existing hypomethylating-based protocols: a) high gene specificity b) lower cytotoxicity and c) absence of drug based off-target side-effects. In the short term, this research can lead to the identification of novel key regulators of leukemogenesis and new targets for therapeutic treatments; in the long term pave the way for development of RNA-based gene demethylating agents for cancer treatment.

PB1665

JQ1 AND CURCUMIN COMBINED TREATMENT SHOWS SYNERGIC EFFECTS IN MLL-REARRANGED LEUKEMIA CELL LINES

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Background: MLL-rearranged leukemia accounts for □70% of infant and □10% adult acute leukemias, featuring a particularly poor prognosis and high risk of relapse. Our main field of study is AML, in which nearly 50% of total cases accounts for t(9;11) translocation, the remaining 50% predominantly includes t(6;11)(q27;q23), t(10;11)(p12;q23), t(11;19)(q23;p13.1) and t(11;19)(q23;p13.3). A 2% of AML total cases, however, is characterized by t(4;11) translocation, which is a marker of bad prognosis and it's, so far, poorly characterized. A key feature of MLL-rearranged leukemia is cMyc overexpression, a well-known oncogene involved in several types of cancer. JQ1 is a novel molecule, which prevents cMYC expression binding an important bromodomain protein, BRD4. Moreover, Curcumin, a natural compound, inhibits HATs enzymes preventing lysine 14 acetylation on histone H3 (AcH3K14), a particular residue which is bind by BRD4 to exert its function.
Aims: We would like to explore a potential synergic effect between JQ1 and Curcumin molecules in the attempt to develop a novel therapeutic alternative to standard chemotherapy and to deeply investigate features underlying the molecular pathogenesis in pediatric MLL-rearranged pediatric AML.
Methods: Four human leukemia cell lines with MLL fusion protein have been employed in this study: RS4:11, MV4:11 expressing MLL-AF4 and THP1, MOLM13 expressing MLL-AF9 fusion genes. 5μM and 10μM Curcumin were used to treat MLL-AF4 and MLL-AF9 cell lines respectively, while 250nM JQ1 was used to treat all the cells lines. After 2 days of treatment, either with single and combined drugs, cell number quantification, based on metabolic activity, was detected through XTT assay. In order to assess the cMYC, CDKN1A, BCL2 transcripts levels and mir-99a expression a quantitative RT-PCR analysis was carried out, while we used western blotting to detect the expression of cMYC, PARP, Caspase3 and AcH3K14. Apoptosis and cell cycle were evaluated by flow cytometric analysis.

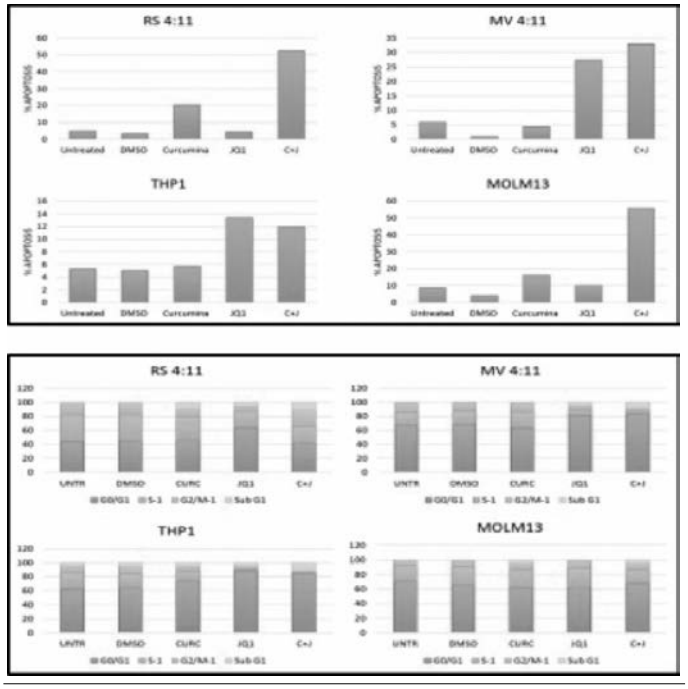

Figure 1.

Results: In apoptosis analysis, a synergic effect was detected for all 4 cell lines, similarly cell cycle evaluation showed a significant accumulation of cells at SubG1 phase (2-8 fold) (Figure 1). XTT metabolic assay showed a reduction in proliferation percentage: 65±5 for curcumin and JQ1 single treatment and 59±5 for combination of drugs in both MLL-AF4 cell lines, meanwhile in MOLM13 cells it was 64±2 and 87±2 for curcumin and JQ1, respectively and 76±2 for their combination (P<0.005). The THP1 cells did not shown any significant modulation in the proliferation. We decided to focus our study on t(4:11) translocated cells, considering the more intense effect of the combined drugs on previous analysis. qRT-PCR and western blot experiments revealed a synergic effect of the 2 experimental drugs on both apoptosis and proliferation gene related (bcl2, caspase3, Parp, cdkn1a) as well as on the direct targets of the drugs (cMyc, AcH3K14). Finally, in MLL-AF4 cell lines, curcumin and JQ1 together induced a significant decrease in mir-99a expression.
Summary/Conclusions: Our data demonstrated that curcumin and JQ1, inhibiting HATs and BRD4 respectively, exert a more intense synergic effect on MLL-AF4 than in MLL-AF9 cells. Increased apoptosis together with a reduced proliferation rate, prompted us to investigate on molecular pathway in which targets of these drugs are involved. Intriguingly, we found a significant decrease in cMyc, bcl2 and AcH3K14 expression, confirming that both curcumin and JQ1 have a synergic effect. Additionally, we revealed a significant reduced expression of mir-99a, a well know oncomiR reported to act as negative regulator of differentiation and involved in drug-resistance, typically upregulated in pediatric AML and ALL.

PB1666

TP53B AND TP53Γ EXPRESSION LEVELS IN RELATION TO NPM1 AND CEBPA MUTATIONS.

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Background: Acute myeloid leukemia (AML) is a heterogeneous clonal disorder with the presence of diverse genetic abnormalities in hematopoietic stem cells. The most frequent alterations in normal karyotype AML (NK AML) are mutations in exon 12 of nucleophosmin gene (*NPM1*). Until now 56 different mutations of *NPM1* exon 12 have been described, mostly insertions. The NPM protein plays an important role in cell cycle and apoptosis control. It cooperates with several proteins, among them with p53 and ARF. The median levels of functional nuclear p53 protein are reduced in *NPM1* and *FLT3 ITD* mutant samples. *TP53* encodes a tumor suppressor protein which consists of transactivation, DNA-binding and oligomerization domains. Due to alternative splicing it may exist in 13 different isoforms. Alternative splicing of intron 9 leads to production of 2 different proteins, p53β and p53γ, without oligomerization domain (stop codon is localized in exon 9b). These isoforms can be present in acute myeloid leukemia (AML) cells. p53β binds to *BAX* promoter and can induce apoptosis independent from p53 wt. p53 has influence on activation of *CEBPA* which is associated with cell cycle regulation, especially cell cycle arrest and plays also role in cell differentiation. Generally, it is a transcription factor expressed during myeloid lineage development, from progenitor cells to mature granulocytes. Various mutations of *CEBPA* gene are described. Among them N-terminal and C-terminal mutations, mostly insertions and deletions, are often present.
Aims: The goal of the study was to assess mutational status of *NPM1, CEBPA* and *FLT3* in association with *TP53*beta and *TP53*gamma expression evels.
Methods: 75 NK AML patients were included in the study. *NPM1, CEBPA* and *FLT3* gene mutations were analyzed by direct sequencing. *TP53*β and *TP53*γ expression levels were assessed with real time PCR. Expression levels were analyzed with ΔΔCt method, with *ABL* as a control gene and K562 cell line as a calibrator.
Results: In all 75 cases, *TP53*β and *TP53*γ transcripts were detected. 36 patients had *NPM1* mutations, 25 had *CEBPA* mutations or known polymorphisms, and 25 had *FLT3* ITD mutation. Assessed median expression level of *TP53*β was much higher (ΔΔCt 43,11) than *TP53*γ (ΔΔCt 10,85; p<0,05). Furthermore, expression level of *TP53*γ in *CEBPA* mutated group (ΔΔCt 11,4) was significantly lower than in *CEBPA* wt group (ΔΔCt 17,7) (p=0,03). We have not found any other important correlation between mutations of studied genes and *TP53*γ or *TP53*β expression. We also classified patients, according to median expression value of *TP53,* to two groups: with overexpression or with low expression. Haematological and clinical features, such as white blood cells count (WBC), blasts count in bone marrow or patient age did not depend on *TP53* isoform expressions. However, statistical analysis showed important difference between WBC count in *NPM1*mutated and *NPM1*wt groups.
Summary/Conclusions: Obtained results may suggest a clinical importance of simultaneous analysis of *TP53* isoform expression and mutations in *CEBPA* gene. It may be hypothesized that a changed sequence of the latter gene might influence *TP53* isoform expression and in consequence regulate the cell cycle.

PB1667

EXPRESSION PROFILE OF EPIGENETIC MODULATORS IN ACUTE MYELOID LEUKEMIA OF INTERMEDIATE RISK

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Background: Whole-genome sequencing has revealed acute myeloid leukemia (AML) as a very complex and dynamic disease.Epigenetic modulation is among the functional categories of the mutational landscape in AML.According to recent reports, suppression of the epigenetic reader BRD4 with small-molecule inhibitors (BET-i) results in antileukemic activity. Clinical trials are being developed, however, so far, identification of those patients that may benefit from this therapy is not possible as changes in mRNA BRD4 levels seem to be very subtle. It has been recently suggested that antileukemic effect of BET-i could be due to c-myc suppression and also that high Bcl-2 levels may target those patients that would benefit of BET-i. We believe that establishing the expression profile of epigenetic modulators in AML may help in the identification of patients that could benefit from BET-i.
Aims: We wanted to get a better insight regarding the expression profile of epigenetic modulator in AML of intermediate risk by studying: 1)expression levels of EZH2, ASXL1, BRD4, c-myc and Bcl-2 in a consecutive series of AML patients; 2) correlation between mRNA and protein levels; 3) Determining BRD4 binding to the c-myc promoter through chromatin immunoprecipitation (CHIP).
Methods: Our series consisted of 104 consecutive patients with a mean age of 55.8 years (range 15-79 years) diagnosed and treated between 2005-2016 at the Hospital Universitario de Gran Canaria Dr. Negrín with a median follow up of 12 months. Gene expression analysis was carried out through real time PCR in a LightCycler 480 Instrument II (Roche) using GUS a control gene.Results were normalized with a cDNA pool from bone marrow of 10 healthy donors which was introduced as internal control in each experiment. Western blot were performed to determine protein levels for BRD4, c-myc and Bcl2. CHIP studies for BRD4 were carried out in HL60 cell line. For statistical analysis the SPSS (v.15.0) software was used.
Results: ASXL1 levels were positively associated with EZH2 (Spearman's= 0.285, p=0.021) and BRD4 withc-myc (Spearman's coefficient=0.420, p<0.001), Bcl2 (Spearman's= 0.471, p<0.001) EZH2 (Spearman's= 0.4565, p=0.008) and ASXL1 (Spearman's=0.949, p<0.001). Survival analysis considering 50th percentile as a cut-off value for BRD4 expression indicated that patients with higher levels shows better overall survival (median overall survival, OS, of 27 months, 95% IC 15.1-38.9) compared to those with low expression (median OS 12 months, 95% IC 0.4-23.7), although the association was not statistically significant (p=0.196) probably due to the limited series size. Protein levels of Bcl2 and c-myc correlated with those of mRNA, but not for BRD4, although other antibodies should be tested in order to confirm these results. CHIP analysis in HL60 cell lines confirmed the binding of BRD4 to c-myc promoter.
Summary/Conclusions: The positive association observed between EZH2 and ASXL1 agrees with the fact that both cooperate in the epigenetic repressive complex PRC2.The association of BRD4 expression levels with c-myc and Bcl2 is in accordance to the reported binding of BRD4 to the c-myc and Bcl2super-enhancer regions and our CHIP analysis also support so. Further studies in a larger series are necessary to confirm the relationship between higher BRD4 levels and better overall survival. Finally, future analysis should be done to determine whether patients with higher BRD4 expression levels determine a subgroup with better response to BET-i.

PB1668

FLOW CYTOMETRY IMMUNOPHENOTYPING IN CEBPA-DM *DE NOVO* AML. BIOLOGIC AND PROGNOSTIC RELEVANCE.

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Background: CEBPA is a transcriptional co-factor of RUNX1 which play a major role in the fate decissions associated with physiologic myelopoiesis. Biallelic CEBPA mutations (dm) define an homogenous molecular subgroup which is associated with a favorable outcome. CEBPA mutations may be transmitted in the germ line giving rise to clusters of familial leukemias.
Aims: To analyze the immunophenotypic findings assessed by multiparametric flow cytometry in a consecutive series of *de novo* CEBPAdm AML.
Methods: Thirty-nine adult patients with *de novo* AML and CEBPAdm who where enrolled on the AML-03 and AML-12 protocols of the Spanish CETLAM cooperative group were included in this study The immunophenotypic analysis was performed on erythrocyte-lysed bone marrow (BM) samples obtained at diagnosis. Antigenic expression of leukemic cells was systematically analyzed by multiparametric flow cytometry using four-color staining. The antigens studied were: CD45, CD34, HLA-DR, CD10, CD20, CD19, CD2, CD33, CD7, CD117, CD66, CD13, CD64, CD36, CD56, CD14, CD123, CD61, CD42b, glycophorin, CD71, CD11b, myeloperoxidase, CD79a, CD3, TdT, lysozyme and lactoferrin. At least 10.000 events/tube were measured. Analytical gates were established according to CD45 reactivity and to FSC/SSC pattern.Positivity thershold was established at 20%. The FACS-DIVA,Paint-a-Gate and Infinicyt software programs were employed for analysis. Amplification of overlapping PCR products covering the whole CEBPA coding sequence followed by Sanger sequencing were used to investigate CEBPA mutations. FLT3-ITD, NPM1, MLL-PTD, WT1 and GATA2 mutations were also investigated by conventional PCR-based molecular methods.
Results: Antigen reactivity was as follows: CD45 (39/39,100%), CD15 (35/39, 90%), CD34 (36/39,92%), HLA-DR (39/39,100%), CD33(39/39,100%), CD2 (2/39,5%), CD7 (36/39,92%),CD117(39/39,100%), CD13(37/39,95%), CD56 (6/39,15%), CD36 (6/39, 15%), CD123(39/39, 100%), CD14 (1/39,0.02%), CD71(38/39,97%), myeloperoxidase (38/39, 97%).In nine cases CD36 and/or CD56 expression on leukemic blasts was greater than 20% Those CD36/CD56+ cases had a shorter overall survival and leukemia free survival (see graph). Four out five tested CD36/CD56+ cases also showed GATA 2 mutations. An additional CD36/CD56+ case had a FLT3-ITD. In three out 39 cases (7%) a population showing cytoplasmic CD79a reactivity was detected (8%, 11%,14% of the neoplastic population, respectively). Two of those cases had also a FLT3-ITD.

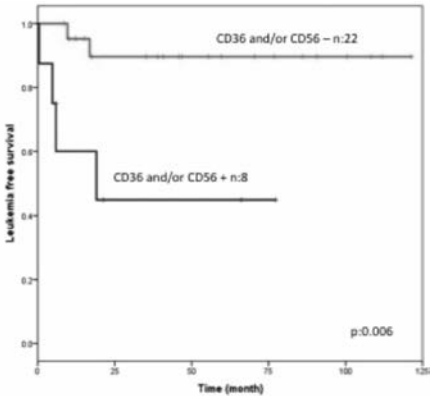

Figure 1.

Summary/Conclusions: CEBPAdm cases showed an homogeneous immunophenotype with positivity for CD45, CD7,CD34,CD123,CD117, HLA-DR, CD71,CD33,CD13 and CD15. CD36 and/or CD56 overexpression was detected in a subgroup of cases (9/39, 23%) with an adverse outcome. The current findings suggest that CD36 and CD56 reactivity should be investigated in larger series of CEBPAdm AML cases. Small leukemic populations with B-cell markers are not uncommon in CEBPAdm AML (3/39, 7%).

PB1669

PROTEOME CHANGES IN ACUTE MYELOID LEUKEMIA PATIENTS BEFORE AND AFTER INDUCTION TREATMENT

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Background: Acute myeloid leukemia (AML) is a malignant disorder of hematopoietic stem and progenitor cells (HSPCs), characterized by the accumulation of immature blasts in the bone marrow and peripheral blood (PB) of affected patients. Standard induction therapy, based on cytarabine and an anthracycline, leads to complete remission in approximately 50% to 75% of patients, depending on prognostic factors, such as age or the presence of certain gene or chromosomal changes. In spite of favorable primary response rates, only approximately 20% to 30% of the patients enjoy long-term disease survival.
Aims: Our aim was to compare the protein expression profile of peripheral blood mononuclear cells (PBMCs) of AML patients at time of diagnosis and after induction therapy.
Methods: PB samples were taken from seven AML patients in Medellin-Colombia at time of diagnosis and after concluding the induction therapy. Informed consent was obtained prior to sample collection. PBMCs were isolated from the 14 blood samples using a Histopaque-1077 solution. Cells were resuspended in lysis buffer (0.5% Triton x-100, 50 mM Tris-HCL pH 8.0, 150 mM NaCL, 1 mM EDTA, protease inhibitor) and proteins precipitated with trichloroacetic acid. Proteins were separated by 2D SDS-PAGE (pI 3–10 NL), and stained with SYPRO®Ruby. The proteomes were compared using PDQuest™ Advanced 8.0.1 Software. Protein spots of interest were those with a fold change of +/- 1.5 and p <0.05.