extremely poor-prognosis, not suitable for intensive chemotherapy. The study was registered at EMA with the EUDRACT number 2012-000334-19. *Acknowledgements.* Chroma is gratefully acknowledged for providing Tosedosatat for the patients. The study was supported in part by AIL Pesaro Onlus.

PO-045

EXPRESSION AND PROGNOSTIC ROLE OF MENINGIOMA-1 (MN1) GENE EXPRESSION AND CORRELATION WITH NPM1 MUTATION AND BAALC EXPRESSION IN PATIENT WITH ACUTE MYELOID LEUKEMIA

Toffoletti E,[1] Tiribelli M,[1] Maccari G,[1] Chiarvesio A,[1] Meneghel A,[1] Cavallin M,[1] Michelutti A,[1] Candoni A,[1] Fabbro D,[2] Damante G,[2] Fanin R,[1] Damiani D[1]

[1]Clinica Ematologica; [2]Dipartimento di Scienze Mediche e Biologiche, Università degli Studi di Udine, Udine, Italy

Introduction. Molecular markers are necessary for prognostic stratification and monitoring of minimal residual disease in acute myeloid leukemia (AML). Cytogenetic aberrations have long been recognized as the most important prognostic variable in AML, and are still the major determinant for post-remission therapy. Unfortunately, only 50-60% of AML patients presents an abnormal karyotype. Meningioma 1 (MN1) gene has been found to be over-expressed in AML with inv(16), and high MN1 levels seems to have prognostic impact in cytogenetically normal AML patients. Others molecular markers are known to have a prognostic role in AML, such as nucleophosmin (NPM1) gene mutation and expression of brain and acute leukemia, cytoplasmic (BAALC) gene. To test the possible prognostic role of MN1 in AML, irrespectively of karyotype status, we studied MN1 expression in a cohort of 171 consecutive AML patients, treated at the Division of Hematology of Udine between 2004 and 2013. We also compared the expression of MN1 with mutation of NPM1 and expression of BAALC. *Methods*. To assess MN1 expression, we designed a quantitative PCR assay with TaqMan chemistry, using ABL as housekeeping gene. To determine the relative quantification of MN1 expression, we used the 2-ΔΔCt method, based on a calibrator obtained on a control group of 12 healthy donors. Results MN1 resulted expressed in all the tested cases, with a median value of 1.1, that was thus considered the cut-off for defining positive (MN1+) and negative (MN1-) cases. The MN1+ cases was not significantly different across the various cytogenetic subgroups (7/10, 70% in favourable karyotype, 38/84, 45% in intermediate karyotype and 22/37, 59% in unfavourable karyotype), but expression level of MN1+ cases was significantly higher in unfavourable (median 7.0, range 1.2-64.7), compared to intermediate (median 2.98, range 1.17-61.9, p=0.007) and favourable (median 1.9, range 1.1-49.9, p=0.01). High MN1 expression was associated with lower frequency of NPM1 mutations (5/70 *vs* 31/71, p=0.0001) and higher BAALC expression (30/68 *vs* 7/69, p=0.0001); no other clinical or biological characteristics were significantly associated with MN1 expression. Complete Remission (CR) rate of the entire cohort was 58%, and MN1 status was not associated with CR achievement. With a median follow-up time of 12 (range: 1-95 months), 3-years disease free survival (DFS) and overall survival (OS) were 45% and 32%, respectively. MN1 expression per se had no impact on DFS and OS; however, considering MN1 in association with other molecular markers, we found that MN1- cases with NPM1 mutation and low BAALC expression had a significantly longer 3-years OS (51% *vs* 28%, p=0.05) (Figure 1).

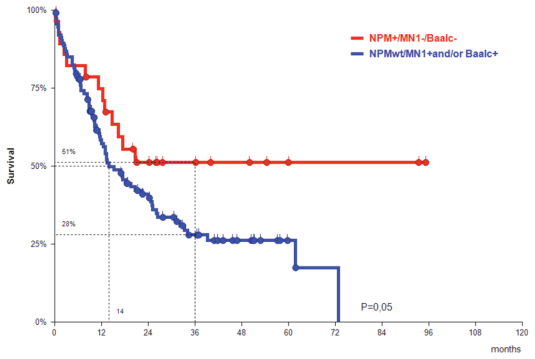


Figure 1. Low expression of MN1 (MN1-), BAALC (Baalc-) and mutated NPM1 (NPM+) predict longer Overall Survival.

Conclusions. The MN1 status, in our cohort, is not associated with a specific genetic subset and do not represent a prognostic factor in terms of CR and OS. On the other hand, MN1 seems identify a defined subgroup with better prognosis in association with NPM1 and BAALC.

PO-046

MYELODYSPLASTIC SYNDROMES: HOW AMELIORATE THE ACCURACY OF DIAGNOSIS BY APPLYING AN INTEGRATED MOLECULAR/CYTOGENETIC WORKUP

Ciabatti E, Grassi S, Ferreri MI, Valetto A, Guerrini F, Guazzelli A, Azzarà A, Metelli MR, Simi P, Petrini M, Galimberti S

Department of Clinical and Experimental Medicine, Hematology Section, University of Pisa, Pisa, Italy

In MDS, cytogenetics is fundamental for the risk stratification, but in this setting non-informative karyotypes represent up to 15-20% of cases. The aCGH is able to detect new abnormalities in up to 80% of cases already tested by conventional karyotype, but it is not enclosed in the routinary diagnostic workup. More recently, the whole genome sequencing methods detected in MDS relevant mutations involving TET2, ASXL1, EZH2, CBL, IDH1/IDH2, DNMT3A. TET2 mutations have been related to a better survival in patients receiving 5-azacitidine, whereas ASXL1, TP53, and EZH2 mutations have been associated with worse outcome. High WT1 expression levels have been related to shorter OS. In this study, we assessed 50 new MDS cases by different techniques: a) conventional cytogenetics; b) FISH for chromosome 5, 7, PDGFRA, and PDGFRB rearrangements; c) aCGH; d)real-time PCR assay for ASXL1, EZH2, TP53, and TET2 mutations. The aim of the study was to determine the adjuctive value offered by FISH, aCGH, and somatic mutation assays in respect of the conventional cytogenetics and to determine if the proposed new diagnostic workup will reach good sensitivity and specificity, necessary for a routinary application. After Giemsa banding, one third of our samples showed chromosomal aberrations, including +8, del(7), del(5), -Y, +6, del(13), +14, del(20), and complex karyotypes. After the FISH analysis, 17% of patients showed chromosomal abnormalities, including 5q- and del(13) that were not detected by the Giemsa banding. The aGCH allowed to detect quantitative chromosomal aberrations in 46% of cases (del(13), -7, del(12), del(16), del(17), del(11), del(8), dupl(14), 5q-). After the RT-PCR assessment, 22% of patients resulted mutated for TP53 gene; the involved nucleotides were 844 (C>T), 733 (G>A), 742 (C>T), and 853 (G>A). Four of these TP53 mutated patients showed normal karyotype, and resulted unmutated also by FISH and aCGH . The WT1 gene was over-expressed (in comparison to healthy subjects) in the 25% of the assessed cases; 50% of these patients presented with RAEB and IPSS intermediate-2/high and had a worse outcome. The half of these patients had a normal karyotype. The RPS14 gene was under-expressed in 77% of cases, analogously to the percentage already reported by our group. Clinical correlations and evaluation of the outcome of these patients are being performed at this time, but we already reported that lower RPS14 and higher WT1 levels did negatively impact on the outcome. In conclusion, these data sustain the fundamental role of the integrated diagnostic work-up for MDS: indeed, 2 cases, correctly classified as affected by the 5q- syndrome after the FISH analysis, received lenalidomide. In one third of the remaining cases, the identification of TP53 and ASXL1 mutations, in addition to the abnormalities of chromosome 17, 8, 11, and 16 detected after aCGH, allowed us to scoring patients as at higher risk. These patients are now candidate to receive azacitidine.

PO-047

AKT PROMOTES THE ONCOGENIC NOTCH SIGNALING IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

Platonova N,[1] Manzo T,[1] Mirandola L,[1] Vigolo E,[1] Cermisoni G,[1] De Simone D,[1] Colombo M,[1] Cecchinato V,[1] Garavelli S,[1] Lazzari E,[1] Galletti S,[2] Neri A,[2] Chiaramonte R[1]

[1]Department of Health Sciences, Università degli Studi di Milano, Milano; [2]Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Hematology, Fondazione Cà Granda IRCCS Policlinico, Milano, Italy

Introduction. Notch and AKT/PI3K signaling are two key oncogenic pathways closely associated in T-cell acute lymphoblastic leukemia (T-ALL). These pathways collaborate in controlling proliferation, survival

and migration of T-ALL cells and are deregulated in 60% (Notch pathway) and 48%(AKT/PI3K) of T-ALL patients. Recent evidences indicate that, in T-ALL cells, Notch and AKT/PI3K pathways collaborate through a reciprocal positive control. Here we identified a novel way by which AKT regulates Notch1 activity and investigated the underlying mechanism. *Methods.* T-ALL cell line, Molt4, and HEK293T cell line were grown in RPMI-1640 and DMEM respectively, supplemented with 10% heat-inactivated FBS. 1 ug RNA isolated from cells was retro-transcribed in 20 ul by M-MuLV reverse transcriptase using random hexamer primers. RT-PCR analysis was performed using primers for Notch1, HES1, preTCRa, GAPDH. Apoptotic cells were identified by Annexin-V and propidium iodide staining. Protein expression was detected by Western blot analysis of whole cell lysates. Immunoprecipitation (IP) of ubiquitin-conjugated proteins was performed using the UbiQapture-Q Kit (Biomol, Exeter, UK), as described by the manufacturer. Co-immunoprecipitation (Co-IP) analysis was performed using Protein G Agarose beads, eluted immunoprecipitates were analyzed by Western blot. Immunofluorescent staining was done on HEK293T cells incubated with anti-Flag or anti-c-Cbl primary antibodies and the appropriate AlexaFluor-conjugated secondary antibodies. Images were acquired with a Leica TCS SP2 confocal microscope. A colocalization area was determined based on a 2D cytofluorogram and density analysis performed by Multicolor Analysis Leica Confocal software. *Results.* The influence of AKT signaling on Notch1 levels was investigated by an inhibitory approach using the PI3K inhibitor LY294002. The LY294002-mediated withdrawal of AKT reduced Notch1 protein levels and activity, without affecting Notch1 transcript. We showed that Notch1 protein decrease was due to lysosomal degradation of the Notch1 membrane-bound form. IP and Co-IP analyses revealed that AKT withdrawal resulted in an increased tyrosine phosphorylation of Notch1 followed by binding to an E3 ubiquitin ligase, c-Cbl, that mono-ubiquitinated Notch1 directing it to lysosomal degradation. *Conclusions.* To our knowledge, these provide the first evidence of mechanism by which AKT pathway controls Notch1 activity reducing the amount of protein undergoing to lysosomal degradation. Given the crucial role of Notch1 in T-ALL, our findings suggest that hyperactive AKT signaling in T-ALL may contribute to increase the oncogenic Notch signaling in T-ALL independently from mutations in Notch1.

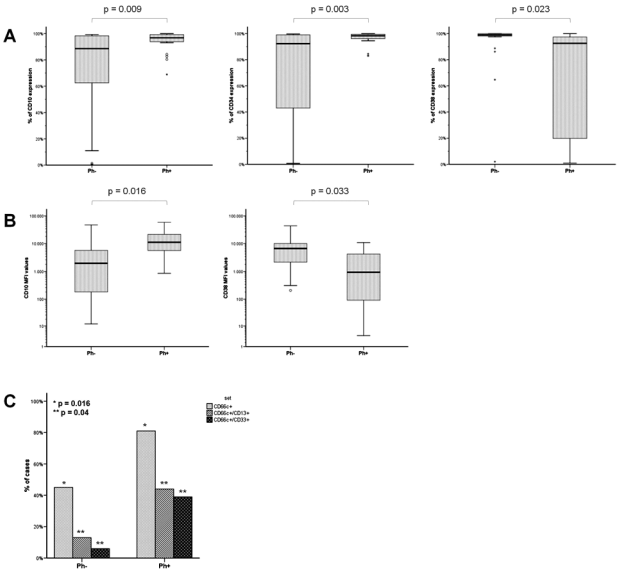
PO-048

ROLE OF FLOW-CYTOMETRIC IMMUNOPHENOTYPING IN PREDICTION OF PHILADELPHIA CHROMOSOME IN ACUTE LYMPHOBLASTIC LEUKEMIA IN ADULTS

Bellesi S,* Corrente F,* Metafuni E, Marietti S, Ciminello AM, Laurenti L, Sorà F, Giammarco S, Pagano L, Sica S, De Stefano V, Chiusolo P

*Department of Hematology, Catholic University of Sacre Heart, Policlinico "A. Gemelli", Rome Italy; *BS and CF equally contributed to this work*

Introduction. Among B-cell precursor acute lymphoblastic leukemia (B-ALL), t(9;22) (q34;q11) responsible for BCR/ABL1 fusion transcripts is the most common cytogenetic abnormality that occurs in around 20-30% of adult patients (pts). Philadelphia-positive (Ph+) B-ALL has been typically associated with high expression of myeloid antigen such as CD13, CD33, CD66c, CD25. We analyzed immunophenotypic pathway of 44 adult patients with B-ALL diagnosed in our center from December 2004 to December 2013, with a median age of 46 ys (range 13-78), 23 males and 21 females. *Methods.* Immunophenotyping was performed using a standard lyse/wash technique. Antibodies definition panel: FITC-CD45; PE-Cy7-CD19; APC-HLA-DR; FITC-CD20; PE-CD22; FITC-CD58; PE-CD10; APC-CD34; PE-CD13; APC-CD33; FITC-CD66c; APC-CD38; FITC-CD24; FITC-NUTdT; PE-NG2; FITC-CD65 FITC-CD15 FITC-MPO PE-cyCD79A. Data were acquired on FACSCanto cytometer using BDFACSCanto software (BD Biosciences). Levels of BCR-ABL fusion transcript were quantified in a multiplex RT-PCR assay. Data were analyzed using SPSS 15.0 version. As the low sample size, the statistical analysis were performed by using both parametric (Student's t-test) and non-parametric (Mann-Whitney) tests. Reported p-values showed a comparable significance in both type of statistics. The Chi-squared test or Fisher exact test were applied for categorical variables. Arbitrary cutoff of 20% analyzed events that were brighter than the control stain, was required for an antigen to be considered positive. *Results.* BCR/ABL transcript was identified in 21 pts (47.7%). Patients with Ph+ ALL were significantly older than their counterpart (mean age, 52 *vs* 39 ys, p=0.015). The mean leukocyte count at diagnosis was higher in the Ph+ pts than others (48.771 *vs* 11.325 x 10^9/L, p=0.010). Ph+ pts had a greater mean percentage of CD10 (94% *vs* 72%, p=0.009), and CD34 (96% *vs* 69%, p=0.003) expression, and a lower mean percentage of CD38 expression (68% *vs* 92%, p=0.023). Considering Median Fluorescence Intensity (MFI), we confirmed that Ph+ pts presented an higher CD10 MFI (mean MFI, 18059 *vs* 6285, p=0.016) and a lower CD38 MFI (3201 *vs* 8012, p=0.033) than their counterpart. CD66c positivity was more frequent in Ph+ pts than in the others (81% *vs* 45%, p=0.016). The co-expression of CD66c with CD13 or CD33 was more frequently present in Ph+ pts (44% and 39%, respectively) than in other cases (13% and 6%, respectively)(p=0,04). The sensitivity of CD66c as sole marker was 81%. The co-expression of CD66c and CD13 or CD33 presented a greater specificity (88% and 94%, respectively) and a positive predictive value (80% and 88%, respectively), Figure 1. *Conclusions*. Based on our analysis, we suggest a possible screening panel for Ph-positive prediction on B-ALL pts by using the combination of CD38, CD10, CD34, CD66C, CD13 and CD33 expressions. These data need to be confirmed by a larger sample size, in order to establish also a possible prognostic role of these markers.



(**A**) Comparison by box-plots of CD10, CD34 and CD38 expression percentage between Philadelphia-positive (Ph+) and Philadelphia-negative (Ph-) B-ALL patients. (**B**) Evaluation of differences in Median Fluorescence Intensity values (MFI) of CD10 and CD38 expression distribution in the two cohorts. (**C**) Frequency bar graph of CD66C positivity (cases with an expression percentage more than 20%) and CD66C co-expressed with CD13 or CD33 between Ph+ and Ph- patients.

Figure 1.

PO-049

AZACITIDINE IN HIGH-RISK MYELODYSPLASTIC SYNDROMES: RETROSPECTIVE ANALYSIS OF 28 PATIENTS TREATED WITH THE AZA 5-2-5 REGIMEN

Clissa C,[1] Follo MY,[2] Stanzani M,[1] Mongiorgi S,[2] Manzoli L,[2] Cocco L,[2] Cavo M,[1] Finelli C[1]

[1]Institute of Hematology, Policlinico S.Orsola-Malpighi, Bologna; [2]Department of Human Anatomical Sciences, Cellular Signalling Laboratory, University of Bologna, Bologna, Italy

Introduction. The currently approved azacitidine (AZA) regimen for myelodysplastic syndromes (MDS) is 75 mg/sqm/die subcutaneously (SC) or intravenously (IV) for 7 days every 28 days. Tree different AZA dosing regimens, which avoid week-end dosing, have shown to induce therapeutic responses consistent with the currently approved schedule (Lyons, 2009). However, the community-based study of Lyons mainly involved lower-risk MDS patients (pts). These data prompted us to investigate the therapeutic effect of the more convenient AZA 5-2-5 regimen (50 mg/m2/d subcutaneously for 5 days, followed by 2 days no treatment, then 50 mg/m2/d for 5 days) in higher-risk MDS pts (*i.e.*: IPSS risk: high or intermediate-2). *Methods.* From December 2007, in our Institution, 28 IPSS high-or-intermediate-2 risk MDS pts. (20 males), with a median age of 70 (37-83) yrs, were treated with the AZA 5-2-5 regimen. Moreover, as our group (Follo, 2009) previously demonstrated that phosphoinositide-phospholipase C (PI-PLC) beta1, may repre-