were performed and germinal origin of IDH2 mutation were exluded.

Summary / Conclusion: Our data indicate that, in the majority of AML patients, status of IDH2 mutation correlate with the clinical course of disease. Thus mutation c.G419A in IDH2 gene seems to be suitable marker for MRD moni-
toring in AML patients. However, we have shown that discrepant cases (20%) further analy-
ses were performed and germinal origin of IDH2 mutation were exluded.

B1238

ASSESSMENT OF WT1 TRANSCRIPT REDUCTION FOR PROGNOSTIC IMPLICATION IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Wilm’s tumor 1 (WT1) gene is over-expressed in most cases of acute myeloid leukemia (AML). It is one of proposed markers for minimal resid-
ual disease (MRD) monitoring.

Aims: Our study aims to estimate the utility of WT1 transcript reduction for pro-
gnostic implications in acute myeloid leukemia, including acute promyelocytic leukemia (APL).

Methods: Real time quantitative RT-PCR was made according to the ELN guidelines. WT1 transcript level was determined in BM samples of adult patients with AML and APL. We examined the samples of 122 patients with AML and

510 | haematologica | 2013; 98(1)
25 patients with APL at diagnosis. We also analyzed 384 specimens of AML and APL patients during follow-up. The obtained WT1 values were normalized with respect to the βGUS transcripts.

**Results:** The median value of WT1 levels at diagnosis was 755 (range 2.4-16283) in AML patients and 3528 (range 547-8103) in APL patients. The median value of WT1 levels of patients in complete remission was 8.5 (range 0.3-4.3) for AML (n=142) and 8.9 (range 0.4-2.3) for APL (n=24) (p=0.3). In the non-remission group, 70% of AML patients showed the expression of WT1 one log higher the median values in complete remission. WT1 transcript reduction after induction above 2 log showed 69% of patients in favorable subgroup with APL + AML with AML1/ETO, CBFB/MYH11, and utilizing 25% of patients in subgroup of poor prognosis - AML with MLL (n=89) (p=0.08). In full group (all AML patients + APL) we assessed the correlation between the value of reduction in WT1 after induction and unfavorable event (relapse, failure in complete molecular remission after induction in APL patients) during 12 months; in subgroup of non-remission group above 2 log only 34% of patients had unfavorable event, whereas in subgroup with failure in WT1 reduction (under 2 log) 50% of patients had unfavorable event; P=0.02 (Fisher Exact test).

**Summary / Conclusion:** Quantitative assessment of WT1 transcript reduction after the first cycle of chemotherapy can be used to assess treatment response. Failure in WT1 reduction is predictor of subsequent relapse risk.

### B1239

**ABERRANT EXPRESSION OF LYMPHOID-ASSOCIATED ANTIGENS IN ACUTE MYELOID LEUKEMIA: INCIDENCE AND CLINICOClinICAL IMPLICATIONS**

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**Background:** A variable proportion of patients with acute myeloid leukemia (AML) express lymphoid-associated antigens (LAAs). The exact incidence and clinical significance of this phenomenon remains unclear due to inconsistencies between series, likely related to methodological aspects and/or case selection biases.

**Aims:** We aimed at obtaining insight into the prevalence and clinical impact of LA expression in AML by retrospectively evaluating the expression of LA in blast cells of selected AML patients, and using external controls.

**Methods:** The patient cohort included 203 males and 140 females with a median age of 61 years (range, 10-88); 183/343 (53%) cases were above the age of 60. Within this cohort, 235 cases (68%) had de novo AML, whereas the remaining 108 cases (32%) concerned secondary AML (sAML) to either hematologic or myelodysplastic syndromes.

**Results:** At diagnosis 89% of AML patients + APL (n=57) were treated uniformly according to age with Aracytin/Idarubicin induction regimens (weekly alternating cycles). The induction therapy of primary AML patients (n=235) revealed significant associations (P<0.05) between: (i) CD7 or CD10 expression and adverse cytogenetics; and (ii) CD4 expression and short-prognostic indicators (P=0.05) for DFS. Regarding OS, only sAML, advanced age, and an age of 61 years (range, 10-88); 183/343 (53%) cases were above the age of 60. Within this cohort, 235 cases (68%) had de novo AML, whereas the remaining 108 cases (32%) concerned secondary AML (sAML) to either hematologic or myelodysplastic syndromes.

**Conclusion:** Cells sensitive to AKN-028 showed a higher overall TK-activity than more resistant ones. The TK-activity was inhibited by AKN-028 in a dose dependent manner in all samples tested, whereas the overall STK-activities were low in all cells. The results suggest that the difference in cytotoxic activity of AKN-028 may be due to the variation in basal overall TK-activity among the cell lines. A larger number of patient samples will be analyzed to confirm these findings.

**Summary / Conclusion:** Cells sensitive to AKN-028 showed a higher overall TK-activity than more resistant ones. The TK-activity was inhibited by AKN-028 in a dose dependent manner in all samples tested, whereas the overall STK-activities were low in all cells. The results suggest that the difference in cytotoxic activity of AKN-028 may be due to the variation in basal overall TK-activity among the cell lines. A larger number of patient samples will be analyzed to confirm these findings.

### B1240

**AML CELLS SENSITIVE TO THE NOVEL TYROSINE KINASE INHIBITOR AKN-028 SHOW A HIGHER OVERALL TYROSINE KINASE ACTIVITY THAN MORE RESISTANT SAMPLES**

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**Background:** AKN-028 is a novel tyrosine kinase (TK) inhibitor with preclinical activity in acute myeloid leukemia (AML), presently undergoing investigation in a phase I/Ii study. AKN-028 is a potent inhibitor of FMS-like kinase 3 (FLT3) and has shown in vitro activity in a wide range of AML samples, irrespective of FLT3 mutation status or quantitative FLT3 expression.

**Aims:** To further characterize the mechanism of actions involved in the AML cell response to treatment with AKN-028.

**Methods:** TK-activity profiles in AML cell lines HL60 and MV4-11 and three primary AML samples (UPN 1-3) were determined using the PamChip® tyrosine kinase peptide microarray system. For the primary AML samples, serum/threonine kinase (STK) activity profiles were generated on the PamChip® using the corresponding kinase array.

**Results:** Previous characterization regarding the cytotoxic properties of AKN-028 in vitro showed that the patient sample UPN1 and the cell line HL60 represent relatively resistant samples, UPN2 an intermediate variant and UPN3 and MV4-11 are relatively sensitive to AKN-028. The TK-activity profile between the cell lines resistant and sensitive to AKN-028 showed a relative difference (p-value<0.05). Student’s t-test in basal phosphorylation level of 57 of the 141 peptide substrates tested. For primary AML samples, this pattern was even more pronounced with 73 out of the 141 peptides showing significantly higher phosphorylation rate compared to the HL60 and MV4-11 samples. The differences in STK-activity between the samples were in general less pronounced. The TK-activity in lysates from AML cell lines and from the three primary patients was also analyzed in the presence of AKN-028. In all samples tested, 10 µM of AKN-028 reduced tyrosine phosphorylation to almost 50% of the parent value. Similar TK-activities were tested against four different concentrations of AKN-028 (0.25-µM), showing a dose-dependent inhibition in all samples. STK-activity in UPN2 and UPN3 was not affected by 10 µM of AKN-028 whereas UPN1 showed slight inhibition on some peptides.

**Summary / Conclusion:** Cells sensitive to AKN-028 showed a higher overall TK-activity than more resistant ones. The TK-activity was inhibited by AKN-028 in a dose dependent manner in all samples tested, whereas the overall STK-activities were low in all cells. The results suggest that the difference in cytotoxic activity of AKN-028 may be due to the variation in basal overall TK-activity among the cell lines. A larger number of patient samples will be analyzed to confirm these findings.
Background: Among prognostic factors associated with outcome in acute myeloid leukemia (AML) cytogenetic aberrations, although detectable in only half of the patients, have long been recognized as clinically highly important. During recent years several distinct gene mutations, preferably detected by PCR, have also been linked to treatment response and long-term prognosis. Subsequently, involving the FMS-like tyrosine kinase 3 (FLT3) and nucleophosmin 1 (NPM1) genes. There is, however, still a need to discover additional disease-related molecular signatures, including aberrant gene expression patterns that may serve as novel biomarkers and/or as targets of novel therapeutic regimens in AML.

Aims: Using global genomics to identify biomarkers at the molecular level, predictive of clinical response to chemotherapy, by comparing AML patients with a poor vs. those with a better clinical outcome.

Methods: We analysed mononuclear cells obtained from 42 patients at AML diagnosis, using Affymetrix U133 Plus 2.0 and the GeneSpring GX 10.0 software.

Subsequent pathway assessments were performed through IngenuityPathwayAnalyses. Patient characteristics and clinical outcome were collected. All patients entered complete remission (CR) after high-dose chemotherapy, typically consisting of an anthracyclin and cytarabine. The median CR duration was 161 (range 7-3701) days. The patients were subdivided into two groups according to their CR duration: those with “short CR duration” (<180 days, n=24) and those with “long CR duration” (>180 days, n=18). The groups were equivalent regarding age, gender, with blood cell count, cytogenetic status and presence of secondary leukemia.

Results: Gene array analysis revealed markedly differences with 383 genes to be up-regulated and 616 genes down-regulated more than 2 fold, in pooled samples from patients with short CR vs. those with long CR duration. Among the differentially most expressed genes we identified RUNX1T1 to be 116-fold up-regulated, while ANXA1 was 58-fold down-regulated in those with short CR. These gene expression data were confirmed by using real time (qPCR). Thus, a strikingly higher expression of RUNX1T1 was observed in individual patient samples with short CR duration (n=10) as compared to those with long CR duration (n=10); median relative expression 0.01 vs. 0.59 (P=0.0002). The transcription factor gene RUNX1T1 is a part of the well-known t(8;21)(q22;q22) cytogenetic aberration, creating the RUNX1-RUNX1T1 fusion gene that translates into a protein known to block normal hematopoietic differentiation. It is observed in 5-12% of adult AML patients and is associated with a favourable clinical prognosis. Our pathway analyses identified connections between RUNX1T1 and CD34 and TCF3. In Figure 1, we showed that cell viability of the bulk blast population and the more immature CD34+ leukemic population remained unaffected in the presence of G-CSF or AMD3100. G-CSF upregulated RUNX1T1 expression, while AMD3100 treatment induced a 0.71-fold reduction of CXCR4 surface expression at the highest dose (1ng/ml) (P<0.0001). Additionally, AMD3100 also induced a significant downregulation of the VLA-4 and VCAM-1 surface expression (P=0.0004 and P=0.02 respectively). AML clonogenic capacity was significantly reduced in a dose-dependent manner after treatment with G-CSF and AMD3100 (Figure 1). At the highest concentration, G-CSF (100 mg/ml) and AMD3100 (1 mg/ml) decreased the clonogenic capacity by 41% (P=0.0004) and 44% (P=0.0002), respectively.

Summary / Conclusion: The addition of G-CSF or AMD3100 impairs remarkably the clonogenic capacity of primary AML cells. These results suggest that the use of these two molecules as priming agents may contribute to eradicate the LSC population. Our findings support the design of further studies aimed to explore a potential synergistic effect of these agents in combination with standard chemotherapy against LSCs.

B1243 TREATMENT WITH G-CSF AND AMD3100 DIMINISH THE CLONOGENIC CAPACITY OF PRIMARY ACUTE MYELOID LEUKEMIA BLASTS IN VITRO

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Background: The simultaneous administration of G-CSF and chemotherapy as a priming strategy has resulted in a clinical benefit in determined subsets of patients diagnosed with acute myeloid leukemia (AML) (Lowenberg et al, NEJM 2011; Pabst T, et al, Blood 2012). On the other hand, the CXCR4 antagonist AMD3100 has shown to prolong survival in AML-bearing mice (Nervi Blood 2009). However, the mechanism responsible for these anti-leukemic effects is not fully characterized. We hypothesized that these observations could be explained based on a higher sensibility of leukemic stem cells (LSCs) to these priming regiments.

Aims: The main goal of this project is to study the in vitro effect of G-CSF and AMD3100 on LSCs and the bulk leukemic population from primary AML samples.

Methods: Primary peripheral blood cells from 10 AML patients were treated with G-CSF or AMD3100 at different doses for 72 h. Cell viability was measured by 7-AAD (eBioscience) exclusion; cell surface phenotype and volumetric cell count were obtained by flow cytometry (FACSVerse, BD) and analyzed using the FlowJo software (TriStar). For the clonogenic assays, primary AML cells were treated for 18 h with G-CSF and AMD3100 at increasing doses and cultured on MethoCult H4034 Optimum (StemCell Technologies) for 14 days.

Results: Cell viability of the bulk blast population and the more immature CD34+ leukemic population remained unaffected in the presence of G-CSF or AMD3100. G-CSF treatment upregulated CXCR4 expression in a dose-dependent fashion. A 1.44-fold increased expression was observed at the highest G-CSF dose (100 µg/ml). However, the expression of VCAM-1 and VLA-4 did not change. On the contrary, AMD3100 treatment induced a 0.71-fold reduction of CXCR4 surface expression at the highest dose (1ng/ml) (P=0.0001). Additionally, AMD3100 also induced a significant downregulation of the VLA-4 and VCAM-1 surface expression (P=0.0004 and P=0.02 respectively). AML clonogenic capacity was significantly reduced in a dose-dependent manner after treatment with G-CSF and AMD3100 (Figure 1). At the highest concentration, G-CSF (100 mg/ml) and AMD3100 (1 mg/ml) decreased the clonogenic capacity by 41% (P=0.0004) and 44% (P=0.0002), respectively.

Summary / Conclusion: The addition of G-CSF or AMD3100 impairs remarkably the clonogenic capacity of primary AML cells. These results suggest that the use of these two molecules as priming agents may contribute to eradicate the LSC population. Our findings support the design of further studies aimed to explore a potential synergistic effect of these agents in combination with standard chemotherapy against LSCs.

B1244 CLINICAL AND BIOLOGICAL CHARACTERISTICS OF ID1 AND 2 MUTATIONS IN ACUTE MYELOID LEUKEMIA PATIENTS WITH NORMAL KARYOTYPE (NC-AML) IN SERBIA

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Background: Acquired mutations in exon 4 of both isocitrate dehydrogenase 1 and 2 genes (IDH1 and IDH2) were recently reported in patients with acute myeloid leukemia (AML). Numerous studies have tried to reveal the association of these mutations with clinical features, prognosis and the outcome of the disease.

Aims: To evaluate the frequency and examine the impact of IDH1/2 mutations on outcome, as well as their association with other molecular and clinical prognostic markers in adult de novo NC-AML patients.
**B1245**

**DOWNREGULATION OF PROGRAMMED CELL DEATH LIGAND-1 PROTEIN INHIBITS MIGRATION,ADHESION AND INVASION OF LEUKEMIA HL-60 CELLS**

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**Background:** Programmed cell death ligand-1 (PD-L1) is a newly discovered apoptosis gene, which was highly expressed in children with leukemia, and it was considered as a predictive marker for leukemia micrometastasis.

**Aims:** To investigate the impacts of programmed cell death ligand-1 (PD-L1) downregulation on cell abilities of migration, adhesion and invasion of leukemia HL-60 cells.

**Methods:** PD-L1-siRNA, control-siRNA and blank-siRNA were transfected into leukemia HL-60 cells through liposome as experiment group, negative control group and blank control group, respectively. The transfection efficiency was observed by fluorescence microscope. Forty-eight hours after transfection, reverse transcription PCR and immunocytochemistry were employed to detect the expression of PD-L1 in three groups. Cell experiments were performed to assess cell migration, adhesion and invasion respectively, when PD-L1-siRNA was transfected into HL-60 cells for 48 hours.

**Results:** The transfection efficiency of HL-60 cells was 90%, PD-L1 mrRNA and protein expression of PD-L1-siRNA group were 0.03±0.002 and 532.3±2.5, which were significantly lower than those of control-siRNA group and blank-siRNA group (P<0.05, t=1.767, P<0.05). The number of migrated cells in PD-L1-siRNA group was 23.7±2.06 fewer than those of control-siRNA group and blank-siRNA group (P=0.015, t=2.755, P<0.05). The number of invasion cells in PD-L1-siRNA group was 23.17±2.06 fewer than control-siRNA group and blank-siRNA group (P=0.015, t=3.965, P<0.05). The number of invaded cells in PD-L1-siRNA group was 37.65±3.12, significantly fewer than those of control-siRNA group and blank-siRNA group (P<0.05, t=2.619, P<0.05).

**Summary / Conclusion:** Downregulation of PD-L1 expression in HL-60 cells can inhibit the abilities of migration, adhesion and invasion which suggests that PD-L1 plays an important role in the metastatic potential of leukemia cells.

**Methods:** To clarify the expression profiles of TRIM32 in human leukemia cell lines, we compared the protein levels of TRIM32 by immunoblot analysis in various human leukemia cell lines. Next, to verify whether TRIM32 drives RARA-mediated transcription in human leukemia and cancer cell lines, we performed a luciferase assay using a RAR promoter-driven luciferase construct (RAR-Luc) and we performed immunoblot analysis to verify whether TRIM32 stabilizes the expression level of endogenous RARα in HL60 cells without ATRA. We also examined whether similar morphological features of granulocytic differentiation were observed even in some of the ATRA-un-treated HL60 cells in which TRIM32 was transiently transfected. In addition, we calculated the percentage of CD11b-positive HL60 cells in which TRIM32 was induced without ATRA treatment.

**Results:** Expression of TRIM32 protein was observed in leukemia cell lines and its expression level of TRIM32 was varied in various cell lines. Immunoblot analysis showed that endogenous RARα was more highly expressed in HL60 cells in which TRIM32 had been expressed than in mock cells. We also found that similar morphological features of granulocytic differentiation were seen in some of the ATRA-un-treated HL60 cells in which TRIM32 was induced. The percentage of CD11b-positive cells was significantly higher in HL60 cells in which TRIM32 was induced than in mock cells (1.4±1.0% for mock versus 21.9±5.1% for TRIM32 (P<0.01)). We performed cell proliferation assay using HL60 cells after induction of TRIM32 or empty expression vectors. The effect on HL60 cells after induction of TRIM32 was characterized by significant suppression of cellular proliferation compared with that of mock cells (Figure).

**Summary / Conclusion:** Results of cell proliferation assay suggest that TRIM32 induced granulocytic differentiation via RARA-mediated transcriptional activity in the absence of ATRA, and proliferation of HL60 cells after induction of TRIM32 showed slow proliferation because of their disappearance of self-division potential. In conclusion, it is important to analyze TRIM32 protein that would be helpful for patients suffering from malignant diseases, whose cancer cells are induced to differentiate by retinoic acid-dependent RARA-mediated transcriptional activity.
B1247

EXPRESSION LEVELS OF WT1 AND NPM1 AND NOVEL NONSPECIFIC MARKERS AS TOOLS FOR EVALUATING MINIMAL RESIDUAL DISEASE IN AML
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Background: Monitoring of minimal residual disease (MRD) is an important tool in the medical management of acute myeloid leukemia (AML). Of the specific molecular markers, mutations of the nucleophosmin 1 (NPM1) gene represent the most frequent aberration. This makes NPM1 a favorite gene for MRD detection. Approximately one half of AML patients do not have a suitable specific molecular marker for MRD monitoring. Therefore, development of sensitive assays for quantification of nonspecific leukemia-associated antigens (LAA) presents a significant challenge for the medical management of AML. The Wilm’s tumor gene (WT1) has been suggested as a possible molecular marker of MRD in AML as it is overexpressed in 80% of AML patients at diagnosis. The LAAAs that we focused on were PRAME, MSLN, ST18, XAGE1, CSPG4, CA9 and BAALC in genes.

Aims: To examine selected LAAs as potential tools for MRD monitoring in AML.

Methods: Established IVD CE protocols of fluorescence-based quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) and protocols based on TaqMan Gene Expression Assays® were used for quantification of selected targets.

Results: Of the 475 patients treated at the Institute of Hematology and Blood Transfusion in Prague in 2002-2012, 113 (23.8%) had a mutation in the NPM1 gene. Of the 45 patients with NPM1 mutation and follow-up+4 months, 7 nev er showed molecular disease while sustaining hematological remission and 41 suffered hematological relapse. MRD positivity preceded hematological relapse by a median of 3.23 months. Overexpression of WT1 in peripheral blood was detected in 199 of 211 patients (94.3%) at diagnosis. Thirty-three experienced relapse. Median time from MRD positivity to hematological relapse was 1.8 months. MRD quantities monitored by NPM1 on mRNA level were compared to WT1 expression in 60 AML patients with a median follow-up of 12.3 months (range 1.32–110.4). Both methods for MRD detection correlated significantly (P<0.0001). Levels of selected LAAs (MSLN, ST18, XAGE1, CSPG4, CA9) were evaluated in 153 AML patients at diagnosis. Overexpression of MSLN was demonstrated in 86 patients (56.1%). However, the high expression of MSLN in normal peripheral blood makes this marker unsuitable for MRD monitoring. This is not the case of ST18, XAGE1, CSPG4 and CA9, which were overexpressed in 50.3%, 17.9%, 45.7% and 36.4% respectively. In 12 patients (7.8%) with high expression of a particular LAA at diagnosis, it was possible to trace MRD during the course of disease. Furthermore, 19 patients positive for the molecular marker revealed that within the PML-RARA (+) patients, 42.3% bared the Short form and 57.7% the Long form of the transcripts, while FLT3-ITD was detected in 7/15 (46.7%) patients. In contrast, all PML-RARA (+) AML patients were positive for the Long form and only one was positive for FLT3-ITD. No differences in the mean age, gender, leucocyte and platelet counts, and hemoglobin levels were observed between patients with and without molecular abnormalities related to the origin of the disease (dnaml vs. tAML).

Summary / Conclusion: In this study we found a relatively high incidence of “favorable” fusion transcripts in patients with tAML similar to that in de novo AML, compared to only myeloid morphology M2 (3) and M2e (1) in tAML. CBFB-MYH11 were found in M1eo (1); M2 (2); M4 (8); and M4eo (7) cases.

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B1249

UNFAVORABLE PROGNOSTIC IMPACT OF WT1 MUTATIONS IN PEDIATRIC ACUTE MYELOID LEUKEMIA IN KOREA
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Background: Acute myeloid leukemia (AML) is a clinically and biologically heterogeneous disease. Recently, about 65% of pediatric AML patients show long-term survival. The relevance and prognostic value of mutations in FLT3, NPM1, and WT1 for novel classification and risk stratification have been studied mainly in adult AML and to a lesser extent in patients with pediatric AML.

Aims: The aim of this study was to show the clinical implications of these genetic variants on childhood AML in Korea.

Methods: We have evaluated the frequency and prognostic significance for the mutation status of FLT3-ITD/TKD, NPM1, KIT, CBEBPA, WT1, NRAS, BAALC and IDH1/2 in childhood AML (n=104). Mutations were analyzed by PCR amplification of genomic DNA. WT1 and BAALC expression levels were measured using a quantitative assay using normalized expression of MBL. The Kaplan-Meier method was used to calculate the survival and the log rank test was used to evaluate the significance. Among 104 patients, 52 patients were WT1 wild type and 52 patients were WT1 mutated. The median age was 6.5 years and 38% were below 1 year. The median age of WT1 wild type was 6.5 years while WT1 mutated was 4.5 years. The 5-year EFS rate was significantly different according to FLT3-ITD, IDH1/2 and WT1. The 5-year EFS was 52% for WT1 wild type while 31% for WT1 mutated. WT1 mutated patients showed decreased EFS rate (68% vs. 44%, P=0.04). According to WT1 mutational status, the 5-year EFS rates were 65% for WT1 wild type and 51% for WT1 mutated.

Summary / Conclusion: The most common mutation was found in NPM1. The KIT, IDH1/2 and FLT3/TKD mutations were very rare. Patients with High BAALC and WT1 expression levels were associated with poor prognosis, but...
statistically not significant. W77 mutated patients with normal karyotype showed a poor prognosis. The result of this study was not consistent with previous published data because of small sample size, selection bias, high rate of SCTs, and ethnic differences. However, these data suggest that some clinical implications of molecular genetic alterations in Korean children with AML. Further retrospective and prospective multicenter studies are needed to unravel how these genetic abnormalities affect the leukemogenesis, treatment and prognosis in pediatric AML.

B1250 MANAGEMENT OF FIT OLDER PATIENTS WITH AML: MAJOR IMPACT OF CYTOGENOTICS
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Background: Median age of Acute Myeloid Leukemia (AML) patients at diagnosis is 67 years and represents a real clinical challenge. Major problems of older AML remain co-morbidities, poor performance status and biological features increasing resistance to chemotherapy. However, some patients are cured with chemotherapy.

Aims: We studied prognostic factors in order to identify those patients deserving intensive treatment.

Methods: We retrospectively reviewed 78 patients over 60 (60-86) years old referred to our centre for treatment. AML was classified according to WHO classification; cytogenetic data were obtained by routine karyotype, additional FISH analysis and molecular biology; fit patients (PS<2, reversible or controlled comorbidities and no genetic syndromes) received intensive chemotherapy according to successive EORTC protocols. Several factors were assessed in univariate and multivariable analysis, in order to define their prognostic significance according to age subgroups. We also looked for factors predicting treatment-related toxicity and/or death and disease related mortality. Kaplan-Meier curves were used to compare significant prognostic factors in terms of complete remission, overall survival and disease-free survival according to prognostic factors and complete remission.

Results: 54% of patients are male. 52% AML are secondary. 50% of all treated patients reached a complete remission post induction. Overall survival (OS) of patients over 80 years old is 48%; 31% and 17% at 2 and 5 years, with a median survival of 9 months. This is higher compared to other studies, since a majority of patients were “fit” enough to be referred to our centre. According to age, OS is significantly better (P<0.0001) for patients below 70 years than above (44% at 2y vs. 16%). In univariate analysis, whatever the age, favourable karyotype provides a better prognosis (2y OS 63% vs. 20%, P=0.007). Taking into account cytogenetic data and age, median survival of patients above 70 with favourable karyotype was very similar to population below 70 (38 and 44% at 2y). In multivariable analysis, OS in patients treated with curative intent is much better than supportive care (62% at 1y vs. 6%, P<0.0001). For patients who received intensive chemotherapy (77%), death due to uncontrolled disease remains a major concern compared to death induced by treatment related toxicity (10%). In multivariate analysis, levels of LDH (P=0.03), liver diseases and prior malignancy (P=0.01) come out as others majors significant prognostic factors.

Summary / Conclusion: In our single-centre experience of ‘fit’ older AML patients, we confirm that cytogenetic data is the prognostic factor with the strongest predictive positive value in terms of outcome whatever the age in univariate analysis. Particularly for patients above 70, individual geriatric assessment combined with major prognostic factors should be refined in prospective studies to improve therapeutic decision making and limit treatment related toxicities. The survival of older AML patients remains poor, more likely due to disease resistance with aggressive biology, than because of treatment toxicity. New alternative treatment approaches using less toxic target therapies are urgently needed.

B1251 A RARE VARIANT TRANSLLOCATION IN APL
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Background: Acute promyelocytic leukemia (APL) is characterized by the t(15;17) patognomic translocation. Although almost all APL patients have the classical balanced translocation, a small proportion have complex or simple variant translocation, such as t(4;15;17) or t(5;17;15). Some of them, such as t(15;17) variants, are resistant to ATRA treatment whereas detection of classical t(15;17) or underlying PML-RARA rearrangement is highly predictive of response to ATRA in virtually 100% of cases.

Aims: The aim of this study was to describe a rare variant translocation in APL patient. A 21 year-old woman was admitted to our hospital because of fever and hemoptosis. Coagulation test revealed that INR was 1.52, normal partial thromboplastin time (PTT) and there was a slight reduction of fibrinogen (94.8 mg/dL). Peripheral blood examination showed 127x10^3/mm^3 white blood cells and 10x10^3/mm^3 platelets. Bone marrow aspirate showed 100% infiltration by promyelocytes. At immunophenotypic analysis 91.1% cells were CD15, CD33, C14, C14B8, C14D4, CD64, CD3, CD4, and bcr3 subtype was detected by reverse transcriptase polymerase chain reaction. A diagnosis of APL high risk was made according to the above data.

Methods: Chromosome analysis was based on G-banded metaphase and the karyotype was described according to the ISCN 2008. A whole chromosome painting (WCP) probe for chromosome 15 and a whole genomic expression probe for PML/RARA were used for fluorescence in situ hybridization (FISH).

Results: The karyotype of the patient was interpreted as 46,XX,15;17(15q23;17p13) by conventional cytogenetic and it was elucidated by fluorescence in situ hybridization that a new variant translocation t(15;17) was identified.

Summary / Conclusion: The t(6;17)(q22;p13) translocation found is, to our knowledge, one of a few series in wich 6p is involved. This case is a contribution to the study of the infrequent variants APL translocation and could provide further insights into biologic and prognostic information provided by cytogenetics.

B1252 DNA METHYLTRANSFERASE FAMILY GENES MUTATIONAL STATUS IN CANIS FAMILIARIS AML: A POTENTIAL MODEL FOR THE DISEASE IN PEDIATRIC PATIENTS
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Background: Acute myeloid leukaemia (AML) is the most widely occurring acute leukaemia in adults with a high prevalence of relapses. Genetic aberrations are often found in AML specimens and recent whole genome sequencing studies identified the most frequent genetic alterations and clinical importance. In addition, several studies revealed that both global DNA hypomethylation and regional hypermethylation occur in tumorigenesis. In this respect, mutations of DNA methyltransferases gained particular interest. Indeed, several studies reported a high occurrence of somatic mutations at codon R882 of DNA methyltransferase 3A in adult AML specimens and recent whole genome sequencing studies identified somatic mutations at codon R882 in DNA methyltransferase 3A and 3B, that play a role in de novo CpG methylation and a functional accessory protein, DNMT3L, that activates the two enzymes by binding to their catalytic domain2. Functional studies performed in vitro on human cells line models or using murine models have associated mutations of DNMT3A with DNA hypomethylation and altered whole genome expression profiles. However, a direct correlation between aberrant DNA methylation and mutated DNMT3 remains elusive. We propose the dog as a model for functional studies of human AML providing the advantage that the disease occurs spontaneously in out-bred dogs.

Aims: The purpose of this study is to explore canine AML as a model of human AML. Canine AML specimens were screened for DNMT3A, DNMT3B and DNMT3L to provide a view on the mutational status of all members of the family of DNA methyltransferase genes.

Methods: Sanger sequencing of the C-terminal domain of DNMT3A, DNMT3B, and DNMT3L was achieved in 16 samples of canine AML. The majority of canine patients were large breed in their middle age (mean age 6.9±2.7 years); the diagnosis of AML was based on clinical evaluation and immunophenotyping. Primers were designed to obtain DNA sequences of the C-terminal domain of DNMT3A, DNMT3B and DNMT3L and sequence were homologous to sites of hot spot mutations in adult human patients with AML. Since the mutation detection limit of Sanger sequencing is 20%, we will also use next generation amplicon deep sequencing to detect mutations down to 1% to more thoroughly analyze mutations including the analysis of all exons of the C-terminal domain of DNMT3A, DNMT3B and DNMT3L.

Results: Diagnosis of canine AML was confirmed by morphology with the major part of specimens classified as M2-M4 with some cases of M5, complete blood count profiles (CBC), white blood cell counts (WBC), and by the immunophe-

Summary / Conclusion: In this study we investigated the dog as a study model of human AML. Acute myeloid leukaemia occurs spontaneously in out-bred dogs suggesting it to be a natural model for clinical and biological studies. Identification of specific hypomethylated regions and of biologically critical mutations in DNMT3A, DNMT3B, and DNMT3Lwhich may indicate that childhood AML also canine AML has no mutations in the family of DNA-methyltransferase genes. Along this line canine AML in spite of middle age of patients seems to resemble pedi-
Aims: To investigate the prognostic significance of MMPs in patients with acute myeloid leukemia (AML).

Methods: The MMP-2 and MMP-9 activity ratios (MMP-2/MMP-9) was investigated in BM plasma of 53 patients with de novo AML. During the collection of BM 33 patients were diagnosed with complete remission (CR) and 20 patients without CR. The method of zymography in polyacrylamide gel containing gelatin as a substrate was used.

Results: MMP-2/MMP-9 ratio was approximately 1.00 (0.91±0.06; from 0.42 to 1.77) in the BM samples of 30 patients in CR (91.0%) and 10 patients without CR (50.0%). The median overall survival (OS) of these patients was 27 months, while the median OS of the patients with the same ratio less than 0.50 was not more than 3 times higher (3.34±0.34); from 1.80 to 5.50 (P=0.001) in 13 patients. In the group with high MMP-2/MMP-9 ratio, 10 patients had resistant variant of AML. Their median OS was only 7 months; P=0.001. Other 32 patients were in CR. The natural history of AML in 3 patients with CR and high MMP-2/MMP-9 ratio was poor.

Discussion: In 1 patient MMP-2/MMP-9 ratio was increased before relapse at 3 months and in other 2 patients have got the sign of minimal residual disease.

Summary / Conclusion: The conclusion is that high MMP-2/MMP-9 ratio >/= 1.80 may be associated with unfavorable prognosis patients with AML/L.

References:
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