approach was able to detect the most frequent *IKZF1*del on chromosome 7p, which could be used as markers for minimal residual disease monitoring. However, precise data concerning *IKZF1* whole-gene Δ1-8 deletions, which account for approximately 30% of all *IKZF1del*, are still lacking.
Aims: We aimed at characterizing *IKZF1* whole-gene deletions at the genomic level in order to improve the current PCR methods for the analysis of the most frequent *IKZF1* deletions in pediatric patients with BCP-ALL.
Methods: Samples of BCP-ALL with *IKZF1* whole-gene deletions (n=30) were included. Deletions of exons 1-8 were first identified by the multiplex ligation-dependent probe amplification (MLPA) kit (P335-A4), and confirmed using specific *IKZF1* MLPA kit (P202). Six samples were analyzed with CytoScan HD array (Affymetrix. Inc., Santa Clara, CA, USA), and the breakpoints were confirmed by multiplex PCR. Afterwards, 24 samples were screened for the hotspot identified by the array (located within *COBL* gene) with a long distance PCR (LDI-PCR) and sequencing approach.
Results: According to the comparative genome analysis, 2 out of 6 samples with BCP-ALL (33.3%) and *IKZF1* whole-gene deletions had a breakpoint on *COBL* intron 5. They presented a ~1.7 Mb and ~18.8 Mb deletion (7p12.2-*COBL* and 7p14.3-*COBL*). Further screening of 24 samples within *COBL* intron 5 led to the verification of three molecular alterations: a 1.1 Mb inversion of *COBL* that also compromised *IKZF1*; and two chromosomal translocations (Xp21.3-*COBL* and C20orf194-*COBL*). In sum, a hotspot on *COBL* intron 5 was found in 16.7% of the samples (five of thirty samples) with *IKZF1* Δ1-8.
Summary and Conclusions: This study identified chromosomal translocations, deletions, and inversions involving *COBL* intron 5 on samples with *IKZF1* whole-gene deletions. This clearly indicates that *COBL* intron 5 is a new hotspot for *IKZF1* whole-gene deletions. Genetic alterations starting on *COBL* intron 5 also promote *IKZF1* deletions due to the proximity between *IKZF1* and *COBL* (~800 Mb). Further analyses covering a larger genomic area within *COBL* are ongoing in order to unravel novel regions associated with DNA breaks and *IKZF1* deletions.
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LB2082

EXPRESSION ANALYSIS OF GENES LOCATED IN THE COMMON REGION OF AMPLIFICATION IN PEDIATRIC B CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA WITH INTRACROMOSSOMAL AMPLIFICATION OF CHROMOSOME 21

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Background: B-cell precursor acute lymphoblastic leukemia (BCP-ALL) cases frequently present high hyperdiploidy (>50 chromosomes) or chromosomal translocations resulting in chimeric gene fusions (*ETV6-RUNX1, BCR-ABL1, TCF3-PBX1*, and *KMT2A-AFF1*). Recently, molecular cytogenetic studies defined a subgroup among BCP-ALL with cryptic intrachromosomal amplification features on chromosome 21 (iAMP21). The iAMP21 has been described in 1.5-3% of BCP-ALL and is associated with a higher relapse risk when patients are treated under standard regimens. Genomic analysis demonstrated the presence of a common region of amplification (CRA), including genes such as *IFNGR2, GART, SON DSCR1, RUNX, DYRK1A, ERG*, and *ETS*. These genes are described to be involved in the leukemogenesis and/or in the therapeutic response of some leukemia subtypes. Our hypothesis is that aberrant expression of these genes could also contribute to the particularities of iAMP21-BCP-ALL.
Aims: We aimed to identify genes that could be potentially relevant in the leukemogenesis of iAMP21-BCP-ALL by evaluating the expression of genes with functions previously described in leukemia and located in the CRA.
Methods: From 368 bone marrow (BM) samples of patients with BCP-ALL diagnosed between 2002 and 2012, 74 were suggestive for chromosome 21 copy number alterations by *Multiplex Ligation-dependent Probe Amplification* (MLPA). In 9 patients an iAMP21 was confirmed by Fluorescence *in situ* hibridization (FISH). Gene expression analysis of these samples was performed by reverse transcriptase quantitative PCR (qPCR) and CT values of the selected genes among BCP-ALL samples either with or without an iAMP21 were calculated. The non-iAMP21 subgroups were: *ETV6-RUNX1* fusion (n=9), hyperdiploid karyotypes (n=3), no recurrent gene fusions (n=7), and non-leukemic controls (n=7). Statistical analysis was performed using *Student's t-tests,* p values <0.05 were considered statistically significant (*Unpaired t-test*), using the GraphPad Prism 1.5 software.
Results: Patients with iAMP21-BCP-ALL had higher expression of *IFNGR2* (*p=0.0331; **p=0.0147; ***p=0.0023), *ERG* (*p=0.0284; ***p=0.0115), *ETS2* (*p=0.0343; **p=0.0058; ***p=0.0021) and *DYRK1A* (***p=0.0008) genes when compared with *ETV6-RUNX1* (*p), no recurrent gene fusions (**p), or with hyperdiploid karyotypes (***p) groups.

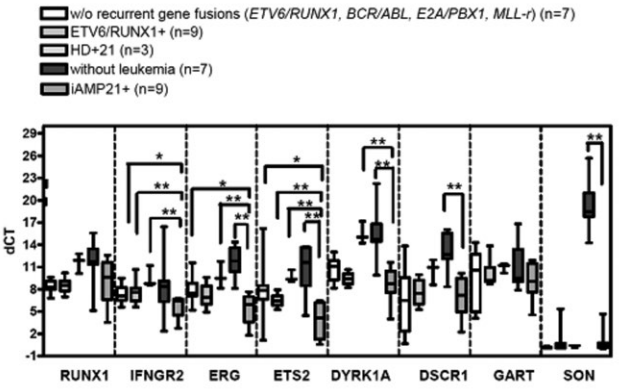

Figure 1.

Summary and Conclusions: Our results suggest that there is a differential expression of some genes in the CRA of samples from patients with iAMP21-BCP-ALL. Thus, these data allow further studies in order to evaluate the contribution of these genes in leukemogenesis and therapeutic response of iAMP21-BCP-ALL.

LB2083

AKT REGULATION OF ONCOGENIC NOTCH PATHWAY IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Notch and PI3K/AKT 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% (PI3K/AKT) of T-ALL patients. Recent evidences indicate that in T-ALL cells, Notch and PI3K/AKT pathways collaborate through a reciprocal control.
Aims: of this work was to investigate the molecular mechanisms of PI3K/AKT-dependent Notch1 activity regulation.
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 mg RNA isolated from cells was retro-transcribed in 20 ml 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. HEK293T cells were transfected with expression plasmid encoding the dominant negative mutant form of AKT (DN-AKT) and with plasmid encoding full length Flag-tagged NOTCH1 using the DMRIE-C reagent (Invitrogen), according to manufacturer's instructions. Molt4 cells were electroporated with 20 μg of pcDNA3.1 DN-AKT or mock pcDNA3.1 using the Neon Transfection System (Invitrogen) according to the manufacturer's guidelines. After 72 h cells were harvested to prepare protein lysates. Immunoprecipitation of ubiquitin-conjugated proteins was performed using the UbiQapture-Q Kit (Biomol, Exeter, UK), as described by the manufacturer. Co-immunoprecipitation 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: Both LY294002-mediated chemical inhibition of PI3K/AKT signaling and transient transfection of a DN-AKT mutant strongly reduced Notch1 protein level and activity, without affecting Notch1 transcription. We showed that downstream AKT regulator, GSK-3β, did not mediate the effect of PI3K/AKT withdrawal on Notch1 protein. We demonstrated that Notch1 protein decrease upon PI3K/AKT inhibition was due to lysosomal degradation of the Notch1 membrane-bound form. IP and Co-IP analyses revealed that PI3K/AKT withdrawal in Molt4 cells resulted in an increased tyrosine phosphorylation of Notch1 and monoubiquitination of Notch1 as detected by ubiquitin capture assay. Co-immunoprecipitation assay and co-localization analysis further showed that E3 ubiquitin ligase, c-Cbl, interacted with Notch1 in order to direct it into the lysosome for degradation.
Summary and Conclusions: To our knowledge, our results provide the first evidence of mechanism by which AKT pathway controls Notch1 activity reduc-

ing 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. Therefore a therapeutic strategy directed to PI3K/AKT signaling in T-ALL could provide advantages to inhibit the dysregulated NOTCH signaling.

Acute lymphoblastic leukemia - Clinical

E870

EVALUATION OF INOSINE TRIPHOSPHATE PYROPHOSPHOHYDROLASE 94 C>A, IVS2 +21 A>C POLYMORPHYSMS IN ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED WITH 6-MP AND PREDICTION OF ITS MYELOSUPPRESSIVE EFFECTS

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Background: 6- Mercaptopurine (6-MP) plays an importante role in treatment of ALL. ITPA is one of the enzymes which is involved in 6-MP metabolism pathway, catalyzes the pyrophosphohydrolysis of inosine triphosphate (ITP) to inosine monophosphate (IMP) and prevents accumulation of toxic ITP metabolits.
Aims: Our objective was to evaluate the *ITPA 94 C >A, IVS2 +21 A >C* gene polymorphisms in patients with ALL under treatment with 6-MP and prediction of its myelosuppressive effects.
Methods: In this case series study the population consisted of 70 patients diagnosed with ALL in the Division of Hematology-Oncology of Tehran Mofid Hospital. PCR was carried out to amplify exon 2, exon 3, intron 2 and intron 3 of *ITPA* gene. All the amplified fragments were subjected to directional sequencing, then association between genotype and 6-MP toxicity was studied.
Results: In this study two exonic variants including *94 C>A* and *138 G>A* showed a prevalence of 8.5% and 36.4% respectively and two intronic variants, *IVS2 +21 A>C* and *IVS3 +60 G>A* were found in 13.5% and 7% of the samples respectively. The rate of myelosuppression in the presence of mutant homozygote and heterozygote alleles (*94 C>A, 138 G>A, IVS2 +21 A>C* and *IVS3 +60 G>A*) was higher compared to wild type (CC, AA and GG) alleles during the use of 6-MP (P<0.05). Hepatotoxicity in individuals with mutant homozygote and heterozygote *94 C>A* and *IVS3 +60 G>A* alleles was higher than individuals with wild type alleles (CC and GG) during the use of 6-MP (P<0.05).
Summary and Conclusions: Our results indicate that individuals who have aberrant ITPase genotype (mutant homozygous or heterozygous), more likely to have myelosuppression and hepatotoxicity during the use of 6-MP. This results also suggest that pre-therapeutic screening of *ITPA 94 C>A*, *IVS2 +21 A>C* and *IVS3 +60 G>A* may help in minimizing the myelosuppression and hepatotoxicity induced by 6-MP in ALL patients.

E871

COMPARISON OF THREE DIFFERENT PRIMING REGIMENS BASED ON IDARUBICIN, ACLACINOMYCIN OR PIRARUBICIN FOR REFRACTORY/ RELAPSE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Refractory/relapse acute lymphoblastic leukemia (ALL) has very poor clinical outcome for the resistance to routine chemical agents. Priming regimen was a promissing choice for refractory/relapse ALL, especially for those eldly patients.
Aims: We were interested and analyzed three different priming regimens based on idarubicin, aclacinomycin or pirarubicin for refractory/relapse ALL. Both the effectiveness and safety were investigated.
Methods: A total of 43 refractory and/or relapsed ALL patients were enrolled in this study. These patients composed of 31 male and 12 female, with the medial age of 26(8~ 85) years old. The salvage regimen was idarubicin, aclacinomycin or pirarubicin combined with cytarabine (Ara-C) and granulocyte colony-stimulating factor (G-CSF). The detailed three regimens were performed as, IAG (idarubicin 10mg/d, d1,4,8,11; Ara-C 10 mg/m^2 twice daily, d1-14; G-CSF 200 mg /m^2 d0~14), CAG(Acla 10mg/d, d1-8; Ara-C 10 mg/m^2 twice daily, d1-14; G-CSF 200 mg /m^2 d0~14) and TAG(pirarubicin 10mg/d, d1,4,8, 11; Ara-C 10 mg/m^2 twice daily, d1-14; G-CSF 200 mg /m^2 d0~14). There were 13 cases in IAG group, 18 cases in CAG group and 12 cases were assigned to TAG group. Effectiveness was measured using objective response and safety was measured using the NCI classification system of common toxicity criteria for adverse events. Some parameters which may influence the clinical outcome, such as age, immunophenotype and white blood cell (WBC) count were also explored.
Results: The overall complete remission (CR) rate for all patients in three groups was 46.5% (20 cases), partial remission (PR) rate was 16.2% (7 cases), and 16 cases (37.2%) got no remission (NR). The overall response rate (ORR) was 62.8%. In IAG group, the ORR was 69.2%, and the CR rate as well as PR rate was 53.8% and 15.4%, respectively. There were 8 patients (44.4%) got CR, 3 patients (16.6%) got PR, and ORR was 61% in CAG group. In group TAG, a total of 5 cases (41.7%) got CR, and 2 cases (16.6%) got PR, with ORR 58.3%. The response rate in three groups had no statistical difference (P=0.837). Compared with patients □35 years old (ORR 71.4%), the ORR for ≤35 years old group was 54.5%, and no statistical difference was found between two groups (P=0.347). There was also no significance about ORR between T-