EUROPEAN HEMATOLOGY ASSOCIATION

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ABSTRACT BOOK

EHA-SWG Scientific Meeting

New Molecular Insights and Innovative Management Approaches for Acute Lymphoblastic Leukemia

April 12-14, 2018 | Barcelona, Spain

EUROPEAN HEMATOLOGY ASSOCIATION

CALENDAR OF EHA MEETINGS

23RD EHA Congress

June 14-17, 2018 | Stockholm, Sweden

Hematology Tutorials

EHA-TSH Tutorial on Acute leukemia
April 28-29, 2018
Chairs: H Öszan, G Ossenkoppele
Istanbul, Turkey

EHA-SHRC Tutorial on Thalassemia
May 10-11, 2018
Chairs: M Karimi, MD Cappellini, A Taher
Shiraz, Iran

EHA-SAH Tutorial on Lymphoid Malignancies and Plasma Cell Dyscrasias
September 14-15, 2018
Chairs: D Fantl, G Kusminsky, R Foà
Buenos Aires, Argentina

EHA-PTHiT Tutorial on Myeloid and Lymphoid Leukemias
September 21-22, 2018
Warsaw, Poland

EHA Baltic Tutorial on Lymphoid Malignancies
October 18-19, 2018
Chairs: E Laane, S Lejniece, V Peceliunas
Tallinn, Estonia

Scientific Meetings

Highlights of Past EHA (HOPE 2018)
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INTRODUCTION

This abstract book contains abstracts submitted for the **EHA-SWG Scientific Meeting on New Molecular Insights and Innovative Management Approaches for Acute Lymphoblastic Leukemia**, to be held on April 12-14, 2018 in Barcelona, Spain.

Considerable progress has been made in the management of ALL in the past decade and a number of exciting new compounds are under development. Furthermore, new biologic subgroups and their respective diagnostic strategies have been described. The activities of European ALL study groups greatly contributed to this success. These rapid developments are a challenge for patient treatment and clinical science.

This meeting aims to bring clinicians and young scientists together with leading international experts to discuss the above-mentioned developments. They have all been given the option to actively participate in this meeting by submitting abstracts under the various topics covered by the program.

We would like to thank the following reviewers for their time and efforts:

- N Gökbuget (Goethe University, Frankfurt, Germany)
- R Bassan (UOC Ematologia, Ospedale dell'Angelo, Mestre Venezia, Italy)
- A Fielding (UCL Cancer Institute, London, United Kingdom)
- JM Ribera (ICO-Hospital Germans Trias i Pujol, Badalona, Spain)

Following review, abstracts were selected for an oral 'pitch' presentation in various sessions throughout the program; a poster presentation during the two scheduled poster sessions on April 12 and April 13 or online publication (in this book) only.

We hope the abstracts in this book will be a valuable source of information, during and after the SWG Scientific Meeting on ALL.

Kind regards,
European Hematology Association
"Towards a world without blood disorders"

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ORAL 'PITCH' PRESENTATIONS

THURSDAY, APRIL 12

SCIENTIFIC SESSION: GENETICS OF ALL-SUBTYPES

O001

Multiomics of Relapsed B-Cell Precursor Acute Lymphoblastic Leukemia

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Introduction: Outcomes of relapsed B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL) remain unsatisfactory, particularly in adult patients (Gökbuget et al, 2016). To gain a higher resolution and a broader molecular view of relapsed BCP-ALL, we performed a multiomics study to reveal relapse driving alterations in pediatric and adult BCP-ALL patients (n=50) lacking typical fusion-genes (B-others).

Methods: We studied a homogeneous multi-omics dataset including DNA-sequencing, RNA-sequencing and methylation data from matched diagnosis and relapse samples of adult (n=24) and pediatric BCP-ALL (n=26) patients. All patients were treated in population based German study trials (GMALL, BFM, CoALL).

Results: We identified significantly more mutations in relapsed compared to diagnosis samples (adult median: 16 vs. 13.5; paediatric median: 19 vs. 10). The most recurrently mutated genes included *NRAS*, *TP53*, *KRAS*, *IKZF1*. *NT5C2*, *SYK* and

CHD1 were exclusively mutated in the pediatric cohort with at least 3 mutations. *NT5C2* was also specific for early relapse. Based on distinct expression signatures and the detection of specific gene fusions, we defined four main subtypes among B-other BCP-ALL: *DUX4*, Ph-like and two distinct aneuploid subtypes. Subgroup assignment remained stable between diagnosis and relapse. 80% of patients (n=40) showed relapse specific alterations in

genes enriched for chromatin modifiers, nucleotide and steroid metabolism.

The dissection of clonal evolution revealed *stable mutations* (present at diagnosis and relapse; 31% of mutations); *ID only* alteration not detected at relapse (22.6%), and mutations *acquired at relapse* (41%). We calculated that 52% of relapsed ALL arose from a *precursors clone* characterized by the loss of a major clone, while maintaining a minor clone from diagnosis. In contrast, 44% of cases relapsed from a major clone present at diagnosis (*successor*). Only 4% of the relapsed ALL samples were classified as *novel*. In addition, analysing gene expression patterns and employing a multi-omics approach, we identified a relapse-specific gene signature for *DUX4* BCP-ALL highlighting chemotaxis and cytokine environment as a possible driver event at relapse.

Conclusions: We present novel insights of relapsed adult and paediatric BCP-ALL based on a comprehensive multi-omics integrated data set. The data uncover novel insights into clonal evolution, age and relapse specific molecular alterations that will allow to explore novel therapeutic concepts.

O002

The mutational and transcriptome landscape of infant B-cell acute lymphoblastic leukemia: The INTERFANT treatment protocol experience

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Background: Infant B-cell precursor acute lymphoblastic leukemia (iBCP-ALL) has dismal prognosis, especially with MLL-gene rearrangements (MLLr) which are hallmark clonal leukemogenic drivers. Molecular pathogenesis of MLLr-iBCP-ALL remain somehow enigmatic and in vivo recreation of MLLr-iBCP-ALL is challenging.

Methods: We performed whole-genome, exome and RNA-sequencing on an Interfant study discovery cohort of 50iBCP-ALLs (27MLL-AF4+, including relapses, 5MLL-AF9+ and 10non-MLL). An independent validation cohort of 82iBCP-ALLs (43MLL-AF4+, 11MLL-AF9+, and 28non-MLL) was used for targeted DNA-sequencing/qRT-PCR.

Result: iBCP-ALL shows an extremely low frequency of somatic mutations, irrespective of the presence/subtype of MLLr, with the predominant leukemic clone carrying a mean of 2.5 non-silent mutations. Recurrent mutations were exclusively found in KRAS and NRAS, which were more frequent in the MLL-AF4+ than in MLL-AF9+/non-MLL iBCP-ALL due to common NRAS mutations found in MLL-AF4+ infants (32%vs6%; $p<0.01$). These mutations were subclonal and frequently lost at relapse, despite a larger number of non-silent but non-recurrent mutations (19.5 mutations/patient). RNA-seq/qRT-PCR validation revealed that the reciprocal fusion AF4-MLL is expressed only in 55% of the t(4;11)+ patients, and

that HOXA cluster genes are uniquely expressed in AF4-MLL-expressing t(4;11)+ patients. AF4-MLL/HOXA-expressing patients displayed higher 2-year event-free survival than patients lacking AF4-MLL expression (65%vs34%, p=0.15). Opposite to pediatric/adult BCP-ALLs, BCR repertoire analysis revealed only minor, non-expanded B-cell clones in t(4;11)+iBCP-ALL.

Conclusions: iBCP-ALL shows a silent mutational landscape regardless the MLL status. The expression of AF4-MLL associates to a better prognosis and specific upregulation of HOXA cluster genes. A pre-BCR early progenitor/stem cell may represent the cell-of-origin for both the t(4;11) and RAS mutations.

O003

A novel subgroup of B cell precursor ALL defined by *PAX5* sequence variants cooperating with *CDKN2A* deletions and RAS activating mutations

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Background: Gene fusions or abnormal ploidy patterns define subgroups in B cell precursor acute lymphoblastic leukemia (BCP-ALL).[1] These founding lesions relate to specific transcriptional and epigenetic programs in a context of cooperating mutations and represent potential therapeutic targets. Yet, up to one third of BCP-ALL patients lack recurrent founding lesions, thus precluding subgroup allocation and the development of target specific strategies. - An unmet need especially for adult patients with relapsed or refractory disease.[2]

Aims: We applied a multi-omics approach to characterize novel driver candidates in BCP-ALL.

Methods: Bone marrow or peripheral blood samples from patients with first diagnosis of BCP-ALL (n=250, age: median 32 years, range 1 – 81 years) treated in German multicenter trials (GMALL, ALL-BFM, COALL) were analyzed by whole exome (WES) / gene panel sequencing (n=56 / n=156), RNA-Seq / fusion breakpoint-specific qRT-PCRs (n=208, n=250), DNA-methylation chip arrays (n=140) and MLPA (n=210).

Results: Established molecular drivers were identified in 204 patients of the total

cohort (n=250): Ph-like (n=47), DUX4 fusion (n=44), Ph-positive (n=31), Aneuploid (n=30), ZNF384 fusion (n=19), KMT2A fusion (n=14), TCF3-PBX1 (n=6), MEF2D fusion (n=5), ETV6-RUNX1 (n=4), PAX5-ETV6 (n=4).

Unsupervised hierarchical clustering of top variable expressed genes and top variable methylated DNA regions identified a novel cluster (n=19) among the 46 patients lacking established molecular drivers. All 19 patients within this cluster harbored sequence variants in the lymphoid transcription factor *PAX5*. The *PAX5* DNA-binding site was affected at least once in all 19 patients, with the majority of mutations being homozygous (n=14/19) and a high frequency of the PAX5 p.P80R (n=14/19) amino acid change, occurring exclusively in this cluster.

Analysis of cooperating events identified deletions in the cell cycle regulator *CDKN2A* (n=18/19) and either *NRAS* (n=9/19) or *KRAS* (n=6/19) activating hotspot mutations as highly enriched in this cluster (p*PAX5* / *CDKN2A* / *RAS* triple alterations. WES karyotyping of PAX5-plus patients (n=8) revealed structural events of chromosome 9 (9p deletion: n=4, chr. 9 or 9p CN-LOH: n=2) leading to homozygosity for alterations of *PAX5* (9p13.2) and *CDKN2A* (9p21.3).

PAX5-plus samples showed differential expression of 1555 genes (FDR<0.05). Gene set enrichment analysis indicated down-regulation of PAX5 activated target genes in PAX5-plus BCP-ALL patients.

Conclusion: We identified a novel subgroup of BCP-ALL (PAX5-plus) by genomic triple-alterations affecting *PAX5*, *CDKN2A* and *NRAS/KRAS* as well as chromosome 9 structural events and specific gene expression and DNA methylation profiles.

O004

Amplicon-based NGS as a tool for the identification of new poor prognosis markers B-ALL

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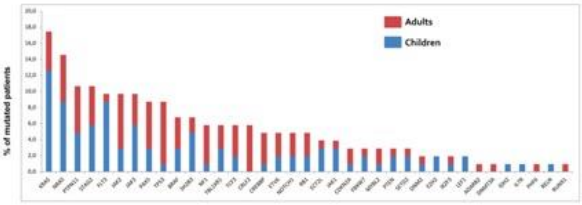
Background: B-cell precursor Acute Lymphoblastic Leukemia (B-ALL) is a neoplasm that can be present in both children and adults, being more frequent in childhood (80%). Despite the increased survival rate, it is still necessary to identify new markers that can explain the relapsing and refractory cases.

Aims: To determine the frequency and prognostic impact of mutations in both children and adults with B-ALL, treated according to PETHEMA and SEHOP protocols.

Methods: A total of 103 samples from children and adults (46 and 57 respectively) with B-ALL were analyzed at the time of diagnosis or prior to treatment. The amplicon-based sequencing (Illumina) allowed the analysis of 52 genes relevant in B-ALL.

Results: 1) A mutation rate of 84.4% (87 of 103) was identified. The most frequently mutated genes were: *KRAS* (17.5%), *NRAS* (14.6%), *PTPN11* and *STAG2* (10.7%) and *FLT3*, *JAK2* and *JAK3* (9.7%). Interestingly, we observed differences between children and adults. While in children the most recurrent mutations were in *KRAS* (28.3%), *FLT3* and *NRAS* (19.6%), *JAK3* and *STAG2* (13%), in adults the most frequently mutated genes were *TP53* (14%), *JAK2* (12.3%), *NRAS*, *PAX5* and *PTPN11* (10.5%) (Figure 1).



2) Mutations in *RAS* pathway were more frequently detected in *BCR-ABL1* negative cases (p=0.001). Considering the whole B-ALL cohort, the univariate survival analysis showed that mutations in *TP53* were related with lower OS (p=0.001), EFS (p=0,005) and RFS (p=0.001), *JAK2* mutations with a decreased RFS (p=0.02) and EFS (p=0.033), while NF1 mutations were associated with lower OS (p=0.006), EFS (p=0.017) and RFS (p=0.016). Combining *JAK2* and *TP53* mutations, the univariate survival analysis showed association between mutations in *TP53* and/or *JAK2* and a lower OS (p=0.005), lower EFS (p<0.0001) and lower RFS (p<0.0001). In a similar way, mutations in *NF1* and *BRAF*, both involved in *RAS* pathway, were linked with a lower OS (p=0.001) and EFS (p=0.007). Of note, mutations in epigenetic regulator and chromatin structure modifiers (*SETD2, PHF6, IDH2, EZH2 Y CREBBP)* were associated with shorter OS (p=0.05). In the pediatric cases, mutations in *TP53* were associated with lower OS (p=0.01), EFS (p=0.01) and RFS (p=0.02), *TP53* and/or *JAK2* mutations were associated with a lower OS (p=0.023) and RFS (p=0.024), and mutations in *NF1* and/or *BRAF* were associated with a lower OS (p=0.032) and RFS (p=0.036). However, in adults, just *TP53* mutations were associated with a lower RFS (p=0.031). In the whole B-ALL cohort, mutations in *TP53*

and/or *JAK2* stood as an independent risk factor associated with shorter EFS (HR=3.4; *p*=0.008) and RFS (HR=5.2; *p*=0.006). By contrast, mutations in *NF1* and/or *BRAF* were independent poor prognosis markers for OS (HR=12.5; *p*=0.034) and RFS (HR=12.2; *p*=0.037) in children.

Summary/Conclusion: Mutations in *JAK2* and/or *TP53*, in epigenetic regulators and chromatin structure modifiers, as well as mutations in *NF1*

SCIENTIFIC SESSION: PERSONALIZED MEDICINE IN ALL

O005

Circular RNAs as novel therapeutic targets in drug resistant childhood leukemia

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Background: Novel therapeutic targets need to be identified for genetic subtypes of acute lymphoblastic leukemia (ALL) that are treatment resistant and associated with poor prognosis. Circular RNAs, a recently described RNA species, present a novel class of therapeutic targets. Similar to linear RNAs, circRNAs are derived from parental genes. They are circularized by backsplicing and are remarkably stable. CircRNAs may play a role in cancer by regulating the expression of genes or microRNAs with tumor suppressive or oncogenic function. Dysregulated circRNA expression has been observed in a variety of human tumors and pathogenic circRNAs derived from leukemia fusion genes have been described recently.

Aims: We aimed at identifying circRNAs that are associated with drug resistance. To this end we compared the landscape of expressed circRNAs in drug resistant *TCF3-HLF*- and drug responsive *TCF3-PBX1*-rearranged ALL.

Methods: We used a bioinformatic approach to identify circRNAs in RNAseq data of *TCF3-HLF*+ and *TCF3-PBX1*+ primary patient samples (n=5 each, matched remission and diagnosis samples). To validate our findings we employed leukemia patient derived xenografts grown in NSG mice from the same cohort of patients and two cell line models: the *TCF3-HLF*+ cell line HAL01 and the *TCF3-PBX1*+ cell line 697. These samples underwent transcriptome, miRNome and circRNome sequencing.

Results: RNAseq patient data revealed 556 putative circRNAs. 249 circRNAs were specifically expressed only in leukemic and not in remission samples. Of those, 109 circRNAs were exclusively expressed in drug resistant *TCF3-HLF*+ leukemia. 9 circRNAs derived from parental genes such as the lysine demethylase *KDM1A* involved in p53 mediated DNA damage signaling were recurrently detected.

Enrichment of circRNAs by RNase R digest and subsequent sequencing led to detection of 14,311 circular RNAs in the cell lines HAL01 and 697. 7,918 of those were described in the circBase database, 6,393 were novel. The circRNAs were derived from 4,652 parental genes. 1 to 39 circRNAs were transcribed per gene. The most highly, but ubiquitously expressed circRNA was encoded by the gene *NRIP1*, a corepressor that modulates a variety of transcription factors. Several circRNAs derived from genes known to be involved in ALL pathogenesis (*PAX5, IKZF1* and *RUNX1*), but no fusion gene encoded circRNAs were detected. CircRNAs derived from the drug transporter gene Multidrug resistance-associated protein 1 (*MRP1*) were highly expressed in drug resistant but not in sensitive cells. MRP1 detoxifies

chemotherapeutic drugs by export of these substances out of the cells. Clinically relevant substrates of MRP1 are anticancer drugs, including vincristine, etoposide, topotecan and methotrexate. Drug screening of *TCF3-HLF*+ patient derived xenografts previously demonstrated explicit resistance against these drugs. Overexpression of *MRP1* and similar transporters is considered a major cause of multidrug resistant tumor phenotypes. Upregulation of these transporters up to 10 fold has been observed in *TCF3-HLF*+ leukemia. CircRNA knockdown and overexpression were carried out to analyze the mechanism of *MRP1* upregulation in detail and will be presented.

Summary - Conclusion: Our results indicate circular RNAs as interesting biomarkers or therapeutic targets for patients with therapy resistant ALL.

O006

Advanced diagnostic approach in the new AIEOP protocol for Acute Lymphoblastic Leukemia

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Background: In the past years the cure rate for ALL patients continuously improved however, there are high risk cases of relapse that so need early treatment.

ALL is characterized by distinct genetic alterations and clinical features. In the new protocol AIEOP-BFM-ALL2017 it's necessary a better stratification of patients and to develop more efficacious therapies directed on specific genetic lesion.

Objectives: The main rationale is to provide a rapid and multi-comprehensive tool for clinical decision making, in particular for actionable lesions, either for precision medicine.

New advanced technologies methods contribute to the understanding of the important relationship between prognostic markers and personalized therapy, in a cost- and time-effective manner.

Methods: At diagnosis, CRLF2 alterations are investigated by immunophenotypic analysis (FACS), in addition to hypodiploidy by DNA index.

Next Generation Sequencing (NGS) is required to identify clonal IG/TR gene rearrangements for Minimal Residual Disease (MRD) quantification at day +33. NGS-digital-MLPA (dMPLA) on DNA has been developed to recognize the *IKZF plus* patient subgroup within day +33.

Moreover, RNA-target-NGS has been setup to identify translocations of recurrent genes with any partner gene, by using the *Trusight RNA Pan Cancer Library Prep* with probes complementary to 1385 cancer-associated genes. Finally, multiplex-RT-PCR used in routine diagnostics for known fusion genes, will also detect *TCF3-HLF*/t(17;19) transcripts associated to poor prognosis and needing early intensive therapy.

Results: In a pilot study within the Euroclonality-NGS Consortium, NGS identified more markers than conventional methods. In 226 patients analysed since May 2017 in our laboratory, NGS identified 1439 IG/TR rearrangements, with a mean of 6.37 clones/pt (range 0-15), with a mean

response time of 14.6 days from diagnosis (range 7-26 days). In 5 out of 226 (2.2%) cases, no IG/TR markers were identified: 3/5 were very immature T-ALL and 2/5 were BCP-ALL (1 BII, 1 BCP-ALL w 2.5% blasts).

A total of 86 samples were analysed in parallel with conventional MLPA and dMLPA to detect *Ikaros-plus* patients, , obtaining 85% concordant results.

In a cohort of patients, selected by either MRD at TP1 (d33) ≥5x10-4 or relapse, RNA-targeted analysis detected 109 fusions, involving recurrent genes such as ETV6, NUP214, BCL9, EBF1, MLL, TCF3 (two cases with TCF3/HLF), ZNF384, PAX5 and JAK2.

Conclusion: Overall, NGS showed itself to be able to identify translocations as well as IG/TR rearrangements; on the other hand, digital MLPA was able to detect copy number alterations (CNAs) associated to Ikaros-plus. This new protocol optimizes the risk-based stratification of ALL patients for a more specific therapeutic interventions, in particular in intermediate and high risk patients. By this combined new methods, in addition to routine diagnostics, it's possible to fine-tune risk stratifications and treatment for genetically defined subgroups, for which a specific experimental arm will be available within a controlled clinical protocol.

SCIENTIFIC SESSION: MANAGEMENT OF BCR-ABL + ALL

O007

Enhancing Management of Philadelphia-Positive (Ph+) ALL by Monitoring with an Analytically Validated Multiplex Assay for BCR-ABL1 minor Breakpoint (e1a2) with Highly Sensitive Detection of 1:40,000 (0.0025% or 4.6 Logs)

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Asuragen, USA

Background: Management of ALL requires MRD quantification to assess response dynamics. For Philadelphia-chromosome-positive acute lymphoblastic leukemia (Ph^+ ALL), such assessment of tumor burden can be achieved through flow cytometry and molecular monitoring (PCR-based) of the BCR-ABL1 fusion transcript of t(9;22), frequently e1a2 (minor breakpoint, m-BCR). MRD is valuable post-induction and to assess eligibility for stem cell transplantation (SCT) after consolidation. For Ph^+ ALL, prolonged monitoring of BCR-ABL1 MRD levels is recommended by the ESMO Clinical Practice Guidelines to guide changing TKI. As therapeutic progress results in deeper clinical responses, analytical sensitivity has become a critical topic for reliable measurement of complete molecular remission (MolCR), the most important prognostic factor for disease-free and overall survival. This level requires an MRD assay for minor breakpoint that confidently and consistently calls molecular responses of ≥1:10,000 (≥4 logs or ≤0.01%).

Aims: We describe the analytical validation and method comparison of a CE-marked IVD multiplex system reporting continuous BCR-ABL1:ABL1 %ratio values via automated analysis.

Methods: Encapsidated RNA molecules form blends of nuclease-resistant BCR-ABL1 and ABL1 transcripts to calibrate and control the system. Multiplexed 4-point curves using such blends provide copy values and account for the relative run-specific efficiency of the RT step. Controls (high, low, negative) were also developed using encapsidated RNAs. cDNA generation and qPCR were optimized to allow high mass of nucleic acid without inhibition to drive analytical sensitivity. Cell line RNA was diluted into non-leukemic human RNA to create challenge panels for most validation studies. RNAs negative for e1a2 tested specificity. Peripheral blood from e1a2-positive ALL patients were collected (n=13) with informed consent from age range 33-73 (median 59) across the 4 groups: 15-35 (1), 36-55 (5), 56-70 (6), and >70 (1). They were tested against a second CE-marked IVD kit capable of e1a2 detection as a comparator. The software includes a logic algorithm that flags any specimen requiring further review. Statistical analyses were carried out after log transformation to achieve normal distributions. Specifically, we introduced "Log Reduction" (LR) values as the log reduction from theoretical totality of 100% ($LR = \log_{10}(100\% \div \%ratio)$), similar to Molecular Response (MR) values for Major breakpoints.

Results: Our gains in analytical sensitivity allowed detection of background e1a2 mRNA in non-leukemic specimens. Specifically, an LOB was determined both for BCR-ABL1 copy number of 1 copy/qPCR and for %ratio of 0.0010% (LR5.00, 1:100,000). The LOD (classical parametric) and LOQ were statistically distinct from LOB at 0.0025% (LR4.61, 1:40,000) and 0.0039% (LR4.45, 1:26,000), respectively. Despite deep analytical sensitivity, this system maintains

analytical specificity ("below LOD" for BCR-ABL1-negative samples); however, it does demonstrate the expected low-level cross-talk from contrived clonal specimens of extremely high BCR-ABL1 Major breakpoint transcripts. Linearity was validated to encompass 4 logs, from 0.0025% to 25% (LR4.61 to LR0.61).

Conclusions: The newly developed test kit quantifies deep response dynamics to 1:40,000 (4.6 log) and improves workflow with streamlined reagent formulation, multiplex format, and automated software analysis.

O008

Long term analysis of a randomized study comparing prophylactic and MRD-triggered, pre-emptive imatinib after SCT for Ph+/BCR-ABL1 positive ALL

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Background: Appearance of bcr-abl transcripts after allogeneic stem cell transplantation (SCT) for Ph+ ALL is highly predictive of recurrent disease. We hypothesized that initiation of imatinib (IM) when residual leukemic cells are detectable only by molecular analysis, i.e. in the setting of minimal residual disease (MRD), may prevent relapse and improve outcome. The optimal time point for starting interventional IM has not been established.

Aims: To determine the impact of IM administration after HSCT (prophylactic IM versus IM triggered by PCR positivity) in terms of tolerability and duration of molecular and hematologic remission.

Methods: In this prospective, multicentre trial by the GMALL study group, adult pts. (≥ 18 y) with Ph+ ALL in CR at SCT received IM prophylactically after SCT or pre-emptively upon detection of MRD. Inclusion criteria included engraftment, sufficient hematopoietic and organ function, no uncontrolled GVHD or infections. Target dose of IM was 600 mg/d, 400mg/d recommended as starting dose. All pts. were followed by frequent serial MRD analysis after HSCT. Bcr-abl transcripts were assessed in peripheral blood and bone marrow

samples every 3 and 6 weeks, respectively. An interim analysis was reported previously. We here provide results of the final analysis of this trial, with long-term follow-up of up to 11 years after HSCT.

Results: 74 pts. were evaluable, 36 received prophylactic and 38 pts. pre-emptive IM. Median age was 41 y (18-69) and 44 y (19-68), respectively. Disease status at SCT was CR1 (n= 67), CR2 (n=5), CR3 (n=1), unknown (n=1). Most pts. received a PBSC graft (n=71) and myeloablative TBI-based conditioning (n=65), 8 pts. underwent RIC with 2Gy or 4Gy TBI (n=6) or non-TBI RIC (n=2). Median time from SCT to starting IM was 48d and 77d. IM dose was 600 mg/d in 22% of pts., remaining pts. received 400 mg. Treatment was prematurely discontinued in 56% and 59% of pts., (median time to discontinuation: 251d and 192d). Median follow-up of surviving pts. is 5.6 y (2.4-10.8) and 6.9 y (1.8-11). Relapse rate (14% *vs.* 18%), NRM (12% *vs.* 11%) and ongoing CR (69% *vs.* 71%) were not significantly different between arms. Probability of DFS and overall survival at 10 years was 64% vs. 69% and 68% vs. 71% with prophylactic and pre-emptive IM, respectively (p=ns). MRD levels were significantly predictive of relapse: BCR-ABL1/ABL1 (B/A) ratio ≥ 10-3 within 6 weeks prior to HSCT was associated with a higher cumulative incidence of relapse (CIR) at (47.5% vs. 10.6%, p=0.006) and inferior DFS (45% vs. 79%, p=0.027) at 10y. B/A ratio ≥ 10-4 within 100d after HSCT was likewise associated with a higher CIR (45% vs. 13%, p=0.0046) and inferior DFS (55% vs. 71%, p=0.054) at 8 y.

Summary: Both interventional strategies are associated with a low rate of hematologic relapse and durable remissions. Use of imatinib after SCT may improve long-term outcome in pts. with Ph+ ALL and should be considered standard of care in Ph+ ALL pts. undergoing SCT. Reappearance of *BCR–ABL1* transcripts early after SCT or at higher levels identifies a small subset of patients who do not benefit sufficiently from imatinib, and in whom alternative approaches should be explored.

FRIDAY, APRIL 13

WORKSHOP B: ALGORITHMS FOR DETECTION OF BCR-ABL LIKE ALL

O009

Workflow for prospective screening of BCR-ABL1-like genetic alterations in ALL in the GRAALL-2014 trial

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Ph-like or BCR-ABL1-like ALL can be defined as Ph-negative ALL bearing genetic alterations that directly target cytokine receptors, tyrosine kinases or other mediators of growth factors signaling. As such, this group of leukemias offers opportunities of targeted therapies. Although Ph/BCR-ABL1-like ALL have been primarily described by whole-genome expression profiling, this technology is difficult to apply for diagnostic purposes. Therefore, some alternative strategies should be developed to identify those patients who may benefit from treatment including tyrosine kinase inhibitors.

In the context of the French-Belgian-Swiss GRAALL-2014 trial, molecular diagnostics of all patients is centralized in a unique laboratory. This has allowed to develop a dedicated workflow for prospective screening of BCR-ABL1-like genetic alterations. It is based on conventional cytogenetics (Lafage-Pochitaloff et al, Blood 2017) associated with detection of multiple fusion transcripts, deregulated CRLF2 or EPOR transcripts. Ultimately, RNA-seq is performed for unresolved cases. At 2 years from trial beginning, approximately 200 patients with Ph-negative B-ALL have been enrolled and more than 70% could be genetically classified, including 22% with a BCR-ABL1-like genetic alteration. The introduction of tyrosine kinase inhibitors for patients with targetable alterations is currently being discussed in the GRAALL.

Results of genetic workup for the first 200 patients enrolled in the GRAALL-2014 will be presented as well as real-life examples of TKI use in patients with BCR-ABL1-like alterations.

O010

Custom targeted RNA sequencing for the classification of BCR-ABL1-like acute lymphoblastic leukemia and the identification of druggable mutations and fusions

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Background: *BCR-ABL1*-like (Ph-like) B-precursor (BCP) acute lymphoblastic leukemia (ALL) displays a gene expression profile closely related to B-precursor ALL with t(9;22)(q34;q11). Ph-like ALL patients are characterized by distinct genetic alterations and inferior prognosis. Some genomic alterations respond to specific treatment approaches and provide hope for tailored therapies. A standard approach for diagnosis is missing and currently mainly based on gene expression analysis. The genomic alterations shown to be responsive to targeted treatment approaches are heterogeneous and usually missed by currently used routine diagnostics.

Aim: The purpose of this study was to evaluate a customized targeted RNA sequencing (RNAseq) panel for the classification of Ph-like ALL and the detection of genomic alterations that were shown to respond to specific treatments.

Methods: Sixteen samples from diagnosis (n=14), relapse (n=1) and remission (n=1) of adolescents and adults (median age 32 years, range 17-76 years) with BCP ALL were included in the study. Eleven patients had Ph-like ALL (identified by genetic alterations or gene expression profiling by Affymetrix Array; n=1 from remission) and five patients had BCR-ABL1 (n=2), TCF3-PBX (n=1), KMT2A-AFF1 (n=1) or ETV6-RUNX1 (n=1) fusions. Samples were selected to provide a representation of the known genetic subtypes of BCP ALL and especially the Ph-like subgroup.

A targeted RNAseq custom panel (SureSelectXT, Agilent) of 111 genes (n=33 gene expression only, n=24 mutational hot spots and 54 complete coverage) was carried out and sequenced on a HiSeq 1500 instrument (Illumina) as 100 bp paired end reads. Additionally, a selection of 131 genes known to be recurrently mutated in ALL were studied by targeted amplicon sequencing (Haloplex, Agilent) in 12 patients. The resulting libraries were sequenced on a MiSeq instrument.

For the purpose of gene expression, the samples were aligned to the human reference genome using STAR after adapter clipping and quality trimming with Trimmomatic. The genes were counted using featureCounts and the differential expression analysis was performed using edgeR TMM-normalization followed by limma. Fusion search was performed using FusionCatcher. Finally, variants were called using VarScan and were annotated functionally using Annovar.

Results: Sequencing delivered a mean of ~25 million mappable reads/sample. The mean sequencing coverage of the target region across all samples was 90-fold. By gene expression analysis the classification of the Ph-like subtype could be verified. All already known fusions (n=6) could be easily detected by the targeted RNAseq panel, whereas no fusion was identified in the remission sample. Additional fusions in several genes were identified and could be validated by cytogenetics and PCR. These included fusions predicted to respond to *ABL1* inhibitors and novel fusions like e.g. ZCCHC7-SLC29A1. Furthermore, recurrent gene mutations in e.g. *JAK2*, *CRLF2*, *IKZF1* were identified by targeted DNA and RNA sequencing as well.

Conclusion: Our study shows that it is feasible to identify the Ph-like ALL subtype by targeted RNAseq. In addition, a customized panel is able to identify common mutations or fusions associated with Ph-like ALL that were shown to respond to specific treatments. Targeted RNAseq could be

instrumental to improve routine diagnostic and design clinical trials that focus on this.

O011

Frequency of ABL-class fusions and gene rearrangements activating JAK-STAT signalling in 331 adults with 'B-other' acute lymphoblastic leukaemia enrolled on the UKALL14 and 60+ trials

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Background: B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) is a genetically diverse disease with a number of well characterised primary chromosomal abnormalities. However, 25% of adults with BCP-ALL do not fit into one of these cytogenetic categories (termed 'B-other ALL')[1]. In a proportion, the leukaemia is driven by ABL-class fusions (involving *ABL1*, *ABL2*, *PDGFRB* or *CSF1R*), rearrangements resulting in JAK-STAT pathway activation (affecting *JAK2* or *CRLF2*)[2], or rarer translocations involving the *ZNF384* and *MEF2D* transcription factors[3,4].

Aims: We sought to identify the frequency of ABL-class fusions, *CRLF2*, *JAK2*, *ZNF384* and *MEF2D* rearrangements in B-other ALL patients enrolled in the national UKALL14 (age 25-65 years) and UKALL60+ (age 55 years and older) adult clinical trials and to correlate specific abnormalities with patient outcome.

Methods: We identified cases based on cytogenetic analyses at diagnosis. Using dual colour break apart probes, fluorescence *in situ* hybridisation (FISH) for *CRLF2*, *PDGFRB/CSF1R*, *JAK2*, *ABL2* was performed on diagnostic samples from B-other patients with suitable material. Any samples with abnormal *BCR-ABL1* signals not due to *BCR-ABL1* fusion were also tested with an *ABL1* break apart probe. Interphase nuclei were scored by 2 independent assessors and an abnormality classed as significant if present in >10% of nuclei. In the absence of *ABL*-class fusions, *CRLF2* or *JAK2* rearrangements, *ZNF384* and *MEF2D* break apart probes were also used to identify rarer translocations.

Results: Among 769 patients with BCP-ALL enrolled onto UKALL14 (N=678) and UKALL60+ (N=91), 331 (43%) lacked a known recurrent chromosomal abnormality and were classed as B-other ALL. *CRLF2* rearrangements were identified in 22/203 (10.8%) patients (median age 47 years, 48% male). The partner gene was *IGH* (15/22, 68.2%), *P2RY8* (5/22, 22.7%) or unidentified (N=2). *PDGFRB/CSF1R* rearrangements (adjacent genes, detected by the same breakapart probe) were found in 3/205 (1.5%) patients (aged 26, 44, 54 years, 33% male), *ABL1* rearrangements in 2/316 (*JAK2* rearrangements in 3/205 (1.5%) (46, 49, 51 years, 66% male). No *ABL2* (0/202) rearrangements were detected.

In UKALL14 patients with *CRLF2* or *JAK2* rearrangements (N=25, median age 48 years), minimal residual disease (MRD) was detected (>$1x10^{-4}$) in 15/17 (88%) after phase 1 induction and in 12/15 (80%) after phase 2 induction (see table – UKALL14 patients only). Overall survival at 3 years for patients with JAK-STAT pathway abnormalities (N=25) was 30% (11-52%) compared to 57% (50-64%) for remaining B-other patients (N=298) (logrank p=0.0006).

Further FISH screening identified *ZNF384* rearrangements in 9/155 (5.8%) patient

samples (median age 26 years, 78% male) and *MEF2D* rearrangements in 2/145 (1.4%) (40, 47 years, both female). *ZNF384* rearrangements were associated with a surprisingly favourable outcome with no events (median follow up 1.8 years).

UKALL14	BCP-ALL	B-other	ABL-class	JAK-STAT	ZNF384
Number of cases	678	298	5	25	9
Median age (yrs)	46	45	28	48	26
Percent male	54%	59%	40%	52%	78%
Median WCC (x109/L)	8.1	5.3	8.2	8.1	4.6
Percent MRD positive post phase 1	59%	59%	33%	88%	55%
Percent MRD positive post phase 2	35%	24%	33%	80%	38%
Overall survival	51% (46-56%)	57% (50-64%)	60% (13-88%)	30% (11-52%)	100%

Conclusion: A gene rearrangement was identified in nearly a quarter of cases tested. The most prevalent abnormality, resulting in *CRLF2* deregulation, was identified in 11% of patients and associated with high MRD and reduced overall survival. ABL-class fusions have previously been reported in up to 10% of adults with Philadelphia chromosome-like ALL[5], thought to account for 50% of adults with B-other ALL. These were particularly rare (N=5, 2.1%) in our study, suggesting some variance with published cohort.

SCIENTIFIC SESSION: MINIMAL RESIDUAL DISEASE IN ALL

O012

Molecular profile refines the MRD-based prognostic assessment in adults with Philadelphia negative B-cell precursor acute lymphoblastic leukemia

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Background: Minimal residual disease (MRD) is the most important prognostic factor in acute lymphoblastic leukemia (ALL) at all ages (1,2). However, around 25% of MRD negative adult patients ultimately relapse (3), suggesting that MRD testing techniques have limitations for prognostic assessment. Molecular characterization may contribute to a more precise risk stratification. Unfortunately, reliable molecular prognostic markers are still warranted, especially for Ph-negative B-cell precursor ALL (Ph neg BCP-ALL).

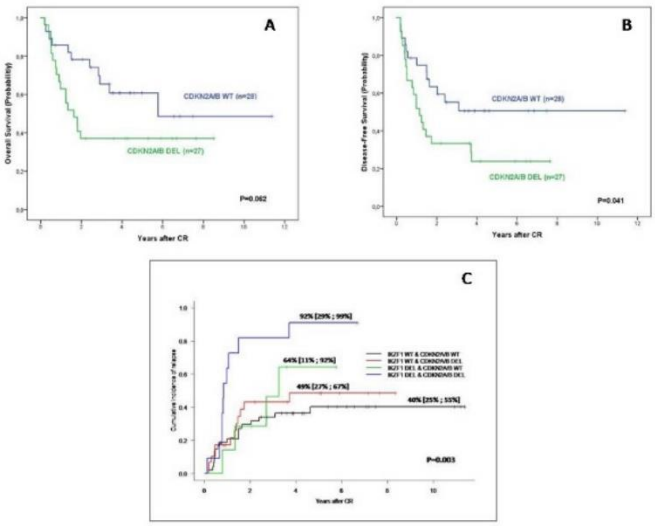
Aim: To identify molecular abnormalities with prognostic significance in a series of homogeneously treated adult Ph neg BCP-ALL patients.

Methods: All patients were treated with PETHEMA protocols. Multiplex Ligation-dependent Probe Amplification (MLPA) was performed for the most recurrent copy number alterations (CNA) in a series of 128 adult Ph neg BCP-ALL patients. MRD was centrally evaluated by flow cytometry and 10-3 (0.1%) was the cut-off established to determine low MRD level at the end of induction.

Results: Patients with partial IKZF1 deletion showed higher cumulative incidence of relapse (CIR) than patients without deletion (5-year CIR 83% [45%; 96%] vs. 43% [31%; 54%]. p=0.005). Patients with CDKN2A/B deletion had poorer outcomes than those without deletion (5-year OS 34% [20%; 48%] vs. 57% [43%; 71%], p=0.042; 5-year DFS 25% [12%; 38%] vs. 47% [33%; 61%], p=0.029; 5-year CIR 56% [40%; 70%] vs. 41% [27% ; 54%], p=0.090).

MRD data at the end of induction were available for 75/128 patients. Patients with MRD ≥0.1% (n= 19/75, 25%) had a trend for a poorer OS than those with MRD<0.1% (5-year OS 26% [5%; 47%] vs. 56% [35%; 63%], p=0.175). Among patients with MRD <0.1% at the end of induction, there were no significant differences in outcome depending on the IKZF1 status. In contrast, patients with CDKN2A/B deletion had lower OS (Figure 1A) and DFS (Figure 1B) than those without CDKN2A/B loss (5-year OS 37% [19%; 55%] vs. 61% [42%; 80%], p=0.062 and 5-year DFS 24% [7%; 41%] vs. 51% [32%; 70%], p= 0.041). Patients with both IKZF1 and CDKN2A/B deletions, had an extremely high CIR (5-year CIR 91% [29%; 99%] vs. remaining, p=0.003) (Figure 1C). Results of multivariate analyses are shown in Table 1.

Outcome variable	Variables	HR (IC95%)	P
Overall Survival	Age	1.037 (1.019 ; 1.055)	0.001
	WBC	1.005 (1.002 ; 1.009)	0.002
	CDKN2A/B	1.793 (1.017 ; 3.160)	0.044
Overall Survival (including MRD in the model)	Age	1.036 (1.013 ; 1.060)	0.002
	WBC	1.007 (1.033 ; 1.011)	0.001
	MRD	2.223 (1.121 ; 4.410)	0.022
Disease Free Survival	Age	1.028 (1.013 ; 1.044)	0.001
	WBC	1.007 (1.003 ; 1.010)	0.001
	CDKN2A/B	1.670 (1.000 ; 2.820)	0.050
Cumulative Incidence of Relapse	WBC	1.005 (1.002 ; 1.009)	0.006
	IKZF1	2.044 (1.033 ; 4.043)	0.040



Summary: Molecular markers such as CDKN2A/B and IKZF1 deletions add prognostic information to the MRD in adult patients with Ph-negative BCP-ALL. Combined MRD and molecular data should be used for risk stratification and treatment assignment.

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SCIENTIFIC SESSION: TARGETED IMMUNOTHERAPY - RECENT DATA

O013

Planned and current immunotherapy trials from the French-Belgian-Swiss Group for Research on Adult ALL (GRAALL)

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The GRAALL is developing a general strategy for Ph-negative ALL patients aiming to incorporate new agent into front-line therapy in high-risk patients and select candidates for allogeneic hematopoietic stem cell transplantation (HSCT) based on early minimal residual disease (MRD) response. Immunotherapy is part of this strategy evaluating blinatumomab front-line in younger patients with high-risk Ph-negative ALL (QUEST trial) and inotuzumab ozogamicin (INO) front-line in older patients with Ph-negative CD22-positive ALL in collaboration with the EWALL group (EWALL-INO trial).

QUEST study

This Phase 2 trial includes patients aged 18 to 59 years old with CD19-positive B-cell precursor Ph-negative ALL: i) enrolled at diagnosis into the front-line younger GRAALL-2014 trial and ii) presenting high-risk criteria on the basis of oncogenetic features and/or unsatisfactory early MRD response. High-risk genetics is defined by *KMT2A/MLL* gene rearrangement or *IKZF1* focal gene deletion, while unsatisfactory MRD response is defined by Ig-TCR MRD level ≥10-4 after first induction (MRD1, week-6). These patients, if still in hematological CR and not presenting contra-indication to blinatumomab, will receive continuous 28 microg/j blinatumomab infusion prior to allogeneic HSCT if poor MRD response (very high-risk patients, defined here as MRD ≥10-3 and/or week-12 post-consolidation MRD2 ≥10-4) and a matched sibling or unrelated donor. If not indication or donor for allogeneic SCT in first remission, they will receive continuous alternating cycles or chemotherapy and blinatumomab for a total of 5 blinatumomab cycles (2 during the consolidation phase and 3 during the maintenance phase). A total of 95 patients will be included with relapse-free survival as primary endpoint.

EWALL-INO study

This Phase 2 trial includes patients with CD22 positive, Ph/BCR-ABL negative de novo ALL aged above 55 years old. Patients will receive an amended EWALL backbone (induction part 1, 4 cycles of vincristine and dexamethasone; induction part 2, 2 cycles of dexamethasone and 3 days of fractionated cyclophosphamide) in combination with 5 injections of INO (three injections of INO 0.8 mg/m2 day 1 and 0.5 mg/m2 days 8 and 15 during induction part 1 and 2 injections of INO 0.5 mg/m2 days 1 and 8 during induction part 2). Patients in CR will receive the consolidation backbone and maintenance without INO. Primary endpoint of the trial is overall survival at 1 year (ITT analysis). Secondary end-points include EFS, CR rates, MDR1 and MRD2. Participating countries include Czech Republic, Finland, Belgium, Spain, Poland and France. A total of 130 patients will be included. The first patient has been included last December in France and is in CR after induction part 1.

The following two abstracts were presented as one, under the title: ***GIMEMA trials with Blinatumomab in Ph- negative and Ph-positive ALL***

O014

The front-line D-ALBA protocol (GIMEMA LAL2116). A dasatinib-blinatumomab combination approach for the treatment of adult patients with newly diagnosed Ph+ ALL. Overview of the trial, enrollment and preliminary findings, on behalf of the GIMEMA Acute Leukemia Working Party

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The management of adult patients with Ph+ ALL, including the elderly, drastically changed since the introduction of TKI. Their use led to overall survival (OS) rates currently approaching 50%, and are comparable to those achieved in Ph- B-lineage ALL. Notably, a significantly better survival is observed in patients who obtain a status of MRD negativity: thus, MRD negativity should be considered the primary endpoint of treatment.

To increase the rate of patients who become MRD-negative, we designed a chemo-free trial (D-ALBA) based on the combination of the second-generation TKI dasatinib with the bispecific monoclonal antibody blinatumomab. This is an open-label, multicenter, phase II study in which Ph+ ALL patients ≥18 years, no upper age limit, are treated front-line with dasatinib followed by blinatumomab. Subsequent treatment is open, but data will be collected. Prior to dasatinib initiation, patients receive a 7-days steroids pre-phase: steroids are continued for 24 days and then tapered and finally stopped at d31. Dasatinib (140 mg/day) is administered as induction for a 12-week period. Thereafter, patients who obtain a complete hematologic response (CHR), regardless of the MRD levels, receive a post-induction consolidation treatment with blinatumomab (28 μg/die). A minimum number of 2 cycles is mandatory, while the administration of up to 3 additional cycles is dependent on the response to therapy with blinatumomab and medical decision. During treatment with blinatumomab, dasatinib is not discontinued; in case of toxicity deemed related to dasatinib and/or the combination of the two compounds, it can be reduced. Dasatinib is continued for the whole study duration. Patients not achieving a CHR upon induction receive blinatumomab only. CNS prophylaxis is carried out with 6 medicated lumbar punctures during induction and then at the end of each cycle of blinatumomab. The primary endpoint is the achievement of a complete molecular remission (CMR) after two cycles of blinatumomab; secondary endpoints include disease-free survival (DFS), OS, cumulative incidence of relapse (CIR) and safety. Translational research includes evaluation of MRD by digital-droplet PCR (dd-PCR), evaluation of response according to i) different fusion types (p190 vs p210) and ii) presence of additional genomic lesions (*IKZF1*,

CDKN2A/B, etc). Sixty patients will be enrolled.

The trial opened to enrollment in May 2017: at January 2018, 28 patients were enrolled and 27 eligible. Median age is 53 years (range 28-73); 10 patients are males and 17 females, with a slight prevalence of women (59%). The median WBC is 3.6 x 10^9/l (range: 2.9-63.4); the p190 fusion product was detected in 14 patients, p210 in 10 and in p190/p210 in 3 cases. So far, 9 patients completed the induction phase, 6 the 1st cycle of blinatumomab and 1 has completed both blinatumomab cycles. All patients achieved a CHR and no deaths in induction were recorded. Upon induction, 1 patient became MRD-negative and the remaining are still MRD+; after the 1st cycle of blinatumomab, 2 patients became MRD-negative 2 positive non quantifiable (PNQ), while 2 are still MRD+; finally, the only patient who completedcycle 2, though still MRD+, showed a further MRD reduction.

This is the first chemo-free induction-consolidation trial for adult Ph+ ALL patients (of all ages) based on a combination of a targeted and immunotherapeutic strategy open to recruitment. Further results will be given during the meeting.

O015

New GIMEMA chemotherapy-blinatumomab protocol for untreated adult Ph- B-precursor ALL (LAL 2317), on behalf of the GIMEMA Acute Leukemia Working Party

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In adult Ph- ALL, the early reduction of MRD is a primary determinant of outcome. GIMEMA and NILG merged under the GIMEMA flagship to explore new strategies aimed at improving MRD response and survival. In the earlier NILG study 10/07 (ClinicalTrials.gov Identifier NCT-00795756), MRD was tested in 95 of 139 patients achieving a complete remission (CR), with a sensitive molecular marker. End of induction (w4) MRD <10-4 ("negative") was documented in 53.3% of cases. Most w4 MRD responders (95.4%) and many in the 10-4-10-3 w4 MRD group (71.4%) were MRD-negative at w10, identifying three distinct prognostic groups: MRD-negative at both time points, relapse 14% and 5-year RFS 74%; MRD-positive at one time point, 43% and 49%; MRD-positive at both time points, 44% and 31% (P=0.04 for relapse and P=0.005 for RFS). More recently, after successfully testing a modified N10 protocol that incorporated pegylated-asparaginase (LAL 1913, n=204, CR 91.5%), GIMEMA launched a new frontline protocol (LAL 2317) for patients with Ph- B-precursor ALL aged 18-65 years, adopting the same chemotherapy backbone plus two blinatumomab (bispecific CD3 X CD19 monoclonal antibody) courses, and having as primary study endpoint an improvement in the rate of early MRD negativity.

To build up on the prior GIMEMA/NILG experience, the GIMEMA LAL 2317 trial will assess the impact of the first blinatumomab course in increasing the rate of early MRD negativity at the end of w14, i.e. 4 weeks after the prior MRD assessment (w10) carried out after induction/early consolidation chemotherapy. All CR patients with CD19+ Ph- B-precursor ALL, regardless of the MRD status detected at w4/w10, will

be eligible to blinatumomab administration. Thus, the potential therapeutic benefit associated with blinatumomab will be offered not only to patients with persistent MRD but also to MRD-"negative" cases, since they may harbor very small amounts of residual ALL not measurable by the current laboratory techniques (<10-5) and have an incidence of relapse of 20-30%.

The sample size of the new study has been calculated on the basis of the historical experience of an early MRD negativity after standard induction/consolidation at week 10 of 60% (P0). The number of patients required to evaluate an increase of MRD negativity from 60% (P0) to 75% (P1), with α = 0.05 and power (1-β) = 0.90, is 85. Considering that approximately 85% of observed patients will be in CR after induction, with approximately 75% of these patients evaluable for MRD analysis and taking into account an expected loss of 10% of patients mainly due to ineligibility or refusal before treatment initiation, the total number of patients to enrol is 149. The design of the study will be presented in detail, including risk stratification criteria and MRD analysis for risk and MRD-directed therapy (with or without allogeneic SCT). Allocation to allogeneic SCT will be determined by high-risk diagnostic features and/or w10 MRD, and will not be influenced by MRD response to blinatumomab. The study has received EC approval at the coordinating Institution and is expected to recruit soon.

O016

Ongoing and planned immunotherapy trials from the European study groups. The Spanish PETHEMA Group

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Background: Immunotherapy is an effective therapeutic strategy for patients with relapsed or refractory (R/R) acute lymphoblastic leukemia (ALL) and is currently investigated in early phases of the disease. Monoclonal antibodies (MoAb)(naked, immunoconjugated or bispecific) and CAR T cells are the most effective therapies for these patients. Blinatumomab and inotuzumab ozogamycin are approved for adults with R/R ALL and Tisagenlecleucel (Tisa-cel), a CD19 CAR T cell developed by the University of Pennsylvania, has been approved in USA for children and young adults with R/R ALL.

Aim: To present the ongoing clinical trial with immunotherapy from the Programa Español de Tratamientos en Hematología (PETHEMA) Group aimed to evaluate the efficacy of Blinatumomab administered during the early and delayed consolidation in reducing the MRD level in adult patients with high-risk (HR), Philadelphia chromosome-negative (Ph-) ALL in first complete remission (CR1) with good minimal residual disease (MRD) clearance after the induction period.

Method: Phase II, open label study. The main objective will be to evaluate the frequency and deepness of MRD reduction, assessed by multiparameter flow cytometry (MFC) after consolidation by substitution of two out of the 6 consolidation cycles of chemotherapy by two cycles of blinatumomab in adult patients with HR Ph- ALL in CR1 with MRD level <0.1% after induction. The number of patients will be 38, from 13 Spanish centers.

Current status: This trial (BLIN01) is currently being submitted to the Institutional Review Boards (IRB) from the participating centers, and is expected to be activated for patients inclusion in the second trimester of 2018.

Expected results: In the current PETHEMA HR ALL-11 trial, 80% of patients attained a MRD level <0,01% (<1x10-4) after consolidation (in 40% of whom the MRD level was <0.001% [<1x10-5]). An additional 15% of patients are expected to achieve the MRD level <0,01% (<1x10-4) after consolidation with the inclusion of blinatumomab. This improvement could be translated into a lower probability of relapse of these patients, as compared with the parallel ongoing study (ALL-HR11) with only chemotherapy without blinatumomab.

O017

Planned and current immunotherapy trials from the German Multicenter Study Group for Adult ALL (GMALL)

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The GMALL conducts and plans several trials with the CD19 antibody Blinatumomab and the CD22 antibody Inotuzumab. First-line setting only will be discussed.

Molact1-Trial: The trial includes Ph/BCR-ABL negative patients with quantifiable MRD positivity after at least induction and consolidation I. MRD level has to be ≥ 10-4 with an assay sensitivity of at least 10-4. Patients with MRD detection after relapse and / or stem cell transplantation can be included. The study has no upper age limit. Patients receive at least one cycle of Blinatumomab at standard dose without dose step. CNS prophylaxis is performed by intrathecal therapy. The molecular response after one cycle is the primary endpoint. Treatment can be continued for up to four cycles. In patients who are not candidates for SCT a maintenance treatment will follow.

Initial-1 Trial: The trial includes patients with CD22 positive, Ph/BCR-ABL negative de novo ALL aged above 55 years. Patients will receive 3 cycles of Inotuzumab for induction therapy. In the first cycle Inotuzumab is dosed with 0.8 mg/m2 day 1 and 0.5 mg/m2 day 15 and the cycles includes two blocks of dexamethasone. In further induction cycles inotuzumab is dosed with 0.5 mg/m2. This is followed by conventional chemotherapy consolidation and maintenance. The primary endpoint is event-free survival after 12 months. Hematologic CR rate and molecular CR rate is evaluated as well as further secondary endpoints.

Trial: The trial includes patients with CD19 positive, Ph/BCR-ABL negative de novo ALL aged between 55 and 75 years. Patients will receive a dose reduced and shortened phase I of induction which is followed by induction with Blinatumomab with standard dose-step. Alternating with HDMTX/ASP,

HDARAC and reinduction patients will receive 3 further cycles with Blinatumomab without dose-step. This will be followed by maintenance therapy. The primary endpoint is hematologic CR after induction. Further endpoints include overall survival, event-free survival, remission duration and molecular response at different timepoints. French centers will participate in the trial.

In all GMALL trials MRD is measured by PCR of cloncal IG/ TR rearrangements in a central reference laboratory (University of Kiel, Germany).

EWALL-PH03: The GMALL plans to participate in the EWALL PH03-trial for Ph/BCR-ABL positive ALL.

O018

Single cycle of blinatumomab followed by high-dose chemotherapy in the induction therapy for Ph-negative acute lymphoblastic leukemia in adults

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Result from multiple studies in BCP-ALL have proven a high efficacy of blinatumomab monotherapy in both relapsed/refractory and MRD-positive settings. The number of complete molecular responses in patients achieving CR reaches 80-88%. However, majority of patients who have reached MRD negativity after blinatumomab and were not transplanted eventually relapsed. As the rapid MRD clearance at early stages of the therapy significantly affects survival and relapse rate, we infer, that blinatumomab needs to be incorporated into polychemotherapy protocols early to induce durable responses and/or cure.

The Czech Leukemia Study Group - for Life (CELL) has designed a protocol which incorporates blinatumomab to the frontline therapy as a part of induction treatment. Patients aged 18-65 years with newly diagnosed, previously untreated, Ph-negative BCP-ALL are eligible. Other inclusion criteria include lymphoblasts positive for CD19 and ECOG performance status ≤2. Subjects with initial CNS involvement are eligible if they reach negativity in cerebrospinal fluid after up to 4 intrathecal applications of chemotherapy within the first 10 days of therapy.

Therapy starts with a run-in phase composed of dexamethasone, cyclophosphamide, vincristine and daunorubicin. Patients are screened to the study on day 11 when bone marrow aspiration is performed. Substantial reduction of disease burden at this stage is expected.

Induction cycle I is running on days 12-40. It is composed of blinatumomab monotherapy 28 µg/day (9 µg/day on days 12-15 if >50% of bone marrow blasts at screening). Induction cycle II starts on day 50, and is composed of dexamethasone, vindesine, high dose methotrexate and cytarabine.

The primary endpoint of the study is a number of complete molecular responses (CMR) after two cycles of induction therapy composed of a Bassan of blinatumomab followed by chemotherapy. It is evaluated at week 11. We hypothesize that the number of CMR will improve from 60% in our recent protocol to 85%.

Key secondary endpoints include MRD at the end of blinatumomab infusion, EFS, OS,

and the rate of alloSCT in the first complete remission.

Consolidation chemotherapy is composed on 6 cycles of polychemotherapy in patients reaching CR with MRD <10-4. Subjects in CR with MRD ≥10-4 will be administered further 1-2 cycles of blinatumomab followed by alloSCT.

The study will be running at 5 centers in the Czech Republic. Fifty subjects are planned to be treated in the study.

O019

CART19-BE-01: a pilot trial on the use of a ARI-0001 cells (i.e. CART19 cells transduced with a new construct comprising A3B1:CD8:4-1BB:CD3z) in patients with CD19+ relapsed/refractory acute lymphoid leukemia

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Background: The prognosis of relapsed/refractory acute lymphoid leukemia (R/R ALL) is very poor, particularly in patients relapsing after allogeneic hematopoietic cell transplantation (alloHCT). New agents such as inotuzumab or blinatumomab, have improved the complete response rate (CRR) in R/R ALL, but with a progression-free survival (PFS) shorter than 6 months [1,2]. In the last decade, several CAR19 constructs have been developed. One of them (tisagenlecleucel) was approved by the FDA for pediatric or young adults with R/R ALL. The approval was based on a CRR around 80% with a 6-month PFS around 70%.

Aims: To develop our own CAR19 construct for clinical use.

Methods: From our institution we selected our anti-CD19 A3B1 hybridoma, identified the scFv sequence and incorporated the CD8, 4-1BB and CD3z modules next to it. We cloned it into a 3rd generation lentiviral vector and transduced PBMCs from buffy coats after activation with CD3 and CD28 dynabeads (ARI-0001 cells). Once cytotoxicity and specificity were confirmed in vitro and in vivo (in NALM6-xenograft murine models), we scaled-up both lentiviral and cell production, the latter using the CliniMACS Prodigy System (Miltenyi). To test for consistency and robustness, 3 apheresis products from healthy donors were activated, transduced with our vector and cultured in media containing IL-7/IL-15 until the desired product was achieved. We reached all pre-specified acceptance criteria in all 3 procedures, which led to the Spanish Agency of Medicines approval of our IND and also our first pilot clinical trial (clinicaltrials.gov NCT03144583) on May/2017. Eligibility criteria included R/R ALL (adult and pediatric), NHL and CLL, but in this abstract we will only report the outcome of patients with R/R ALL.

Results: As of January 2018, we have recruited 8 patients with R/R ALL, all relapsing after alloHCT. Median age was 19.5 years (range 3-34) and 50% were female. Patients were included in the trial in first (1), second (5), and third (2) relapse. Two patients were in CR with negative minimal residual disease (MRD) at study inclusion. The median percentage of blasts in bone marrow was 92% (range 73-96%) for the remaining 6 patients. We successfully prepared ARI-0001 cells in 7/8 patients,

although we eventually managed to prepare enough cells for the eighth patient in a second attempt (8/9 = 11% production failure rate, globally). After fludarabine (90 mg/m^2) and cyclophosphamide (900 mg/m^2) chemotherapy, we infused 0.5-5 x10^6 ARI-0001 cells/kg to 6 patients. The remaining 2 patients are still awaiting treatment. Only 4 patients are evaluable at this time (one was in MRD negative CR upon recruitment, one has not been restaged yet), and all achieved CR with negative MRD (100% CRR) with a median follow-up of 94 days (range 15-190 days). All 5 patients have developed absolute B-cell aplasia, and none has relapsed so far. Cytokine release syndrome (CRS) has been observed in all 6 patients, but it was grade I-II in all of them. Tocilizumab has not been required so far. Grade I neurotoxicity has been observed in 2 (33%) patients but resolved spontaneously in both cases.

Conclusion: It was feasible to prepare ARI-0001 cells in a purely academic setting using the automated CliniMACS Prodigy System and home-made lentiviral vectors. The treatment was safe, with no cases of severe CRS or neurotoxicity so far, and efficacious, with a 100% CRR, although with a very limited follow-up.

SCIENTIFIC SESSION: T-ALL - THE FORGOTTEN SUBTYPE?

O020

Methylation clusters in adult T-ALL reveal distinct molecular characteristics

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Introduction: A relevant percentage of adult T-ALL patients relapse with a consequently poor outcome. In contrast to BCP-ALL with options of immunotherapies in the relapse or MRD-positive setting, targeted therapeutic options for T-ALL patients are still limited. Thus, improved individualized treatment strategies and a better understanding of molecular inter-leukemic heterogeneity is important. Comprehensive analyses of the epigenetic background in adult T-ALL are lacking.

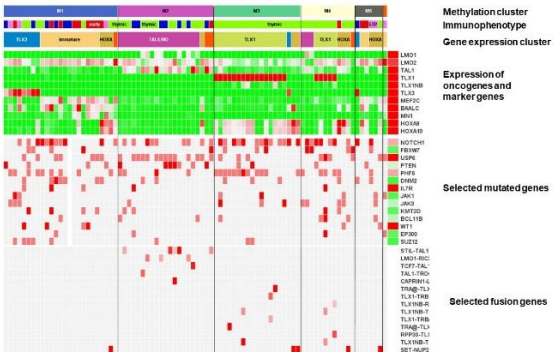
Patients and methods: We studied eighty-four adult T-ALL patients at first diagnosis (age: median 32, range 17-59 years) with different immunophenotypes: 19 early, 14 mature and 51 thymic T-ALL. The epigenetic profile of each sample was determined by Infinium 450k methylation bead arrays. In addition, deep targeted DNA sequencing data, using a gene panel covering 206 genes (HighSeq 1500, 100 bp, average ~800 reads/bp), and RNAseq data (HighSeq 2000, 125 bp, ~30 million reads/sample) were available for all samples.

Results: On the epigenetic level, the 50.000 most variant CpGs were used for unsupervised clustering. Clustering identified five sub-clusters (M1-M5) characterized by distinct molecular alterations. Combining the methylation data with the transcriptional expression profiles (defining subgroups based on the expression of oncogenes), a distinct methylation pattern was identified. The methylation cluster M1 (n=25) included immature (n=13), as well as TLX3-driven cases (n=8) and some samples with HOXA activation (n=4). Mutations in epigenetic modifiers, including *KMT2D*, *DNMT3A*, *SUZ12*, and *EP300*, were enriched in cluster M1. The M2 signature (n=21) was driven by overexpression of TAL1/LMO with a higher rate of mutations for *PTEN* (33%) and *USP6* (67%). In M3 (n=19), mainly cases with a TLX1-overexpression (n=16) were included. These showed a higher rate of mutations for *NOTCH1* (68%) and *PHF6* (63%). The subcluster M4 (n=12) contained cases with HOXA activation and a thymic immunophenotye (n=4), as well as few samples with TLX1-overexpression (n=5). M5 (n=6) comprises a more heterogeneous subgroup with single cases of an early thymic precursor (ETP)-ALL immunophenotype.

Overall, the most frequently mutated genes included *NOTCH1* (51%), *PHF6* (32%), *DNM2* (18%), *PTEN* (18%), *FBXW7* (16%), and *JAK3* (14%). *NOTCH1* was most frequently altered in cluster M3 (68%), the lowest rate of *NOTCH1* mutations was

found in the immature cluster M1 (46%). Noteworthy, multiple *NOTCH1* mutations were present in various patients, frequently on a subclonal level pointing towards a late event in leukemogenesis. The JAK/STAT pathway was affected in all subgroups with two hits in individual patients, e.g. 6/7 (86%) *JAK1* mutations co-occurred with *JAK3* mutations.

Conclusion: We characterized epigenetic subgroups of adult T-ALL in combination with genomic and transcriptional profiling and found a high concordance for all subgroups across the platforms. Epigenetic defined subgroups showed distinct molecular characteristic, which could substantiate targeted therapies, such as JAK/STAT inhibitors or epigenetic modulators.



O021

CI-FISH: a powerful tool for the genetic diagnosis of T-ALL to improve precise diagnosis and address new treatments

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Background: Integrated genomic analysis has greatly enriched our knowledge on T-ALL pathogenesis and progression, identifying concurrent genetic events, in individual case, which co-operate to induce overt leukemia.[1,2] Primary genetic abnormalities, which are mutually exclusive, deregulate transcription factor oncogenes, such as *TLX1*, *TLX3*, *HOXA*, *MYB*, and the *TAL1/2*, and *LMO1/2*, playing a pivotal role in hematopoiesis and T-cell lineage differentiation. Secondary changes affect members of transduction signalling pathways, epigenetic modifiers, and genes controlling cell cycle and ribosomal biogenesis. Translation of this biological knowledge into clinical practice remains difficult and no specific molecular entities have been included in the WHO Classification to date. Moreover, it is urgent to develop a reliable and manageable diagnostic approach to place patients with specific lesions into context within clinical trials.

Aim: To investigate a new approach for genetic diagnosis of T-ALL.

Methods: We applied our CI-FISH assay[3-6] to 330 cases of T-ALL enrolled into the Italian (AIEOP and GIMEMA) and UK (MRC) clinical trials. *NOTCH1/FBXW7* hot-spot mutations were studied in 196 cases.

Results: Overall, CI-FISH classified 80% of pediatric (179/224) and adult (85/106) cases into one of the major genetic groups: *TAL/LMO, TLX1, TLX3, HOXA, NKX2-1/2-2*, and *MEF2C*. Age distribution into genetic

categories reflected previously reported data and provided new information. In fact, *TAL/LMO* (40% *vs* 21%) and *TLX3* (23% *vs* 10%) categories were more prevalent in children while the *TLX1* group (22% *vs* 5%) was more common in adults. Interestingly, we found that the *HOXA* group was more common in adults than in children (44% *vs* 22%) (p*NKX2-1/2-2* category was significantly associated with pediatric age (9% *vs* 1%) (p=0.001). According to phenotype, CI-FISH classified 88% of non-ETP and 61% of ETP ALL cases.

NOTCH1/FBXW7 mutations were detected in 60% of unclassified and in 69% of classified T-ALL, where they showed a significant association with the *TLX1/3* and *HOXA* categories (p*LEF1, GRIK2/CASP8AP2*, and *PTEN* deletions and *MYC* translocations was observed in *TAL/LMO* positive cases, while *TCF7* and *NF1* deletions were preferentially associated with *HOXA*. As expected, the *NUP214-ABL1* fusion was exclusively found in *TLX1/3* positive cases and the *PTPN2* deletion in *TLX1/3* and *HOXA* groups. Even the distribution of *CDKN2AB* deletions across groups showed a preferential association with *TAL/LMO* and *TLX1/3* categories (p<0.001).

Conclusion: CI-FISH is a valid surrogate of other advanced technologies as it successfully identified gene fusions, non-fusion producing chromosome rearrangements, promiscuous genes, and genomic gains and losses. It provided a genetic classification of 80% of T-ALL cases into one of the main groups and also recognized distinct subgroups. We created a diagnostic algorithm to be exploited in prospective clinical trials: 1st level, oncogenes and T-cell receptor to classify the disease within one of the major categories; 2nd level, commonly rearranged oncogenes/oncosuppressors. CI-FISH, in addition to NGS for recurrent gene mutations, may provide a comprehensive approach to introduce genetics in clinical trials.

O022

Characteristics and outcome of the early T cell precursor ALL (ETP-ALL) patients treated with high-risk protocols from the Spanish PETHEMA Group

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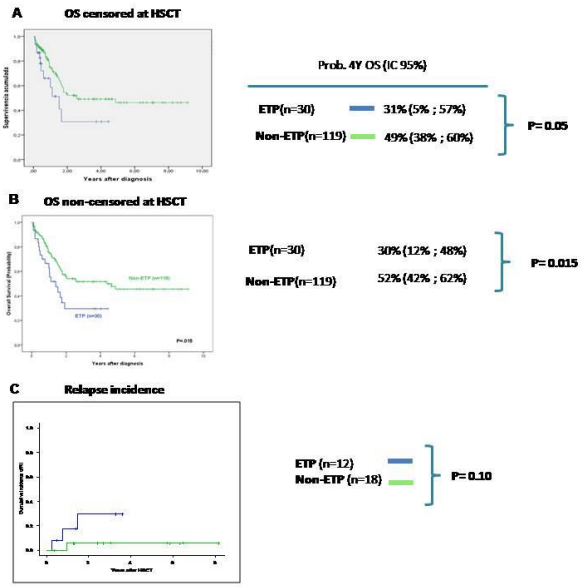
Background: ETP-ALL was included as a provisional entity in the 2016 WHO classification of ALL (1). This subtype was first identified by Coustan-Smith et al in 2009 (2). However, this immunophenotype-based classification does not fully enclose all ETP-ALL cases identified by gene expression profile (GEP). Although initial small series of ETP-ALL suggested that the outcome of ETP-ALL was very poor(2-3), more recent larger series showed improvement in outcome using high-risk treatment in children(4), or incorporating allogeneic hematopoietic stem cell transplantation (HSCT) in adults (5).

Aim: To identify the ETP-ALL subgroup in the Spanish cohort of adult T-cell acute lymphoblastic leukaemia (T-ALL) patients and assess the impact of the treatment on their outcome.

Method: 168 adults with T-ALL and treated according to two consecutive MRD-oriented high risk adult PETHEMA protocols (ALL-HR-2003(NCT00853008) and ALL-HR-11 (NCT01540812) (still ongoing) were included in this study. The EGIL criteria (6) were used to define the immunologic subtype of T-ALL after central review of immunophenotype reports, and the criteria proposed by Zurbier*et al*. were used to define ETP-ALL(7). These criteria consist of a combination of immunomarkers, that resemble those published by Coustan-Smith, with the advantage that enclose most of ETP-ALL identified by GEP, avoiding the use of CD5.

Results: 30 out of 149 (20%) patients with T-ALL were identified as having ETP-ALL. Patients with ETP-ALL were older (median 38.3 vs. 32 yrs; p=0.011), with more frequent lymph node enlargement (78% vs.57%; p=0.05) and lower WBC counts at diagnosis (median, 68.2 vs.95,3 x109/L; p=0.002). ETP-ALL patients showed poorer response to induction therapy: 83% of ETP ALL had poorer early cytologic response (>10% BM blastson day+14)vs. 40% of non-ETP (p<0.001), 21% of ETP-ALL patients did not reach CR vs. 5% non-ETP (p=0.012). Flow MRD data at CR(available in 125 out of 151 patients) showed MRD level ≥0.1% in 65% of ETP-ALLvs. 23% of non-ETP ALL (p=0.001) and MRD level ≥0,01% in 88% vs. 43% (p=0.001). 41% of ETP-ALL patients required a second induction treatment compared with 8% of non-ETP-ALL (p<0.001). As a consequence, more ETP-ALL patients underwent allogeneic HSCT (66% vs. 23%, p<0.001). The OS of ETP-ALL patients was poorer after censoring or not the follow-up at the time of HSCT (Figures 1A and B). A trend for higher cumulative incidence of relapse after HSCT was observed in ETP-ALL patients (Figure 1C).

Summary/conclusions: i)ETP-ALL accounted for 20% of adult T-ALL in the PETHEMA cohort; ii)ETP-ALL patients showed poor initial response to treatment (lower CR rate, poorer MRD clearance) than the remaining T-ALL patients; iii) allogeneic HSCT did not overcome the poor prognosis of ETP-ALL in our series.



Supported by the *"Asociación Española Contra el Cáncer", AECC* (project ref: GC16173697BIGA), 2014-SGR225 (GRE) and CERCA Program from Generalitat de Catalunya, Fundació Internacional Josep Carreras and Amgen.

O023

PTEN alterations predict poor outcome in children with T-cell acute lymphoblastic leukemia treated according to I-BFM protocols

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Background: In T-cell acute lymphoblastic leukemia (T-ALL) risk group stratification relies primarily on response to a glucocorticoid-based prophase therapy and minimal residual disease (MRD). Such strategy is insufficient to recognize high-risk cases immediately after diagnosis. Therefore, the refinement of risk stratification is a prerequisite for future therapy individualization and consequently prompts research on genetic prognostic markers that could assist in clinical decisions.

Aims: To evaluate whether selected genetic features, alone or in combination with FC-MRD, predict long-term outcome in children with T-ALL and should be considered as genetic prognostic markers.

Methods: The study group included 162 children with de novo T-ALL, treated according to I-BFM protocols ALL IC-BFM 2002 (n=91; 56%) and ALL IC-BFM 2009 (n=71; 44%) with the median follow-up of 4.1 years. Mutations in *NOTCH1, FBXW7, PTEN, WT1, IL7R, STAT5B, FLT3, RUNX1* and *DNMT3A* were detected by high resolution melt analysis (HRM) and/or Sanger sequencing. *SIL-TAL1* and *NUP214-ABL1* fusions together with copy number alterations (CNAs) in *LEF1, CASP8AP2, MYB, EZH2, CDKN2A/B, MLLT3, LMO1, LMO2, NF1, SUZ12, PTPN2, PTEN, PHF6* were detected by multiplex ligation-dependent probe amplification (MLPA) (SALSA MLPA P383; MRC Holland). MRD was assessed by multicolor flow cytometry (FC-MRD at d15, 33, 78) and 'FC-MRD positive' result was defined as >=0.01%. Statistical tests included: Fisher exact and Cochran–Mantel–Haenszel (CMH) tests, Kaplan–Meier estimator and log-rank test.

Results: Of all genetic aberrations analyzed, we found PTEN alterations to predict for the unfavorable outcome of T-ALL cases treated according to I-BFM protocols. In patients with PTEN mutations (PTEN.MUT) and deletions (PTEN.DEL) 5-year EFS was respectively: 48% (95%CI:26%-88%) and 40% (95%CI:23%-71%), compared with 74% (95%CI:66%-83%) and 77% (95%CI:69%-86%) (P=0.04 and P=0.00008, respectively). Interestingly, PTEN status divided patients from intermediate risk group into favorable and unfavorable prognosis. In patients classified as FC-MRD-IR

group at d15, 33 or 78, those with PTEN.DEL had lower OS as compared with those without PTEN.DEL: 50% (95%CI:18%-100%) vs 85% (95%CI:72%-100%), P=0.01; 50% (95%CI:22%-100%) vs 90% (95%CI:79%-100%), P=0.006; 0% vs 66% (95%CI:37%-100%), P=0.008, respectively. Furthermore, PTEN abnormalities associated with unfavorable prednisone response (PR) and persistence of MRD at day 78. The odds ratios for the unfavorable PR in patients with PTEN.DEL and PTEN.MUT were: 3.49 (95%CI: 1.21-10.03, P=0.02) and 3.99 (95%CI: 1.13-13.6, P=0.03), respectively. In a stratified analysis for MRD status at day 78 we found that patients harboring PTEN.MUT more frequently had positive FC-MRD results (MRD level ≥ 10-4) (OR: 11,13, 95%CI: 2.38-51.92, P=0.0033) and were more frequently assigned to intermediate FC-MRD risk group (MRD level: 0.1 to <10%) (OR: 8,12, 95%CI:1.63-40.55, P=0.01).

Summary: Out of 25 established T-ALL driver genes analyzed in 162 children treated according to ALL IC-BFM protocols, *PTEN* abnormalities predict worse event-free survival, poorer early response to treatment and complement MRD-based risk analysis. We conclude that *PTEN* mutations and deletions are candidates for genetic risk markers in pediatric T-AL.

O024

Adoptive Cellular Immunotherapy using CD1a CART-cells for Treatment of Cortical Pediatric T-Cell Acute Lymphoblastic Leukemia

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Background and rationale: Seminal (pre)-clinical reports have demonstrated that chimeric antigen receptors efficiently redirect T cells (CARTs) against a variety of malignancies. CD19+ B-cell acute lymphoblastic leukemia (B-ALL) exemplifies the success of this immuno-cell therapy with complete response rates >80% in B-ALL patients after infusion of CAR19 T-cells (1). However, CAR T cell-based immunotherapy for other pediatric malignancies remains more challenging. Pediatric T-cell ALL (T-ALL) is a molecularly heterogeneous clonal disease arising early during T-cell development (2). T-ALL in infants presents a poor prognosis, with treatment refractoriness and relapse being common, thus reinforcing the need for new targeted therapies for T-ALL. CD1a is a cortical T-cell antigen always present in cortical T-ALL but absence in normal circulating T-cells. The choice of

the antigen to be targeted is instrumental for the effective and safe development of adoptive immunotherapies (3).

Aims: We propose to explore pre-clinically, *in vitro* and *in vivo*, the potential CARCD1a-based immunotherapy for refractory/relapsed cortical T-ALL.

Methods: The expression of CD1a was analyzed by FACS in a cohort on primary cortical T-ALL patients and in normal cells from peripheral blood (PB). Second generation CARCD1a was generated and cloned in a pCCL lentivector upstream GFP (pCCL.EF1a.scFvCD1a.CD8TMhinge.41BB.CD3zeta.T2A.GFP. It is stably expressed in primary T-cells and it has been tested in a battery of *in vitro* assays.

Results: Here, we show that CD1a is expressed in 100% of cortical T-ALL patient sample but it is absent in normal circulating cells, included PB-MNCs. PB-MNCs were activated and infected (at day 2), and 48h later CAR transduction was successfully detected by GFP and anti-scFv in activated CD4+ and CD8+ T-cells. CARCD1a-expressing activated (CD69+CD25+) T-cells were expanded extensively *in vitro*, and they exerted robust and specific *in vitro* cytotoxicity against CD1a positive T-ALL cell lines associated to a massive release of pro-inflammatory cytokines including TNFα, INFγ and IL2 detected by ELISA.

Conclusion: We designed and tested *in vitro* CART cells against CD1a, cortical T-ALL specific marker, demonstrating its capacity. Later on, we will study cytotoxic effect of this CART *in vivo* against T-ALL cell lines and T-ALL patient samples. CAR T-based cell immunotherapy for refractory/relapsed T-ALL will be a treatment breakthrough because there are no current alternative treatments.

SCIENTIFIC SESSION: STANDARD DRUGS IN ALL - PRACTICAL HANDLING

O025

The differential effects of chemotherapy on the Acute Lymphoblastic Leukaemia microenvironment suggests a possible mechanism for maintenance chemotherapy

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To investigate the role of mesenchymal stromal cells (MSC) in treatment resistance in acute lymphoblastic leukemia (ALL) we isolated ISCT-confirmed, bone-marrow-derived MSC from 151 samples of adults (22-69 years) with ALL registered on the UKALL14 and UKALL60+ trials both at diagnosis and during therapy.

Tumour-associated macrophages (TAM) were observed in 34/75 (45%) of MSC cultures from follow-up samples (termed M+MSC). By contrast, macrophages were absent (M-MSC) from all diagnostic (0/56) and healthy donor specimens (0/7) tested. TAM were more likely to be present in post phase II specimens as compared to post phase I specimens (64% vs 35%, p = 0.03). ELISA on supernatant from M+MSC showed significantly higher levels of myeloid chemo-attractants IL8 (4961 pg/ml vs 1093 pg/ml, p = 0.01) and CXCL2 (106 pg/ml vs 36 pg/ml, p = 0.03) than from M-MSC. The B-ALL cell line Nalm6 proliferated more rapidly when co-cultured with primary M+MSCs than primary M-MSC (1.93 fold, p = 0.03). Importantly, primary M+MSCs expressed a-smooth muscle actin (aSMA) and had a gene expression profile consistent with a cancer associated fibroblast (CAF) phenotype.

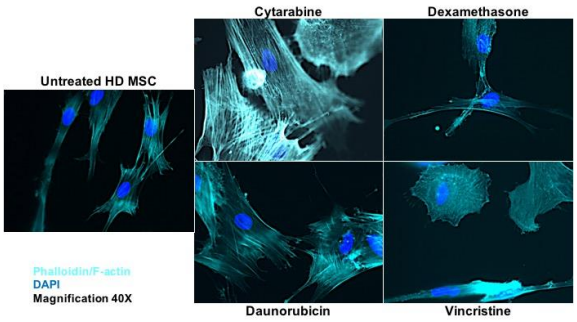
Therefore, we investigated whether chemotherapy drugs used in ALL could induce a CAF phenotype in an MSC cell line HS27a and healthy donor MSC (HD-MSC). Cytarabine induced gross morphological changes in MSC with increased aSMA expression and stress fibres, a gene expression profile consistent with a CAF phenotype and increased secretion of myeloid chemo-attractants, similar to that seen in the M+ MSC primary specimens. In contrast, vincristine induced markedly different morphological changes with the MSCs rounding up without stress fibres or increase in a-SMA expression. Treatment with dexamethasone led to minimal morphological change but did down-regulate many CAF-defining genes and significantly decreased secretion of myeloid chemo-attractants similar to M- MSC.

We then used a niche-like model to re-capitulate treatment resistant disease and explore the functional significance of chemotherapy effects on MSC. HS27a pre-treated with cytarabine significantly increased ALL cell proliferation and reduced chemo-sensitivity in co-culture compared to an untreated HS27a co-culture control. In contrast vincristine and dexamethasone pre-treated HS27a significantly reduced ALL cell proliferation in co-culture and enhanced chemo-sensitivity of ALL cells to all three chemotherapy drugs compared to an untreated HS27a co-culture. This differential effect was lost when the cells were separated by a transwell, suggesting a contact-dependent mechanism.

Subsequent imaging of MSC with ALL cells post treatment with cytarabine revealed MSC adopting a CAF-like appearance with large numbers of ALL cells adherent to the MSC. In contrast post treatment with

vincristine the MSC rounded up and almost no ALL cells attached to the MSC suggesting that inhibition of contact may explain the ability of Vincristine to overcome MSC mediated protection of ALL cells.

We propose that chemotherapy drugs used in ALL have dramatically different effects on the ALL microenvironment. Cytarabine induces a CAF phenotype enhancing ALL cell survival whereas vincristine and dexamethasone reduce MSC-mediated protection of ALL cells, partially through inhibition of contact between MSC and ALL cells. This differential effect on the microenvironment may partially explain the mechanism by which maintenance chemotherapy works in ALL.



O026

Therapy-related acute lymphoblastic leukemia has distinct clinical and pathologic features compared to de novo acute lymphoblastic leukemia

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Background: Therapy-related acute lymphoblastic leukemia (t-ALL) remains poorly defined due to a lack of large data sets highlighting the unique characteristics of this entity.

Aims: To estimate the frequency of t-ALL among adult patients, to evaluate unique clinical and genetic features associated with t-ALL that are distinctive from de novo ALL, and to evaluate the prognostic impact of t-ALL on clinical outcomes

Methods: We retrospectively reviewed all consecutive cases of adult ALL treated at City of Hope Medical Center between 2000 and 2017 and identified cases of t-ALL—defined as ALL preceded by prior exposure to cytotoxic chemotherapy or radiation. Data on prior cancer diagnoses, pathology as well as therapy were analyzed and the clinicopathological features as well as outcomes for t-ALL were compared to *de novo* ALL cases treated at our institution during the same period.

Results: Among 1022 cases of adult ALL, we identified 93 (9.1%) cases of t-ALL The median age of patients was 55 (range 23-85) years and the median latency for ALL onset was 6.8 (range 0.8-50.7) years from the time of original diagnosis. When compared to *de novo* ALL, t-ALL patients were older (*P P P P MLL* rearrangement (17% vs. 4%, *P= <0.01*), lesser incidence of normal karyotype (18% vs. 30%, *P=0.012*), and more chromosome 5 and/or 7 aberrations (16% vs. 8%, *P=0.02*). Remission rates after induction (*P*=0.88) as well as hematopoietic cell transplantation (HCT) rates (*P*= 0.15) were similar between t-ALL and *de novo* ALL. Although the 2-year overall survival (OS) rate was inferior for t-ALL compared to *de novo* ALL (46% vs. 68.1%, *P*=0.001), there was no difference between the two groups when analysis was restricted only to patients who received allogenic HCT (2-year OS: 53.4% vs. 58.9%, p=0.68; non-relapse mortality:28.5% vs. 22.7%, *P*=0.38).

The original diagnosis prior to t-ALL onset was solid cancer in 52 (56%) patients, hematological cancer in 33 (35%) patients,

combined solid and hematological cancers in 2 (2%) patients, and 6 (6%) patients had non-malignant diseases treated with cytotoxic therapies. Breast cancer was the most common prior diagnosis (n = 23, 25%). Thirty-five (38%) patients had chemotherapy alone as prior therapy for the original diagnosis, 26 (28%) had only radiotherapy, and 32 (34%) had a combination of chemoradiation. Among t-ALL, the latency interval from original disease diagnosis to ALL onset was shorter for patients carrying *MLL* gene rearrangement compared to those with other cytogenetic aberrations ($P < 0.01$).

Conclusion: t-ALL represents a relatively large subset of adult ALL and is associated with unique genetic and clinical features. t-ALL cases are enriched with MLL gene rearrangement and chr 5/7q del/monosomy. Although OS was inferior for t-ALL patients compared with *de novo* ALL, the use of allogeneic HCT may overcome this difference in outcomes.

SATURDAY, APRIL 14

SCIENTIFIC SESSION: STANDARD THERAPY OF DE NOVO ALL

O027

Standard Therapy of de novo ALL: Strategy of the Spanish PETHEMA Group.

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Background and aim: To present the strategies and main results of the protocols of standard treatment of adult patients with *de novo* acute lymphoblastic leukemia (ALL) conducted by the Spanish PETHEMA Group.

Methods: Patients with *de novo* ALL are stratified according to their risk factors (standard [SR], high [HR]), Philadelphia chromosome status (Ph+, Ph-), age (adolescents and young adults (AYA), older adults, elderly) and immunologic subtype (mature B, other). Risk-adapted and subtype-oriented protocols have been developed for the main groups and subtypes of ALL. Patients are centrally registered and analysis of the results is performed two times every year.

Results: The main results according to CR, DFS and OS are presented in Table 1

Conclusion: Risk-adapted and subtype-oriented protocols of standard treatment of adult patients with *de novo* ALL from the PETHEMA Group are feasible, with similar results than protocols from other European countries.

Protocol	Group	N	CR (%)	DFS (5-yr, 95%CI)	OS (5-yr, 95%CI)
ALL SR08	AYA with SR ALL	82	98	70 (55-85)	80 (67-93)
ALI HR11	Young adults	209	90	45 (35-55)	50 (38-62)
ALL Ph08	Young adults	118	96	53 (42-64)	55 (43-67)
ALL OPH07	Older adults and elderly Ph+ ALL	86	88	45 (28-62)	46 (32-60)
ALL OLD07	Older adult and elderly Ph-neg ALL	87	67	31 (18-44)	22 (12-32)
ALL OLD FRA	Older adults with frailty	49	55	0	0
BURKIMAB08	Mature B ALL and Burkitt lymphoma	31	85	80 (60-100)	81 (67-95)

O028

Current first-line trials from the French-Belgian-Swiss Group for Research on Adult ALL (GRAALL)

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In Ph-negative ALL patients, the GRAALL is developing a general strategy aiming to incorporate new agent into front-line therapy in high-risk patients and select candidates for allogeneic hematopoietic stem cell transplantation (HSCT) based on early minimal residual disease (MRD) response (GRAALL-2014 trial). In Ph-positive ALL patients, the GRAALL is conducting a front-line nilotinib trial pursuing the goal to reduce chemotherapy intensity and improve MRD response prior to allogeneic or autologous HSCT (GRAAPH-2014 trial).

GRAALL-2014 trial: This trial includes younger patients aged 18 to 59 years old with newly diagnosed previously untreated Ph-negative B-cell precursor (BCP) or T-cell ALL. Chemotherapy schedule is based on the GRAALL-2003/2005 protocols (F.

Huguet et al. JCO 2009 and ASH 2016), but as compared to these previous trials, the ongoing GRAALL-2014 trial includes: i) age-adapted doses of L-asparaginase, prednisone, methotrexate and anthracycline and allogeneic HSCT conditioning in order to improve compliance and reduce toxicity in patients aged ≥45 years old; ii) omission of prophylactic cranial irradiation associated with an increased number of ITs; iii) prospective monitoring of allergy/immunization to L-asparaginase, guiding a switch to erwiniase for delayed intensification; iv) simplified and reduced indication for allogeneic HSCT in first remission, now restricted to very high-risk patients with poor early Ig-TCR MRD response (MRD1 ≥10-3 after first induction at week-6 and/or MRD2 ≥10-4 after consolidation at week-12); and last but not least v) evaluation of incorporation of new agents (QUEST study with blinatumomab in BCP-ALL and ATRIALL study with nelarabine in T-ALL) during consolidation and maintenance phases in patients with high-risk BCP- or T-ALL, defined by bad-risk oncogenetic profiles (based on *KMT2A/MLL* and *IKZF1* gene status and *NOTCH1-FBXW7-RAS-PTEN* gene status, respectively) and/or unsatisfactory MRD1 response ≥10-4. A total of 775 patients will be enrolled. A hierarchical biological workflow, including RNAseq, prospectively identifies Ph-like ALL cases with targetable kinase activation.

GRAAPH-2014 trial: This trial includes younger patients aged 18 to 59 years old with newly diagnosed previously untreated Ph-positive ALL. Four 28-day cycles of combined nilotinib and chemotherapy are given prior to allogeneic or autologous HSCT. Cycles 1 and 3 include nilotinib, vincristine, dexamethasone and ITs. Cycles 2 and 4 include nilotinib, high-dose methotrexate and one IT ± intermediate-dose cytarabine according to front-line randomization (with non inferior post-cycle 4 *BCR-ABL1* MRD response as an end-point). Allowed allogeneic HSC sources include sibling and 9-10/10 HLA matched unrelated donors. Reduced intensity conditioning is planned for patients aged 55 years or more. Autologous HSCT may be proposed to patients with good MRD response, even if a donor is available, based on local investigators' decision. Post-HSCT maintenance with imatinib for at least 2 years is planned in all transplanted patients. A total of 265 patients will be enrolled, with a planned interim analysis after the first 60 patients will be evaluable for post-cycle 4 MRD response.

The following two abstracts were presented as one, under the title: ***CELL treatment approaches for de novo Ph- negative and Ph-positive ALL***

O029

Philadelphia-chromosome positive adult acute lymphoblastic leukemia: standard first-line therapy within the Czech leukemia study group — for life *(CELL)*

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Background: Philadelphia-chromosome (Ph) positive adult acute lymphoblastic

leukemia (ALL) is a distinct entity targetable with tyrosine-kinase inhibitors (TKI) that have helped to significantly improve the survival in younger patients and to reduce the treatment toxicity in the elderly.

Aims: To describe standards of first-line therapy in patients with Ph-positive ALL treated at the centers participating within the Czech Leukemia Study Group – for Life (CELL).

Methods: All patients with Ph-positive ALL diagnosed between 2006 and 2017 and having a data entry in the CELL database were included into this retrospective analysis. These patients were divided into two subgroups – intensive (younger patients with curative approach) and non-intensive (palliative approach, mostly elderly). The data obtained were analyzed for descriptive statistics, hematologic and molecular remission, allogeneic transplant and relapse rates, and survival.

Results: A total number of 81 patients diagnosed with Ph-positive ALL have a record in our database. There were 59 patients with intensive treatment and 22 patients in the non-intensive subgroup.

Intensive subgroup: Median age at diagnosis was 45 years. Slightly more than a half of the patients (56%) had additional chromosomal abnormalities at diagnosis. All patients were treated with imatinib in combination with intensive chemotherapy (93% with a joint CELL protocol, 7% using hyper-CVAD alternating with methotrexate and cytarabine). Complete hematologic remission (CR) after the induction treatment was achieved in 95%, while two patients experienced death in induction and only one was truly refractory. Complete molecular remission (CMR) was achieved in 76%, usually in later treatment phases. Allogeneic stem cell transplant (SCT) was performed in 42 (75%) patients. During the follow-up period, fourteen (25%) patients relapsed. Median progression-free (PFS) and overall survival (OS) were 62.1 months and not reached, respectively, with 5-year PFS and OS 53% and 65%, respectively. CMR and allogeneic SCT were factors associated with a significantly prolonged survival.

Non-intensive subgroup: Median age at diagnosis was 68 years. About one third of the patients (32%) had additional chromosomal abnormalities at diagnosis. All patients were treated with imatinib, either in combination with reduced-toxicity chemotherapy (18%) or with minimal chemotherapy plus steroids (82%). CR after the induction therapy was achieved in 82%, while three patients experienced death in induction and only one was truly refractory, CMR was achieved in 47%. Allogeneic SCT was performed in only two (11%) patients. During the follow-up period, ten (56%) patients relapsed. Median PFS and OS were 8.1 and 12.8 months, respectively, with 2-year PFS and OS 29% and 38%, respectively.

Summary/Conclusion: In Ph-positive ALL patients the intensive treatment led to high CR (95%) and CMR (76%) rates, which resulted in overall survival exceeding 60%. On the other hand, the non-intensive treatment reduced induction deaths allowing extending median overall survival over one year with a very low toxicity and a good quality of life.

Supported by Czech Leukemia Study Group – for Life (CELL).

O030

Philadelphia-chromosome negative adult acute lymphoblastic leukemia: standard first-line therapy within the Czech leukemia study group — for life *(CELL)*

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Background: Adult patients with Philadelphia-chromosome (Ph) negative acute lymphoblastic leukemia (ALL) form a heterogeneous cohort of different age, immunophenotype, molecular and cytogenetic risk groups. A great effort has been made to improve the prognosis with modern risk-adapted treatment protocols. Nevertheless, the outcomes still tend to be quite diverse.

Aims: To describe standards of first-line therapy in patients with Ph-negative ALL treated at the centers participating within the Czech Leukemia Study Group – for Life (CELL).

Methods: All patients with Ph-negative ALL diagnosed between 2006 and 2017 and having a data entry in the CELL database were included into this retrospective analysis. These patients were divided into two subgroups – intensive (younger patients with curative approach) and non-intensive (palliative approach, mostly elderly). The data obtained were analyzed for descriptive statistics, hematologic and molecular remission, allogeneic transplant and relapse rates, and survival.

Results: A total number of 182 patients diagnosed with Ph-negative ALL have a record in our database. There were 159 patients with intensive treatment and 23 patients in the non-intensive subgroup.

Intensive subgroup: Median age at diagnosis was 35 years. All patients were treated with intensive chemotherapy (94% with a joint CELL protocol, 6% using hyperCVAD alternating with methotrexate and cytarabine). Complete hematologic remission (CR) after the induction treatment was achieved in 95%, while four (2.5%) patients experienced death in induction and 4 (2.5%) were truly refractory. Complete molecular remission (CMR) was achieved in 82%, usually in early treatment phases. Allogeneic stem cell transplant (SCT) was performed in 64 (42%) patients. During the follow-up period, fifty-four (36%) patients relapsed. Median progression-free (PFS) and overall survival (OS) were 31.3 months and not reached, respectively, with 5-year PFS and OS 45% and 54%, respectively. We have not found any major differences between various subgroups (age, B or T lineage, immunophenotype, molecular genetics or cytogenetics), except that relapses and deaths occur in the first two years in T-ALL whereas in B-ALL up to year 4 or 5. The only major factors associated with a significantly prolonged survival were CMR and allogeneic SCT.

Non-intensive subgroup: Median age at diagnosis was 65 years. All patients were treated with reduced-toxicity combined chemotherapy (91%) or with minimal chemotherapy plus steroids (9%). CR after the induction therapy was achieved in 74%, while four (17%) patients experienced death in induction and only one was truly refractory. CMR was achieved in 75%, although the MRD data are scarce. Allogeneic SCT was not performed in a single patient. During the follow-up period, eight (47%) patients relapsed. Median progression-free (PFS) and overall survival (OS) were

13.1 and 13.1 months, respectively, with 2-year PFS and OS 39% and 43%, respectively.

Summary/Conclusion: In Ph-negative ALL patients the intensive treatment led to high CR (95%) and CMR (82%) rates, which resulted in overall survival exceeding 50%, reaching up to 70% in specified subgroups. The non-intensive treatment, although being reduced as such, was associated with a higher induction and non-relapse mortality that both, combined with a higher relapse rate, resulted in a shorter survival with the median around one year.

Supported by Czech Leukemia Study Group – for Life (CELL).

O031

Standard therapy for young adults with Philadelphia-negative ALL in Sweden

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Background: In Sweden (a country with 10 million inhabitants) patients with ALL is treated according to national guidelines developed by the Swedish Adult ALL Group, SVALL. For patients with Philadelphia-negative B- and T-ALL, a cooperation is ongoing for children and adults in the Nordic and Baltic countries within the Nordic Society of Paediatric Haematology and Oncology (NOPHO) and patients between 1-45 years are treated according a common protocol "NOPHO ALL 2008", used for adults in Sweden since 2009, proceeded by an earlier NOPHO ALL protocol as a pilot for feasibility. From 2016, also patients aged 46-65 years are treated according to NOPHO ALL 2008 backbone with dose reductions, as best available treatment.

Aims: The aim of the national guidelines and cooperation within NOPHO on the ALL 2008 protocol has been to improve the treatment results for adults with Philadelphia-negative (Ph-neg) B- and T-ALL and to provide a platform for research and future common treatment protocols.

Here we analyze the outcome for the Ph-neg B-and T-ALL patients in the Swedish ALL register to evaluate changes in outcome over time, estimating the effect of changes in the national recommendations.

Methods and Results: The overall survival (OS) for Nordic/Baltic adult patients aged 18-45 years in the NOPHO ALL 2008 study was 78 (72-84)% and the toxicity profile was similar foradults and children > 10 y as previously described (1). In the Swedish ALL Registry, evaluating treatment of adult ALL patient from a population based view, the outcome for the 292 Ph-neg ALL patients aged 18-45 y has improved over time (1997-2000, 2001-2005, 2006-2010, 2011-2015) with a 5 year OS (95%CI) of 51(36-67)%, 44(31-56)%, 66(55-76)% and 62(52-72))% respectively (p=0.02 log-rank). The corresponding early mortality within 30 days was 5, 3, 0 and 1% and the estimated frequency of allogeneic hematopoietic stem cell transplantation in CR1 was 26, 34, 22 and 25% for the same cohorts. The 5 year OS for the 96 patients treated according to NOPHO ALL 2008 was 72 (63-82)%.

Conclusions: Pediatric ALL protocols are effective and deliverable for adult patients

with ALL on a national basis to a population up to 45y. Based on the NOPHO adult experience, we will continue the cooperation within the NOPHO ALL working group by joining an upcoming European study group, the ALLTogether consortium, for the next fist line trial for Ph-negative ALL including both children and young adults.

O032

Current First Line Trials of the German Multicenter Study Group for Adult ALL (GMALL)

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The GMALL group programme is subdivided by age (<= 55 yrs vs > 55 yrs vs > 75 yrs) and by Ph/BCR-ABL (Ph) status. ALL and LBL are treated with slightly modified protocols. The GMALL conducts prospective trials and a national registry. All pts, independent from further trial inclusion, are first entered into the registry and if possible biomaterials are collected in the central biobank. For pts treated in centers, which do not participate in the clinical trials or for treatment situations in which no clinical trials are available, expert recommendations for treatment are provided. This includes treatment of younger, older and frail pts, stem cell transplantation (SCT), relapse therapy and more.

GMALL 08/2013: Risk and MRD adapted trial for de novo ALL and LBL, Ph neg/pos, age 18-55 yrs

The trial evaluates standard ped-based induction with intensification of rituximab (CD20+ and CD20-) and pegylated asparaginase (PEG-ASP) for Ph neg ALL. In B-precursor ALL / LBL the need for CNS irradiation is evaluated in randomisation I. After induction all pts receive standard consolidation I. Afterwards a risk stratification according to minimal residual disease (MRD) and conventional factors take place. All pts with molecular failure become candidates for SCT and an experimental targeted therapy e.g. with Blinatumomab or Nelarabine. Randomisation II evaluates whether SCT can be avoided in pts with initial high risk features but molecular remission. Standard risk pts receive intensified consolidation with additional doses of asparaginase. Pts with T-ALL/LBL receive two cycles with Nelarabin/Cyclophosphamide. After reinduction and a total of 6 consolidation cycles pts receive conventional maintenance therapy up to 2.5 yrs. The feasibility of PEG-ASP maintenance will be tested. MRD testing continues in order to identify molecular relapse which is treated similarly to molecular failure.

Pts with Ph+ ALL will be treated with an innovative dose reduced induction regimen in combination with Imatinib followed by consolidation I. All pts are candidates for a SCT.

GMALL Initial-1 and Bold: Immunotherapy for de novo ALL, Ph neg, age 56-75 yrs

See abstract on immunotherapies

Treatment recommendation for older pts with de novo ALL or LBL, Ph neg/pos, age 56- 75 yrs

Older pts with Ph neg ALL are treated with a dose reduced, ped-based standard induction followed by consolidation with MTX/PEG-ASP, HDAC and reinduction. Patients with molecular failure after consolidation II become candidates for SCT and for targeted treatment.

Older pts with Ph pos ALL are treated with induction based on vincristine/dexamethasone/imatinib only followed by

standard chemotherapy consolidation and Imatinib. In pts with molecular failure the kinase inhibitor is changed after consolidation II. Pts are candidates for SCT with dose reduced conditioning.

A separate protocol is provided for frail pts.

The GMALL plans to participate in the EWALL PH03 trial which is presented separately. Furthermore a trial with first-line Ponatinib is planned for frail pts.

Treatment recommendation for relapsed/refractory ALL and LBL

The GMALL provides recommendations for treatment of hematologic relapse or molecular relapse. Current and planned trials and recommendations include immunotherapies (blinatumomab, inotuzumab) and chemotherapy. Further compounds such as Bortezomib, next generation kinase inhibitors, CD38 antibodies, BCL2-inhibitors, ABL1 and JAK inhibitors are considered.

O033

Optimization of the treatment of adults with acute lymphoblastic leukemia according to Polish Adult Leukemia Group (PALG)-ALL7 protocol

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Treatment of adults with ALL depends on age, immune subtype and the presence of t(9;22). The protocol being currently started in Polish centers (PALG ALL7) stratifies patients accordingly.

Patients with Ph(-) BCP-ALL 0.1% after induction I receive second induction - FLAM (fludarabine+AraC+mitoxantrone) followed by Mtx+Vep+Dexa+/-R consolidation. Patients with primary resistance are intended for blinatumomab as a salvage therapy. Patients with at least one risk factor (MRD>0.1% after induction, MRD>0.01% after consolidation, initial WBC>30 x109/L, *MLL* gene rearrangement, CNS involvement) are candidates for allogeneic HSCT from wither matched sibling, unrelated or haploidentical donor. All remaining patients receive intensification followed by 2 years maintenance or autologous HSCT with maintenance. Treatment schedule for patients with TCP-ALL 55 y.o. the doses of chemotherapy are reduced. In this cohort allogeneic HSCT with reduced intensity conditioning should be considered in all cases.

Patients with Ph-positive ALL 55 y.o. receive the combination imatinib with reduced-dose chemotherapy + imatinib +/-R according to the EWALL consensus. Persistence of MRD after two courses of consolidation implicates switch from imatinib to dasatinib. Allogeneic HSCT is an option for those who do not achieve molecular remission.

PALG ALL7 protocol may be a subject of modifications dependent upon availability of modern methods of immunotherapy.

POSTER PRESENTATIONS & ONLINE PUBLICATION

Genetics of ALL-subtypes

POSTER PRESENTATIONS

P034

The Effect of Polymorphisms of Gamma-glutamyl hydrolase (GGH) Gene on Methotrexate-Induced Toxicity in Acute Lymphoblastic Leukemia

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Context: Acute lymphoblastic leukemia patients show differences in methotrexate-induced toxicity after treatment with this anti-cancer drug. Pharmacogenetics is an important determining factor for toxicity diversity.

Objective: In this study, the effect of +452 CT and - 401CT polymorphisms of Gamma-glutamyl hydrolase (GGH) gene on methotrexate serum levels and its associated toxicity in patients with acute lymphoblastic leukemia was assessed. Furthermore, the frequency of the above polymorphisms was investigated for the first time in Iran.

Material and methods: The prevalence of these polymorphisms was assessed in 83 Iranian patients with ALL using PCR and RFLP. The relationship between the polymorphism and serum methotrexate levels and its toxicity was estimated by calculating the Odds Ratio.

Results: No correlation was found between +452CT polymorphism and serum levels of methotrexate and methotrexate-related toxicity. - 401CT polymorphism was found to be correlated with methotrexate-related toxicity leading to thrombocytopenia (95% CI=0.009-0.019, odds ratio=0.265) and leukopenia (95% CI=0.021-0.042, odds ratio=2.182) in consolidation phase of the treatment.

Discussion: C allele polymorphism of -401C/T allele is a risk factor of leukopenia and thrombocytopenia in patients treated with methotrexate. Moreover, our results suggested that the T allele had a supporting role in prevention of thrombocytopenia.

Conclusion: Evaluation of patients for methotrexate-related polymorphism of GGH gene may be useful to determine the appropriate dose of methotrexate and reducing its toxic side effects.

P035

Acquired marker chromosomes and complex karyotypes in acute lymphoblastic leukemia can emerge from chromothripsis

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Background: Acute lymphoblastic leukemia (ALL) is a heterogeneous, aggressive disease, and is the most common childhood tumor as well as a leading cause of cancer death in young as well as adult. However, about 30% of pediatric and 50% of adult ALL patients still lack defined genetic hallmarks of biological and clinical significance. Complex karyotype-ALL (CX-ALL) promoting a wide spectrum of diverse genetic alterations with striking genomic instability, are overall often neglected by research. Recently, a catastrophic phenomenon was discovered, arising from localized massive chromosome fragmentation and form marker chromosome, called chromothripsis.

Aims: The overall goal of this study was to characterize karyotypes and genetic content of acquired marker chromosomes in 24 ALL cases and to rule out if one single molecular approach may be sufficient to detect chromothripsis in the studied patients.

Methods: Here 24 ALL cases with according to standard GTG-banding normal karyotype (CN-ALLs) were retrospectively studied by multitude multicolor banding (mMCB), fluorescence in situ hybridization (FISH) using selected locus-specific probes and array-comparative genomic hybridization (aCGH).

Results: Most strikingly, 12 of the studied 24 CN-ALLs (~50%) revealed abnormal karyotypes, applying mMCB: structural abnormalities were present in 10 and even numerical ones were identified in 2 cases. Overall, 117 copy number alterations (CNAs) were identified as loss (~79%) and as gains (~21%). Furthermore, chromothripsis was detected by aCGH in 3 out of the 12 aberrant cases. The latter three cases had either deletions in genes involved in lymphoid development, cell cycle and/or DNA repair (*IKZF1*, *CDKN2A/B*, *PAX5*, *BIRC3*, *ATM*, *RB1*), as shown by FISH, and also had acquired marker chromosomes.

Conclusion: Chromothripsis occurs frequently in ALL (12.5%) and impacts for patient prognosis. Thus, genomic instability and complex karyotypes are linked to drug resistance, adverse prognosis and early relapse in ALL.

P036

Chromosome segregation defects as early underlying pathogenic mechanism in hyperdiploid childhood B-cell acute lymphoblastic leukaemia

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Background*:* High-hyperdiploid (HHD) B-cell precursor (BCP) acute lymphoblastic leukaemia (ALL) (HHD-BCP-ALL) is defined by the presence of 51 to 67 chromosomes in leukemic cells. Hyperdiploidy is thought to be the initiating oncogenic event in this subtype of BCP-ALL and even a single abnormal mitosis in BCP might initiate HHD-BCP-ALL. However, despite being a major BCP-ALL subgroup during childhood, the molecular mechanisms leading to HHD remains unknown.

Aim*:* To study the contribution of mitotic and chromosome segregation defects to the origin of HHD in childhood BCP-ALL.

Methods: We set out to study the contribution of mitotic and chromosome segregation defects in both the HHD-BCP-ALL cell line MHH-CALL2 and in HHD-BCP-ALL primary patient samples (fresh or NSG mice-expanded, n=3). Non-HHD BCP-ALL cell lines (SEM and REH) and primary BCP-ALL patient samples were used as controls. Whole-genome transcriptome of both HHD- and normal karyotype (NK)-BCP-ALL primary samples was analyzed by RNA-seq.

Results*:* Our results showed that HHD-BCP-ALL cells grow significantly slower than non-HHD BCP-ALL cells. This was confirmed for both cell lines and primary samples grown *ex vivo* on nestin+ human mesenchymal stem cells. The slower growth of HHD-BCP-ALL cells was associated to cell cycle/mitotic defects as revealed by the accumulation of HHD-BCP-ALL cells in the G2/M phases of the cell cycle. Further immunofluorescence analysis of the different mitotic phases confirmed an accumulation of HHD-BCP-ALL cells in prometaphase/metaphase coupled with defects on chromosome biorientation at the metaphase plate. Supporting these mitotic progression defects, HHD-BCP-ALL cells commonly displayed defects in chromosome segregation such as lagging chromosomes, anaphase bridges and heterogeneous modal karyotypes. Importantly, gene ontology analysis using the genes (n=2842) observed by RNA-sequencing to be differentially expressed in HHD-BCP-ALL primary cells (n=58 patients) versus normal karyotype BCP-ALL primary cells (n=8 patients) supported a "mitotic" and "chromosome segregation" gene signatures in HHD-BCP-ALL cells. Finally, preliminary data on the molecular mechanisms underlying these mitotic defects showed both mislocalization of the chromosome passenger complex (CPC) from the inner centromere in prometaphase cells and cohesion defects revealed by premature chromatid separation in metaphase chromosomes, thus linking the mitotic delay and the chromosome segregation defects observed in HHD-BCP-ALL cells.

Conclusion: Collectively, our results suggest that HHD may arise from defects in mitotic progression and chromosome segregation in human BCP. Future experiments should address whether young (fetal-pediatric) BCP are more prone to such mitotic/chromosome segregation defects than aged BCP or myeloid progenitors, perhaps explaining the almost exclusive existence of hyperdiploidy in pediatric BCP-ALL.

P037

T-cell acute lymphoblastic leukemia from miRNA transcriptome perspective: insights into biology and heterogeneity

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive and heterogeneous subtype of ALL. This heterogeneity was revealed by identification of complex (epi-)genetic landscape of T-ALL with use of high-throughput approaches, incl. next-generation sequencing [1-4]. miRNAs are small non-coding RNAs implicated in complex regulatory networks of gene expression by silencing their target genes. miRNAs might serve as oncogenes or tumor suppressors, thus aberrantly expressed miRNAs contribute to oncogenesis [5]. Exploring miRNA transcriptome with use of next-generation sequencing (miRNA-seq) offers new insights into molecular heterogeneity and pathogenesis of T-ALL and holds potential for improved classification and treatment stratification.

Aims: To identify miRNA transcriptome of pediatric T-ALL and miRNA expression profiles specific to T-ALL subtypes (insights in T-ALL heterogeneity). To identify novel candidate oncogenic/tumor suppressive miRNAs, their target genes and related pathways (insights in pathogenesis). To assess potential of miRNAs as classification and prognostic markers in T-ALL.

Methods: We studied bone marrow samples of 34 pediatric T-ALL patients. As controls we used bone marrow samples of 5 healthy donors and RNA-seq data of thymocyte subsets (CD34+; CD4+/CD8+) of 4 donors [6]. Total RNA was isolated (miRCURY RNA Isolation Kit-Cell & Plant; Exiqon) from T-cells selected immunomagnetically (BD IMag Human T Lymphocyte Enrichment Set; BD Biosciences). NextSeq500 Illumina was used for miRNA sequencing (NGS Service Exiqon), with 10 mln reads/sample, 51bp single-end; reference annotation: GRCh37, miRbase21. Target genes for differentially expressed miRNAs were identified using 8 algorithms and tested for overrepresentation in biological pathways using Fisher's exact test and Benjamini-Hochberg correction for multiple testing.

Results: We identified miRNA expression profiles discriminative between pediatric T-ALL, bone marrow and thymic controls; and miRNA profiles related to T-ALL maturation stages (EGIL immunophenotypic subtypes: II, III, IV). We identified 23 overexpressed miRNAs (potential oncogenic miRNAs) in T-ALL vs. bone marrow controls; 36 overexpressed miRNAs (when bone marrows and thymic subsets were combined as controls) and 10 miRNAs overlapping between those 23 and 36 overexpressed miRNAs, incl. miR-128-3p and miR-20b-5p. We identified 38 underexpressed miRNAs (potential tumor suppressors) in T-ALL vs. bone marrow; 166 underexpressed miRNAs (when control bone marrows and thymocytes were combined) and 33 overlapping underexpressed miRNAs. We showed significant target enrichment in pathways related to: regulation of apoptosis, regulation of kinase activity, regulation of signaling, incl. interleukin-6-mediated signaling pathway.

Summary: We provide novel insights into the heterogeneity of pediatric T-ALL at the miRNA transcriptome level. Expression profiles of miRNAs differentially expressed in patients vs. controls and in T-ALL subtypes (EGIL: II, III, IV) hold potential for

improved classification of T-ALL. Selected differentially expressed miRNAs are tested functionally to assess their oncogenic/tumor suppressive role in T-ALL. Association analysis (survival and treatment response) will be used to assess prognostic potential of miRNAs in T-ALL.

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P038

Gene aberrations in adult patients with Ph-like ALL in the Czech Republic

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Background: Philadeplhia chromosome-like acute lymphoblastic leukemia (Ph-ALL) is a newly identified high-risk B-lineage ALL subtype, accounting for approximately 20% of adult patients with B-ALL. Ph-like ALL is characterized by poor outcome, very high risk of disease relapse and poor overall survival. The gene aberrations detected in Ph-like ALL patients are associated with JAK/STAT, Ras, Ikaros and ABL signalling pathways and include a broad spectrum of sequence mutations, fusion genes (without *BCR-ABL1*) and rearrangements involving locus specific or extensive deletions or duplications. The diverse range of kinase-activating alternations in Ph-like ALL has important therapeutic implications. However, the genetic basis of adult patients has not yet been studied in the Czech Republic.

Aims: The study aims to identify the frequent gene aberrations associated with Ph-like subtype using molecular analysis in 73 adult patients with B precursor ALL from the Czech Republic. The molecular genetic analysis was focused on the "hot spot" region of *JAK2* gene (exon 15), on the detection of presence of *P2RY8-CRLF2* fusion gene and on the detection of copy number variation in genes associated with ALL, in particular on the detection of deletions of the *IKZF1* gene.

Methods: The genomic analyses were performed using several techniques including PCR, real-time PCR, Sanger sequencing, NGS, and MLPA methods.

Results: Patient cohort of Ph-like ALL accounted for 46.6% of young adults (age 15 to 39 years), 35.6% of adults (age 40 to 59 years), and 17.8% of older adults (age 60 to 75 years). Among all patients, increasing age was associated with inferior outcome of disease. The difference in overall survival (OS) was significant for young adults compared with adults and older adults (median: 126.7; 23.5 and 22.6; P=0,0087). The above mentioned gene aberrations were identified in 35.6% of patients with Ph-like ALL to date. The 5-year OS, resp. 3-year OS for patients with and without gene aberrations were 12% and 21%, resp. 36%

and 49%. Copy number alternations were detected by almost a third of patient cohort in many genes (*IKZF1, PAX5, BTG, ETV6, EBF1, CDKN2A/2B* and *SHOX*). *IKZF1* deletions were the most frequent aberration of them (17.8%). Within the patient cohort, the presence of *IKZF1*deletions significantly did not influence the OS. The presence of *P2RY8/CRLF2* fusion gene was identified in 10 patients (16.4%) with markedly inferior median of OS in comparison to patient group without detected *P2RY8/CRLF2* (median: 33.2; 126.7). Sequence mutations of the *JAK2* gene occurred in only one patient (1.6%). This patient harbored a subclonal mutation p.(Arg683Ser) and deletions in *IKZF1, PAX5* and *CDKN2A/2B* genes.

Conclusion: Our findings demonstrate the percent representation of selected gene aberrations in Ph-like ALL adults patients. In addition, they have led to introduction of molecular genetic diagnostics of Ph-like ALL to routine hematological practice for these patients.

P039

Distinct mutational processes causing hypermutation in relapsed pediatric acute lymphoblastic leukemia.

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Introduction: Despite improvements in the treatment of pediatric acute lymphoblastic leukemia (ALL), relapse still represents the most common cause of treatment failure. These relapses are associated with poor prognosis, and better understanding of mechanisms driving relapse is needed in order to improve the outcome. We recently found that a significant proportion of relapsed ALL is hypermutated. In this study, we selected 13 hypermutated relapse cases and studied mutational processes by extracting mutational signatures.

Methods: Eight males and 5 females diagnosed with ALL relapse (9 B-progenitor and 4 T-lineage) were selected. Nine patients had one relapse, while 4 patients experienced two relapses. The median time to first relapse was 1.9 years (range 0.4 to 5 years), and the median time to second relapse was 0.6 years (range 0.2 to 6.9 years). Diagnosis, relapse and matched normal samples were analyzed using exome sequencing and capture validation of identified SNVs/indels. Signature extraction was done with non-negative matrix factorization using the R package MutationalPatterns.

Results: The median number of somatic mutations was 39 (range 22 to 70) in diagnosis, 270 (70-1139) in first relapse and 1029 (102-1699) in second relapse. In order to understand the mutational processes leading to relapse, we performed mutational signature analysis on acquired mutations in first or second relapse. We identified at least three mutational processes to be responsible for hypermutation in these cases at variable levels, including deamination of methylated CpG dinucleotides (COSMIC signature 1), AID/APOBEC (COSMIC

signatures 2 and 13), and mismatch repair deficiency (COSMIC signature 15). One additional signature without high similarity to a particular COSMIC signature was also found. Signature 1 mutations represented the biggest contributor of hypermutation in this cohort, causing the highest mutation load per sample. Thus far signature 1 was described as a spontaneous process driving acquirement of mutations in an age dependent manner, rather than an active hypermutator mechanism. The acquired signature 1 mutations in these hypermutated relapses showed a striking gradual variation in variant allele frequency, suggesting that these mutations arise in an ongoing process during relapse outgrowth.

Conclusions: Taken together our data show that hypermutation in relapsed ALL is caused by various alternative mechanisms of mutation or repair deficiency that are acting separately or in combinations. Further studies are required to elucidate the underlying mechanisms of each of these mutational processes.

P040

Pediatric patients with acute leukemia and MLL rearrangement show a distinctive expression pattern of histone deacetylases

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Introduction: Histone deacetylase inhibitors (HDACi) are promising drugs to reverse aberrant epigenetic changes associated with leukemia. However, their lack of specificity and secondary toxicities limited their use in clinical practice. Therefore, more specific HDACi are needed. The study of *HDAC* expression in pediatric acute leukemia could help to tailor treatment with HDACi in a more personalized approach.

Aim: to analyze the expression of *HDACs*, *MEF2C* and *MEF2D* in a series of pediatric patients with acute leukemia.

Methods: we analyzed *HDAC1-11*, *SIRT1, SIRT7, MEF2C* and *MEF2D* mRNA expression by quantitative PCR in pediatric patients diagnosed with *de-novo* acute leukemia from 2003 to 2017 in our center. Patients were uniformly treated according to the Spanish Society of Pediatric Hematology and Oncology (SEHOP) consecutive protocols. We used a pool of non-neoplastic patients as calibrator and studied also *HDAC* expression in normal bone marrow CD34+ cells, mature CD19+ and T lymphocytes.

Results: We studied 211 patients (57% males, median age 5.8 years, range 0-17.4), including 134 B-cell precursor acute lymphoblastic leukemia (BCP-ALL) cases, 33 T-ALL and 44 patients with acute myeloid leukemia (AML). We observed a global overexpression of *HDACs* and nearly all patients expressed class I *HDACs* homogeneously. In

T-ALL patients, high *HDAC4* expression correlated with a poor prednisone response at day +8 of induction therapy, although not significantly (p=0.053). Interestingly, *MEF2C* expression seemed to reflect the oncogenic pathway of leukemic blasts. Thus, all patients with absent expression of *MEF2C* were *NOTCH1/FBXW7* mutated, while all but one patients with high *MEF2C* expression were wild-type; the only *NOTCH1*-mutated patient who expressed *MEF2C* gene had an immature pre-T phenotype. Noticeably, we identified a distinctive signature for patients with *MLL* rearrangement, with high *HDAC9* and *MEF2D* expression, regardless of age, *MLL*-partner and lineage (p=0.005 and p=0.034, respectively). Regarding outcome, in BCP-ALL patients, low *HDAC2* expression (cut-off 3.29, around percentile 50) and very high *HDAC9* (cut-off 3.62, around p65) impacted negatively on event free survival (EFS) at 5 years (70.5±7% *vs.* 95.4±3%, p=0.002; 69±1% *vs.* 97.9±2%, p*HDAC9* expression was an adverse prognostic factor both in *MLL*-rearranged and in *MLL* wild-type cases.

Conclusions: We observed different profiles of *HDACs* in different subtypes of childhood leukemia, some of them probably reflecting the lineage and maturity and some pointing out the oncogenic pathway of leukemic blasts. We identified an *HDAC* signature for *MLL*-rearranged patients, with overexpression of *HDAC9* and *MEF2D*, regardless the age, lineage and the *MLL*-partner. Moreover, we confirmed previous reports of *HDAC9* as a prognostic biomarker in BCP-ALL. Our results provide useful knowledge on the complex picture of *HDACs* expression in childhood leukemia and support the directed use of more specific HDACi to selected subtypes of pediatric patients with acute leukemia.

P041

The impact of IKZF1 deletions on MRD-clearance and prognosis in adult Ph-negative and Ph-positive B-cell acute lymphoblastic leukemia patients treated in Russian acute lymphoblastic leukemia study.

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Background: *IKZF1* gene deletions (del) are associated with poor outcomes in adults with B-cell ALL, and in combination with minimal residual disease (MRD) monitoring may efficiently predict relapse.

Aims: To evaluate MRD kinetics and prognostic impact of *IKZF1* mutation status in patients (pts) with *de novo* Ph-negative (Ph−) and Ph-positive (Ph+) B-cell ALL.

Patients and methods: The study included 67 pts with newly diagnosed B-cell ALL, 18 were Ph+ and 49 Ph−. Pts with Ph+ ALL (median age 31,5, range 17-68; m:f=9:9) were enrolled in Ph+RALL-2012 since Feb 2010 till Aug 2017. Pts with Ph− ALL (median age 29, range 17-56; m:f=19:30) were enrolled in RALL-2009, RALL-2016 since Aug 2009 till Aug 2017. Intragenic del of *IKZF1* were detected by breakpoint-specific fluorescent multiplex PCR using bone marrow samples. MRD analysis was assessed in 21 cases at day +70 (postinduction time) and +133 (after 3 consolidation) for pts with Ph− ALL by 6 color flow cytometry (sensitivity 0.01%). MRD the *BCR-ABL1* transcript for pts with Ph+ ALL was evaluated at day +70 by RT-

PCR with 10^{-4} sensitivity. MRD-negative status was stated if bcr/abl chimeric transcript was 0.01%.

Results: The *IKZF1* gene del were detected in 9 (18%) of 49 pts with Ph− ALL. The median follow-up time in 49 pts was 18,1 months (range: 1.5-93.4 month). Nine pts died (18%): 2 at induction from infections, 5 after relapse or disease progression. Three pts had refractory disease, 1 of them with *IKZF1* del. Other 44 (89%) pts had achieved complete remission. Among 21 pts with MRD data 6 had *IKZF1* del. They had MRD positivity (+) in 100% cases at +70 day, while pts without *IKZF1* del had MRD+ in 5 (33%) cases of 15 (p=0,0011). Among patients without *IKZF1* del at +133 day, as well as at +70 day, the incidence of MRD+ and its level was lower than in patients with *IKZF1* del (p=0,0021). In pts with *IKZF1* del, MRD level decreased at +133 day, however only one pt achieved MRD negativity (-) (p=0,016). In a group without mutations among 5 pts with MRD+ at +133 day, 1 pt had achieved MRD- and 3 pts had the decrease of MRD (p=0,063) (fig.1). Overall survival (OS) in Ph−ALL pts with *IKZF1* del and without was 100 % and 75,8 % (p=0,149), cumulative incidence of relapse (CIR) 20 and 26,6%, respectively (p=0,831).

The IKZF1 gene del were detected in 10 (56%) of 18 pts with Ph+ ALL, which is more frequent compared to Ph−ALL (p=0,0074). The median follow-up time in 18 pts was 21,2 months (range: 3.53-91.77 month). Seven pts died (38%): 5 after relapse or progression of the disease and 2 pts as a result of complications of bone marrow transplantation. Eleven pts are alive. Pts with IKZF1 mutations were MRD+ in 2 cases (13%) and MRD- in 5 cases (33%). Pts without IKZF1 mutations were MRD+ in 4 cases (26%) and MRD- in 4 cases (26%), p=0,398. OS for pts with Ph+ALL with IKZF1 del and without was 60% and 42,9% (p=0,410), CIR 25 and 60% (p=0,381), respectively.

Conclusion: *IKZF1* del in our study did not seem to be prognostically valuable for both Ph+ ALL and Ph−ALL. The *IKZF1* gene del found before treatment are associated with MRD persistency during therapy in patients Ph−ALL and have no impact on MRD status in patients Ph+ ALL. Regarding Ph−ALL: though the group of patients is small, we can suggest that *IKZF1* del did not appear to influence the survival due to the difference of chemotherapy principal in RALL – 2009 – non-intensive but not-interruptive therapy.

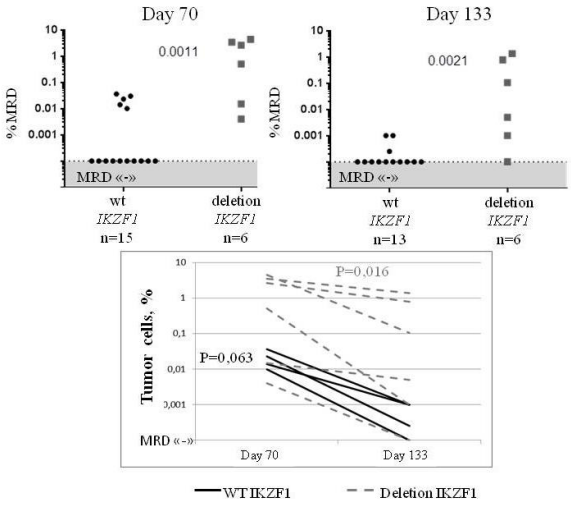

Fig. 1 MRD kinetics of patients with BCR-ABL1-neg at day 70 and 133

P042

Characterization of RAS (K-RAS, N-RAS), JAK2 and CRLF2 genes mutations in Ph-negative B-cell acute lymphoblastic leukemia patients treated in RALL-2009/2016 studies.

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Abstract: Approximately 25% of adults with B-ALL have Philadelphia chromosome (Ph)-like properties. Within the Ph-like ALL cohort, 61% had *CRLF2* overexpression. Next-generation sequencing of the *CRLF2* positive group identified mutations in the *JAK-STAT* and *Ras* pathways in 85% of pts, and in 20% - a *CRLF2* mutations [*1*].

Aim: To evaluate the frequency and impact of *K-RAS, N-RAS, JAK2* and *CRLF2* genes mutations on outcome (CR rate, refractoriness and early death rate) in adult pts with Ph-neg B-ALL treated with RALL-2009/2016 protocols.

Patients and methods: The study included 44 adult pts (median age 33, (17-56); m:f=18:26) with newly diagnosed Ph-neg B-ALL since March 2008 till Oct 2017. 23 pts were enrolled in Russian study RALL2009 [*NCT01193933*], 17 pts were treated by RALL2016. 5 pts treated by other protocols were included only for mutation status evaluation.

The diagnosis was B-I in 10 pts (22,7%), B-II in 22 pts (50%), B-III in 10 pts (22,7%) and B-IV in 2 pts (4,6%). CNS disease was diagnosed in 11,9% (5 of 42 pts), WBC> 30*109/l=28,6% (12 of 42 pts), rearrangement of the *MLL* in 12,5% (5 of 40 pts). DNA for PCR was purified from frozen bone marrow samples by phenol-chlorophorm extraction. The sample preparation for sequencing included: amplification of the exons of the *K-RAS, N-RAS, JAK2*, and *CRLF2* genes and purification of PCR fragments on columns Wizard (Promega). Sequencing was carried out with a set of reagents ABI PRISM®BigDyeTM Terminator v.3.1, followed by analysis of the reaction products on an automatic DNA sequencer ABI PRISM 3100Avant.

Results: Using Sanger sequencing, the nucleotide sequences of the 2, 3 and 4 exons of the *N-RAS* and *K-RAS* genes; 14, 16 and 20 exons of the *JAK2* gene; 2 and 6 exons of the *CRLF2* gene were determined. Incidence of activating mutations in the *N-RAS, K-RAS, JAK2* and *CRLF2* genes, regardless of the codon involved and the gene, was 11 cases out of 44 (25.0%). Two mutations were found in 2 pts: 1 pt in the genes *N-RAS* (Q61H) and *JAK2* (D873N), another pt in the *K-RAS* (G13D) and *JAK2* (L892V) genes.

Mutations in the gene *N-RAS* were found mostly often – in 5 out of 11 (45.5%), and 4 different variants were found (G13D - in 2 pts, G12A, G12C and Q61H). In the *JAK2* gene, activating mutations were detected in exons 16 and 20 (R683G, D873N and L892V). Mutations in the *CRLF2* gene weren't found, only the known polymorphic variant V244M was detected in 7 pts in the heterozygous state. 1 pt was examined both before the treatment and in the relapse of the disease. At the time of the diagnosis the mutations weren't founded. In the recurrence of the disease, the clonal mutation A146T in the *N-RAS* gene was detected. 39 pts were enrolled in the study on the evaluation of the effectiveness of therapy, 8 of them with mutations. Clinical data were available in 7 of 8 pts with mutations and 29 of 31 pts without mutations. At this stage of the study, we have not

found a correlation between the presence of mutations and demographic and laboratory data (sex, age, leukocytosis, LDH activity, CNS disease). The outcome on both groups (mutated & non-mutated) was follows: CR – 85,7% vs 96,6%, primary refractory – 14,3% vs 3,4%, early death – 28,6% vs 3,4%, respectively.

Conclusions: In the sample of pts with Ph-negative B-ALL, mutations in the *N-RAS* gene (6 of 12) prevailed over mutations in the *K-RAS* gene (3 of 12) and *JAK2* (3 of 12).

P043

MiRNA-Seq Analysis in Childhood Acute Lymphoblastic Leukemia Reveals miR-5096 Association with Increased Soluble HLA-G Levels

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Background: Acute lymphoblastic leukemia (ALL) is the most common cancer in children, which can affect B- or T-cell hematopoietic lineage. Despite advance in therapeutic regimens and increased overall survival rates, ALL is still considered an aggressive disorder with poor outcome for patients who relapse. Genetic and immunological factors such as microRNA expression (miRNA) and soluble HLA-G (sHLA-G) levels may influence the severity of cancer and immunosurveillance process.

Aims: This study aimed to evaluate miRNA expression profile in childhood ALL according to their sHLA-G levels (low versus high producers).

Methods: Patients with ALL (n=15) were referred at IMIP Hospital, Recife, Northeastern Brazil, and bone marrow was collected and used for total RNA extraction (Trizol). miRNA expression assays were performed using TruSeq Small RNA Kit and HiSeq 2500 platform (Illumina) for sequencing. Data analysis was performed using FastQC, Cutadapt, miRDeep2, edgeR (False Discovery Rate ≤ 0.05), miRWalk 2.0, and RNAhybrid 2.2. Differential expression (DE) analysis was conducted based on sHLA-G levels determined by ELISA (EXBIO), considering low producers (250 U/mL; median age= 8.5y; 6M) patients.

Results: Bioinformatic analysis revealed 10 miRNAs (miR-1248, miR-205-5p, miR-3196, miR-4485-3p, miR-4488, miR-4516, miR-451a, miR-4532, miR-486-5p, and miR-5096) upregulated in high versus low producers group. miR-5096 was the most prominent miRNA for targeting *HLA-G* 3'UTR, although others also presented binding sites for *HLA-G*. RNAhybrid analysis showed 19 putative binding sites for miR-5096 in *HLA-G* messenger RNA, with all interactions presenting minimum free energy below -20 kcal/mol. Of these, three binding sites mapped after 14 base pair 3'UTR polymorphism, and two overlapped with +3003 CT, +3010 CG, and +3035 CT 3'UTR polymorphisms.

Conclusion: Here we suggest another mechanism for miR-5096 in regulation of *HLA-G* gene, since miR-5096 is upregulated in ALL patients high sHLA-G levels. We propose a mechanism of translation induction for miR-5096 in *HLA-G* mRNA. These findings must be replicated in more samples for confirmation.

ONLINE PUBLICATION ONLY

PB044

The outcome T-Acute lymphoblastic leukaemia(T-ALL) harbouring PICALM-MLLT10 fusion in adult patients following matched unrelated donor allogeneic bone marrow transplant after induction chemotherapy.

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Background: The t(10;11) translocation is a well described recurrent abnormality, occurring in about 10% of T-ALL cases, represent PICALM-MLLT10 fusion [CALM-AF10]; t(10;11)(p13;q14) rearrangement remains a poor prognosis indicator in T-ALL with negative CD3/T cell receptor.

Aim: The retrospective data analysis was performed to identify the outcome for patients with T-ALL and PICALM-MLLT10 fusion with lack of expression of surface CD3 following allogeneic bone marrow transplant after induction chemotherapy on UKALL 14 protocol.

Methods: The data was collected from the Haemto-oncology diagnostic service laboratory to identify all patients with T-ALL with PICALM-MLLT10 fusion from five Hospitals over the East of England region from 2005 till 2018. We identified 2 cases of T-ALL with CALM-MLLT10 fusion with lack of surface CD3/T cell receptor expression with early T cell phenotype. Flow cytometric evaluation of the bone marrow aspirate showed lymphoblast expressing the T lymphoid antigens cytoplasmic CD3, strong CD7, TdT and CD99 in addition to CD10, CD13, CD33, variable CD34 and HLA-DR. They did not express CD1a, CD2, surface CD3, CD4, CD8 or any other myeloid associated antigens consistent with Precursor T Cell Lymphoblastic Leukaemia (pro-T phenotype). Karyotype analysis showed a balanced reciprocal translocation between the short arm of chromosome 10 and the long arm of chromosome 11, consistent with t(10;11) translocation, which represents PICALM-MLLT10 [CALM-AF10]; t(10;11)(p13;q14) rearrangement. T cell rearrangement clonality studies showed the presence of clonal TRD rearrangements, with absent TRB rearrangement and lack of CD3 expression consistent with a TCR negative T-ALL. Both patients were negative for the BCR-ABL1 rearrangement between chromosomes 9 and 22 t(9;22), and negative for rearrangement of MLL (11q23), STIL/TAL1 (1p32), TLX3 (5q35), TRA/TRD (14q11), TRB (7q34) and TRG (7p14). In addition to the above cytogenetic abnormality, in the second patient, FISH together with karyotype identified deletion of TP53 at 17p13, consistent with the presence of an isochromosome of the long arm of chromosome 17. Lymphoblasts showed three complete copies of RARA, indicating partial or complete trisomy of chromosome 17, consistent with the presence of the isochromosome of the long arm of chromosome 17. The next generation sequencing using the Ion AmpliSeq™ Cancer Hotspot Panel v2 identified mutation in exon 5 of the TP53 locus. The mutation was NM_00546 TP53 c.473G>A giving rise to

the p.Arg158His amino acid change. This mutation has been observed in a patient with T-ALL. (COSMIC COSM10690)

Results: The patients were treated according to the UKALL 14 protocol and achieved molecular remission following phase 2 induction. They underwent unrelated allogeneic bone marrow transplant. The first patient has remained in complete remission for 37 months following bone marrow transplant. However, the second patient who had TP53 mutation along with PICALM-MLLT10 fusion relapsed within 3 months of having allogeneic bone marrow transplant and had progressive disease on further nelarabine based salvage chemotherapy.

Conclusion: There is a role for consolidation allogeneic bone marrow transplant for patient with T-ALL with PICALM- MLLT10 fusion to mitigate the poor risk, however the heterogeneous nature of the genetic profile of T-ALL with PICALM- MLLT10 fusion poses a challenge to offer the optimum treatment to have a long-term remission.

PB045

Controversial diagnosis of intrachromosomal amplification of chromosome 21 (iAMP21) in childhood acute lymphoblastic leukemia

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INTRODUCTION: B lymphoblastic leukemia with intrachromosomal amplification of chromosome 21 (iAMP21) is a provisional new entity recognized and incorporated into the WHO classification in 2016, that can occur in approximately 2-5% of pediatric patients with B-cell precursor ALL (BCP-ALL). This abnormality is defined as three or more extra copies of *RUNX1* on a single abnormal chromosome 21 and a total of five or more *RUNX1* signals per cell.

A clinical and molecular cytogenetic analysis of a child with BCP-ALL with a controversial diagnosis of iAMP21 abnormality is presented.

CASE REPORT: An 8-year-old boy arrives to our hospital for the study of mediastinal mass. A physical examination revealed laterocervical, thoracic and abdominal adenopathies significantly enlarged and splenomegaly. No signs of cardiovascular and respiratory abnormalities. The hemoglobin level was 11,4 g/dL, white blood cell count was $2{,}53x10^9$/L, platelet count was $145x10^9$/L and lactate dehydrogenase was 489 U/L. Immunophenotyping by flow cytometry showed a 88% of immature B-cells with a pattern of BCP-ALL: $CD19^+$, $CD22^+$, $CyCD79a^+$, $NuTdT^+$, $CD10^+$, $CD58^+$, $CD81^+$, expression of myeloid-related antigen CD33, and absence of expression of CD45, CD34 Cd20 and CyIgμ.

The patient's bone marrow sample was sent for cytogenetic analysis, at the time of diagnosis, and after 24-hour culture technique the karyotype result in 50,XY,+X,+21,+21,i(21)(q10)x3,+mar[20]. Molecular analysis did not detect BCR/ABL transcript. Fluorescence in situ

hybridization (FISH) was performed on interphase cells using the commercially available LSI ETV6/RUNX1 dual color dual fusion translocation probe and LSI MLL dual color break apart rearrangement probe, according to the manufacturer's instructions. Three-hundred interphase cells were scored for each probe. Interphase FISH analysis showed normal results for *MLL,* no *ETV6/RUNX1* fusion signals and 7 signals for the *RUNX1* probe in 52% of the analyzed nuclei. Metaphase FISH analysis revealed also 7 signals for *RUNX1*, one corresponding to the normal chromosome 21 and the remaining six distributed in two per each isochromosome 21.

DISCUSSION: This case arise us to the question if this patient must be classified as iAMP21 or not, as he present more than 5 copies of *RUNX1* in interphase nuclei, following the description of the alteration, but metaphase FISH analysis reveals that in a single abnormal chromosome 21 there is only two signals, contradicting this part of the definition as there is not three signals or more. We consider necessary a review of the current definition to be able to classify correctly these cases.

PB046

Coexistence of CRLF2 translocation and iAMP21 in childhood acute lymphoblastic leukemia

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Introduction: B lymphoblastic leukemia with BCR-ABL-like is a new entity incorporated into the WHO classification in 2016, which includes translocations involving tyrosine kinases and cytokine receptors. The 50% of this group of patients show cytokine receptor-like factor rearrangements (CRLF2r). It had been postulated that CRLF2r are not driver abnormalities in the process of leukemogenesis. Instead, are secondary lesions and require concomitant mutations to become functionally important and activate JAK/STAT and PI3K/mTOR pathways. These *CRLF2* rearrangements tended to be acquired in B-cell precursors acute lymphoblastic leukemia (BCP-ALL) patients with constitutional trisomy 21 (Down Syndrome), with high hyperdiploidy or with intrachromosomal amplification of chromosome 21 (iAMP21).

A clinical and molecular cytogenetic analysis of a child with BCP-ALL with the coexistence of iAMP21 and *CRLF2* rearrangement is presented.

Case report: A 9-year-old boy with hypoactivity and asthenia of 10 days of evolution was admitted at the Oncohematology Pediatric Service of Hospital Universitari Vall d'Hebron after the detection of pancytopenia. A physical examination revealed that the patient did not present laterocervical, axillar or inguinal adenopathies. No signs of cardiovascular and respiratory abnormalities were found. The hemoglobin level was 5,1 g/dL, white blood cell count was $3{,}3 \times 10^9$/L, platelet count was 20×10^9/L and lactate dehydrogenase was 1717 U/L. Immunophenotyping by flow cytometry

showed a 56% of immature B-cells with a pattern of BCP-ALL: $CD45^{dim}$, $CD19^{+}$, $CD22^{+}$, $CyCD79a^{+}$, $NuTdT^{+}$, $CD34^{+}$,$CD10^{+}$, $CD58^{+}$, $CD123^{+}$, $CD9^{+}$,absence of CyIgμ and other myeloid-related antigen (MPO, CD33, CD13, CD15).

The patient's bone marrow sample was sent for cytogenetic analysis, at the time of diagnosis, and after 24-hour culture technique the karyotype result in 48,XY,-13,+21,iAMP(21),+mar[9]/ 46,XY[11]. Molecular analysis did not detect BCR/ABL transcript. Fluorescence in situ hybridization (FISH) was performed on interphase cells using the commercially available LSI ETV6/RUNX1 dual color dual fusion translocation probe as well as LSI MLL and LSI CRLF2 break apart probe. Three-hundred interphase cells were scored for each probe, revealing 5 signals for *RUNX1* and rearrangements of *CRLF2* in 70% of the analysed nuclei. Metaphase FISH confirmed the existence of iAMP21.

Conclusion: We found in this case report the coexistence of the *CRLF2* translocation and iAMP21 in a BCP-ALL. In the literature, it has been described that the 30% the patients with iAMP21 present *CRLF2* rearrangements, specially the *CFRL2-P2RY8*. Most protocols describe the association of iAMP21 with a poor prognosis; therefore it could be interesting detect *CRLF2* translocations in these patients as it could be used for target therapy in case of relapse.

PB047

UNUSUAL CO-EXISTENCE OF NOTCH1 MUTATION AND MLL REARRANGEMENT IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: A CASE REPORT

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Background: T-cell Acute Lymphoblastic Leukemia (T-ALL) is known to be a clinical and biological heterogeneous disease, with a poorer outcome than B-cell precursor ALL (BCP-ALL). Mixed Lineage Leukemia (*MLL)* gene rearrangements are found in all lineages, but their frequency in T-ALL is very low.

Aims: To report clinical and molecular features of a rare pediatric T-ALL case with t(11;19)(q23,p13.3) and *MLL-ENL* rearrangement.

Case: A 7-year-old girl presented with fever, anemic syndrome, splenomegaly, mediastinal mass and hyperleukocytosis ($196x10^{9}$/L, 91% blasts). Central nervous system infiltration was discarded. By flow cytometry, leukemic blasts expressed CD34, TDT, CD99, CD3cyt, CD3surface dim, CD2 (partially), CD7, CD5 dim, CD8, CD10 and were negative for CD4, CD1a, TCRαβ-, TCRγδ-, NG2, TSLPR and all myeloid antigens tested. The patient was diagnosed with T-II stage according to AIEOP-BFM 2016 consensus guidelines. No metaphases

were evaluable in conventional karyotyping. Fluorescence In Situ Hybridization revealed the presence of *MLL* rearrangement in 67% of nuclei and *MLL-ENL* fusion was confirmed by quantitative RT-PCR (qRT-PCR). We assessed immunoglobulins (IG) and T-cell receptor (TCR) clonality and observed no IGH rearrangement and two clonal peaks in the TCR*γ* assay (Vγfl-Jγ1.1/2.1 and Vγfl-Jγ1.3/2.3). By multiplex ligation-dependent probe amplification (SALSA kit P383 T-ALL) we observed an amplification of *MYB* gene. By qRT-PCR, *HOXA3, HOXA9, HOXA10, MEIS10*, and *FLT3* were expressed, but not at very high levels, and *MEF2C* and *HDAC4* were not expressed. *FLT3* internal tandem mutations were discarded by fluorescent PCR. Interestingly, we found a *NOTCH1* mutation in heterodimerization domain (HD) (c.4723G>C, p.Val1575Leu); no mutations were observed in *FBXW7*, *PTEN*, *NRAS* or *KRAS* genes. The patient started therapy according to intermediate risk SEHOP-PETHEMA 2013 protocol. She presented a good prednisone response and minimal residual disease at day 15 of induction therapy as assessed by flow cytometry was 4.7%. A complete remission was achieved after first induction, with minimal residual disease levels of 0.1% and negative at month 3.

Discussion: We describe a pediatric patient with T-ALL and *MLL-ENL* rearrangement and presence of a *NOTCH1* mutation. To date, around 70 T-ALL MLL+ have been reported, including nearly 50 pediatric cases, with a mean age of 12 years, and some anecdotal infant patients. The partners most frequently found are *ENL* (*AFFT1*) and *AF6* (*MLLT4*). Interestingly, only 4 cases of *NOTCH1* mutations have been previously reported in T-ALL *MLL*-rearranged cases and, like in our case, the mutation was localized in the *NOTCH1* HD domain and the partner gene was *ENL*. *MLL* rearrangements are considered as primary events harboring very few secondary genetic abnormalities. On the other hand, *NOTCH1* mutations have been described both as primary and secondary events in T-ALL.

Conclusions: We report a pediatric patient presenting typical clinical, phenotypical and molecular features of T-ALL, but harboring the rare coexistence of *NOTCH1* mutations and *MLL-ENL* rearrangement.

POSTER PRESENTATIONS

P048

Asparaginase-triggered Hypertriglyceridemia: An Analysis on Germline Gene Variation in Adult Acute Lymphoblastic Leukemia Patients

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Background: Asparaginase (ASP) is considered an essential part of successful treatment for acute lymphoblastic leukemia (ALL) (Inaba et al., 2013). Aside from the aimed antileukemic properties, asparaginase exposes patients to multiple adverse effects including severe hypertriglyceridemia (HTG) (Patel et al., 2017). The molecular background of ASP-induced HTG and its regulators are not known.

Aims: We aimed to investigate constitutional genetic factors as biomarkers for HTG following ASP therapy.

Methods: Twenty-three ALL patients were included in the study (informed consent patients with exome sequencing data, recorded ASP doses, and lipid measurements). Maximum and minimum fasting plasma triglyceride (fP-TG) levels of the study subjects were compared. Patients were divided into three separate study groups according to the difference between maximum and minimum fP-TG value (ΔfP-TG). In group one (N=9) ΔfP-TG was 0-5 mmol/L, in group two (N=6) 5-15 mmol/L and in group three (N=8) more than 15 mmol/L. Clinical characteristics of the patients were retrieved from the Finnish Hematological Registry (FHR) and electronic health records of the hospital. Whole exome sequencing (WES) was conducted from DNA prepared from skin biopsies. SNVs and indels in the exome sequencing data were analyzed using an in-house developed analysis tool. Shared homozygous variants within the patient groups 1 and 3 were identified in the WES data and genes associated to "fatty liver" "fatty liver disease" "hypertriglyceridemia" "lipid metabolism" "steatohepatitis" "triglyceride levels" were prioritized with two phenotype-associated gene prioritization programs.

Results: There was no difference in previous illnesses, cumulative glucocorticoid dose during ALL chemotherapy, BMI, alcohol use, or the prevalence of certain adverse effects (hepatotoxicity, pancreatitis, hyperglycemia or thrombosis) between the study groups. One patient (from group 3) had diabetes before ALL diagnosis. In this cohort, steatohepatosis was more common in group 3 patients. However, the incidence of liver toxicity at any time point after the beginning of treatment was similar in all the study groups. Variants in eleven candidate genes *ATP10B, GABRG3, MYO3A, GBP4, GBP3, KRT19, KRT75, ADAMTSL4, COL6A6, SLC16A2* and *THEM241* were enriched in patients with ASP-induced HTG.

Conclusion: A marked between-patient variation was observed in ASP-induced

hypertriglyceridemia, with extreme increases seen in 35 % of patients. We also found evidence for germline predisposition as putative biomarkers for ASP-induced HTG. The data should be prospectively validated in an independent patient cohort and the biochemical mechanisms studied in detail. If validated, these variants could be clinically useful in selecting patients for personalized preventive measures (e.g. novel TG lowering drugs) and/or dose modifications to ensure safe and effective use of ASP in ALL.

P049

Germline Mutation Analysis in Adult Acute Lymphoblastic Leukemia Patients

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Background: Personalized medicine argues for a thorough analysis of factors affecting the disease. Active collection of family history is important but not a definitive source of information for inherited predisposition to malignancy (Furutani and Shimamura, 2017). Therefore, studying the germline exome in conjunction with somatic exome sequencing is justifiable. Literature is also scarce regarding the familial propensity for adult adult lymphoblastic leukemia (ALL) (Kratz et al., 2016)

Aims: We analyzed germline exome sequencing data from 53 adult patients, who gave their informed consent, diagnosed with acute lymphoblastic leukemia (ALL) during 2010-2017 in Finland in order to identify possible predisposing germline mutations.

Methods: Patients' germline DNA was extracted from a skin biopsy taken in conjunction with bone marrow sampling and exome sequencing was conducted. A panel of sixty-nine genes were chosen for the first-line analysis. The genes were selected to the panel if previously associated with germline predisposition to ALL, often somatically mutated in ALL, or involved in DNA repair. We categorized variants as possibly pathogenic if they were frameshift or nonsense variants with an allele frequency (AF) of <0.01 in The Genome Aggregation Database (Broad Institute; gnomAD Finns). Missense variants were considered remarkable if AF <0.01 in gnomAD Finns and at least two out of three algorithms (SIFT, Polyphen-2, PROVEAN) classified the variant as pathogenic. Clinical characteristics of the patients were retrieved from the Finnish Hematological Registry (FHR).

Results: Our analysis revealed 20 possibly pathogenic variants in 16 genes in 20/53 (38%) patients. The distribution of diagnoses among patients with variants were comprised of 13 pre-B-ALLs (including 4 Ph-like ALLs), two Ph+ ALLs, and five T-ALLs (including two ETP-ALLs). One variant was shared by three and two variants by two unrelated patients. Five patients had two possibly pathogenic mutations. Patients carrying possibly pathogenic variants also included two individuals with a history of an antecedent malignancy (Mb Hodgkin and breast cancer, respectively). Among 20 patients carrying variants, four had first degree relatives who had suffered from cancer.

Conclusion: Our results emphasize that potential germline predisposition to ALL may also be identified in older patients and without family history of malignancies. Germline variants may also contribute to the risk of secondary or second malignancy. Despite the possible anxiety that acknowledging inheritable factors may cause in patients, families, and caretakers, we encourage clinicians to integrate timely and astutely interpreted germline data into patient care with the support of a comprehensive genetic counseling team.

ONLINE PUBLICATION ONLY

PB050

Treatment of relapse pre-B acute lymphoblastic leukemia with Blinatumomab in a Jehova´s Witness patient.

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BACKGROUND: Jehovah´s Witnesses patients refuse to accept blood products transfusions due to their religious beliefs. The treatment of acute lymphoblastic leukemia (ALL) in this group of patients is challenging as most therapeutic regimes used to treat this disease are potencially myelosuppressive. Blinatumomab is a bispecific T-cell engager antibody with two arms, one binding to CD19 and the other binding to CD3, resulting in T-cell-mediated serial lysis of B cells. Blinatumomab has been approved in Europe for the treatment of refractory or relapse Philadelphia chromosome-negative acute lymphoblastic leukemia patients. Hematologic toxicities in ALL patients receiving Blinatumomab are very frequent (>1/10). CASE REPORT: We report a case of a 42 years old Jehovah´s Witness female patient with first relapse ALL who was treated with Blinatumomab in our centre. The patient was diagnosed with pre-B ALL seven years before in other institution and was succesfully treated with standard induction and consolidation chemotherapy (PETHEMA LAL-AR 03). Although at the time of the first diagnosis the patient was already a member of the Jehovah´s Witnesses group, she accepted blood transfusions and she could complete all the chemotherapy scheme. However, at the time of the relapse, she firmly declare her intention of not receiving any blood products transfusions, even in a life-threating situation, so after discussing the therapeutic options with the patient and taking into consideration potencial risks and benefits, we decided to treat her ALL with Blinatumomab. No cytogenetic or molecular abnormalities were detected in the leukemia cells and the patient had not any comorbidities.The patient received two cycles of Blinatumomab at the standard dose achieving complete response after the second one. Adverse events during the first Blinatumomab course were grade 3 hepatic toxicity, grade 3 anemia (Hemoglobin 69g/L), grade 1 thrombocytopenia (platelets 98x10e9/L) and febrile neutropenia. The anemia was treated with subcutaneous Epoetin Beta and oral folic acid and ferrous sulfate.

The second cycle was well tolerated without any complications. Unfortunately she suffered an extramedullary relapse (cutaneous involvement) before a third cycle could be administered. Then the patient received conventional chemotherapy dying due to sepsis after four months from the diagnosis of the fisrt relapse.

CONCLUSION: To the best of our knowledge, this is the firstcase report of Blinatumomab treatment in a Jehova´s Witness patient. Blinatumomab appers to be safe in this setting.

POSTER PRESENTATIONS

P051

Improved outcome for Philadelphia-positive acute lymphoblastic leukemia in Sweden

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Background: Since the introduction of tyrosine kinase inhibitors (TKI) for Philadelphia-positive (Ph+) acute lymphoblastic leukemia (ALL), outcome has improved and Ph+ disease is no longer associated with impaired prognosis [1]. However, in the cited study only 8% of the patients were Ph+ indicating selection bias.

Aims: We wanted to study Ph+ ALL in a large, population based cohort from Sweden and to assess the Ph+ frequency, patient characteristics and outcome in routine care.

Methods: From the Swedish ALL-registry (as previously described), all patients ≥18 years from 1997-01-01 to 2015-12-31 were identified[2]. Vital status was followed until the last day of April 2017. A new registry database was introduced 2007 with additional variables, including Ph+ disease. Prospectively registered cytogenetic- and molecular results were then validated and for the period 1997-2006 retrospectively collected from the six Swedish genetic laboratories providing the analysis. Ph+ disease was confirmed with conventional cytogenetics and/or FISH and/or PCR. To be classified as Ph-negative either a normal karyotype with ≥20 metaphases, a normal FISH or PCR (for minor and major transcript) or another clonal aberration was required.

Trends in proportions were compared with the Chi2- test. Overall survival (OS) was estimated by the Kaplan Meier method and distribution compared with the log-rank test using IBM SPSS package (v 23). Confidence intervals (CI) of 95% were obtained.

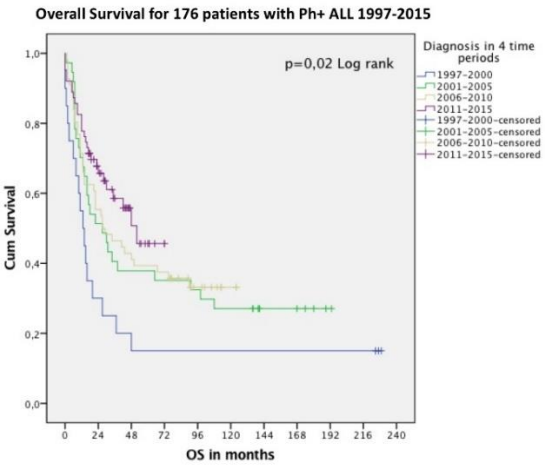
Results: From the registry 934 patients (B-, T-, Burkitt and ALL UNS) were included and 176 (19%) patients were confirmed with Ph+ positive ALL between 1997 and 2015. There was a trend of an increasing Ph+ frequency within the B- and T-ALL cohort throughout the years (p= 0,018). The frequency of Ph+ ALL was 23% of all patients, 27% of tested patients and 35% of tested B-ALL during the latter time period (2006-2015, comprising 50% of the total patient cohort).

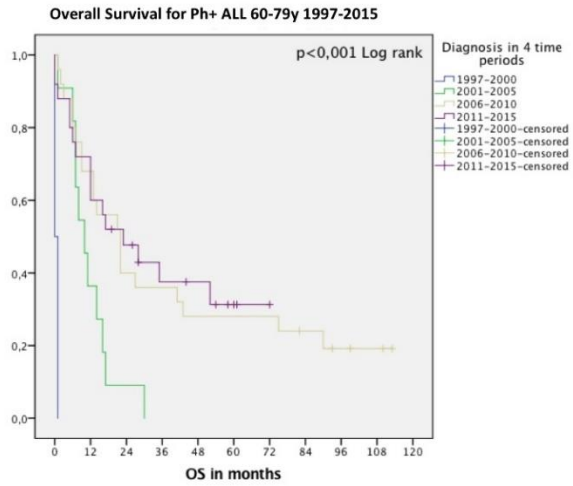
Patient characteristics are summarized in Table 1. Remission inducing therapy was intended in 169 (96%) patients. A date of complete remission (CR) was reported in 81% (in 78% within 90 days from date of diagnosis), no remission achieved in 10% and in the remaining nine percent data was missing. Details on treatment intensity are not presented. The use of imatinib was recommended in the Swedish national guidelines of 2007 but introduced gradually in routine care the years before and combined with age-adjusted chemotherapy protocols changing over the study period. Allogeneic hematopoietic stem cell transplant (hSCT) was recommended in CR1 for

all patients up to the age of 65 fit for the procedure with a suitable donor.

Five-year OS for the Ph+ cohort was 39(CI 32 – 47)% and improved over time (Figure 1A). A marked increase in OS was seen in patients 60-79 years old treated in the TKI-era (2006-2015), compared to the previous period when the use of TKI was sparse (1997-2005) (Figure 1B).

Patient characteristics at diagnosis		
Philadelphia positive ALL, n	176	
Old/New register, n (%)	66/110	(37/63)
Male/Female, n (%)	92/84	(52/48)
Age in years, median (range)	53	(19-87)
B-ALL, n (%)	163	(93)
T-ALL, n (%)	1	(1)
ALL UNS, n (%)	12	(7)
Variables only reported in the new register (110 patients)		
WBC at diagnosis, 109/L , median (range)	29	(0,7-447)
WBC 30 109/L, n (%)	55	(31)
CNS leukemia, no CNS leukemia, missing, n (%)	5,83,22	(5,75,20)





Summary and conclusion: The estimated frequency of Ph+ disease in a population based cohort is approximately 35% of B-ALL with modern diagnistic routines. With the use of TKI in upfront therapy prognosis has improved. This was most evident in the patients not eligible for hSCT, but whether hSCT can be avoided in younger age groups is still an open question.

P052

Low intensity chemotherapy combined with tyrosine kinase inhibitors in elderly patients with Ph+ ALL in the real-life setting. First results of the EWALL-OBS study.

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Background: The EWALL group (European Working group for Adult ALL) has developed the concept of low intensity

chemotherapy combined with tyrosine kinase inhibitors (TKIs) for the treatment of Philadelphia positive Acute Lymphoblastic Leukemia (Ph+ ALL) in patient aged 55y or above. Two consecutive studies were conducted (EWALL-PH-01 with dasatinib and EWALL-PH-02 with nilotinib), showing high CR rates and long-term survival for more than 1/3 of the patient. The EWALL-PH backbone (TKIs and low intensity chemotherapy) is currently the first in choice schedule for aged patients diagnosed with Ph+ ALL in France.

Aims: We decided to initiate the EWALL observatory (EWALL-OBS) in order to collect data from aged patients with Ph+ ALL treated outside clinical trials in a real-life setting.

Methods: Patients aged 55y or above were eligible for registration if they were diagnosed with Ph+ ALL and treated following the EWALL-PH backbone. Patients received four weekly cycles of vincristine and dexamethasone (VCR-DEX) in combination with TKIs during induction followed by 6 cycles of consolidation (ID-ARAC / MTX plus TKIs) and a maintenance (VCR-DEX x7 plus TKIs during 18 months) followed by TKIs alone until progression or death.

Result: We present the 39 first patients registered in the observatory. Median age was 65 years (range 60-83 years). The TKIs used in combination with the EWALL-PH backbone were imatinib (n=24), Dasatinib (n=9), nilotinib (n=5) and ponatinib (n=1). Thirty-seven patients (94.8%) achieved CHR. Sixteen patients (43.2%) achieved a 3-log reduction in BCR-ABL1 transcript levels after the end of induction (MRD1). Ten patients underwent allogeneic hematopoietic stem cell transplantation after non myeloablative conditioning. Twelve patients died during the study (30.7%) (progressive disease (n=7), infections(n=2), organ failure (n=2), allogeneic HSTC (n=1)). Sixteen patients relapsed (41%) after a median CHR duration of 10.8 months (range 0.5-68.6). With a median follow-up of 16.7 months, median EFS was 42.5 months (95%CI: 23.8-60.4). Median OS was not reached and 55% of the patients were alive at 3 years (Figure 1). A trend was observed for a better survival in patient treated with second and third generation TKIs as compared to imatinib.

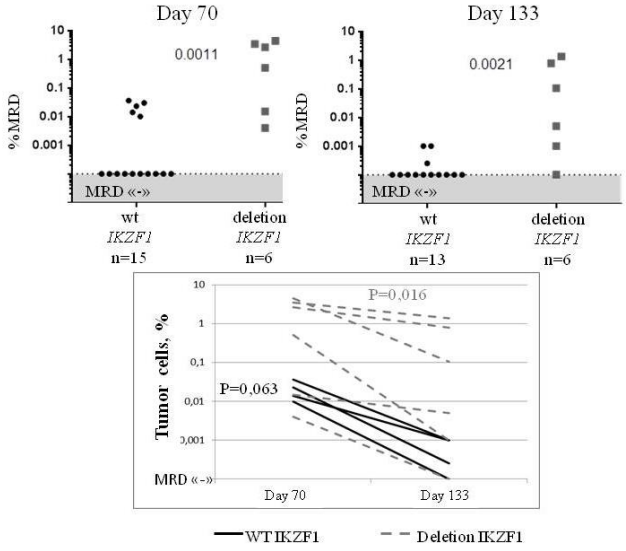

Fig. 1 MRD kinetics of patients with BCR-ABL1-neg at day 70 and 133

Conclusion: The EWALL-OBS study confirms in a real-life setting the good outcomes of aged patients with Ph+ ALL treated with low-intensity chemotherapy and TKIs, in line with the results from the EWALL-PH-01 study. Updated results with more registered patients will be presented.

ONLINE PUBLICATION ONLY

PB053

Treatment outcomes of Philadelphia positive adult Acute Lymphoblastic Leukemia patients: A single institute experience

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Background and aim: The initial treatment of adult Philadelphia chromosome positive ALL (Ph+ ALL) has been dramatically changed by the introduction of Abl tyrosine kinase inhibitors (TKI). However, hematopoietic stem cell transplant (HSCT) is still the only curative therapy. We aimed to study the clinical characteristics, prognostic factors and therapeutic outcome of our adult Ph+ ALL patients.

Patients and method: This is a retrospective study done at NCI – Cairo, in the period between September 2011 and December 2014, the study included 51 adult Ph+ ALL patients representing 24.4% of adult patients diagnosed as B cell ALL. Patients were subjected to the standard work up for diagnosis including morphology and cytochemistry, immunophenotyping, cytogenetics and molecular genetics studies. Patients aged 40 years or less are treated with pediatric derived protocols while those above the age of 40 were treated with BFM modified protocol.

Results: Median age was 35 years (range 18 - 65), Male to female ratio was 2: 1 .Out of 51 patients, 7 cases didn't receive treatment at our center, while 3 cases received only prephase. Induction treatment and evaluation for outcome are available in 41 patients. TKI therapy was given in 21 patients (51.2%). Out of 41 patients received induction of remission, the complete remission rate was 80.5 %. With a median follow-up period of 5.4 months (range 0.1 - 68.7), the median DFS was 7.1 months and the median OS was 5.4 months. Among patients who has been subjected to allogeneic HSCT, the median DFS was not reached. DFS at one, two and three years were 85.7%, 71.4% and 57.1% respectively. P210 type was present in 60.8% of cases while P190 type was present in 35.3% and both types were present in 3.9% of cases with statistically significant prevalence of P210 type in males and P190 type in females (P= 0.003). Patients presented initially with platelet count > 100 x 109/L had a statistically significant better DFS (P= 0.001) and OS (P= 0.002) than patients presented with platelet count < 100 x 109/L. Patients presented with initial hemoglobin level ≥ 10 g/dl had statistically significant better OS (P= 0.04) than patients presented with hemoglobin level < 10 g/dl. Patients presented initially with ECOG PS = 1 had significantly better OS (P = 0.02) than those with PS > 1. The difference in DFS and OS among patients who underwent alloHSCT and patients who continued TKI based consolidation/maintenance treatment was significantly better than patients who received chemotherapy alone (median DFS; not reached and 16.5 vs 3.9 months and median OS; not reached and 21.5 vs 1.4 months respectively, (P < 0.001). Three years DFS rates were 57.1% for cases underwent alloHSCT, 48% for cases continued TKI based consolidation/ maintenance treatment and 0% for cases received chemotherapy alone while 3 years OS rates were 85.7%, 40.2% and 0% respectively. Multivariate analysis revealed that independent factors that significantly affect DFS and OS were initial platelet count (P= 0.035 and P= 0.049 respectively) and type of treatment (P= 0.003 and P= 0.001 respectively) while initial PS was found independently affecting OS (P= 0.027).

Conclusion: Treatment of adult Ph+ALL patients with TKI based chemotherapy and alloHSCT significantly improves DFS and OS. Type of treatment and initial platelet count are independent factors that significantly affect DFS and OS

POSTER PRESENTATION

P058

Minimal Residual Disease in Peripheral Blood during Early Induction Phase in Childhood B-Precursor Acute Lymphoblastic Leukemia

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Background: Minimal residual disease (MRD) in childhood acute lymphoblastic leukemia (ALL) during the early stages of therapy may have significant impact on outcome.

Aim: To study the prognostic significance of MRD by flow-cytometry in peripheral blood (PB) after 1 week of multiagent induction treatment in childhood B-precursor ALL.

Patients and Methods: This study included 359 newly diagnosed pediatric B-precursor ALL patients treated at the Children's Cancer Hospital-Egypt. Patients were risk stratified and received risk directed therapy according to SJCRH total study XV protocol. PB-MRD was measured by flow-cytometry on day 8 in 128 patients and on day 12 in 231 patients as they received 4 days of prednisone pre-phase.

Results: The 5-year event free survival (EFS) of all patients with day 8 or day 12 PB-MRD <0.01% (n=126), 0.01 - <0.1% (n=104) and ≥ 0.1% (n=129) were 89.3±3%, 83.1±3.8% and 70.4±4.2%; respectively (p=0.001), while the relapse free survival (RFS) were 92.9±2.7%, 87.3±3.5% and 78.7±4%; respectively (p=0.007). The median follow-up period of patients who are alive in complete remission was 54.8 months (range, 32-85 months). For provisional low risk (LR) patients, the 5-year EFS and RFS was 92.7±3% and 94.5±2.8%; respectively for PB-MRD <0.01% (n= 101), 83±4.3% and 87.5±3.9%; respectively for PB-MRD 0.01-<0.1% (n= 79), on the other hand it was 72.3±4.9% and 79.6±4.6%; respectively for PB-MRD ≥ 0.1% (n= 91), (EFS p= 0.001 and RFS p= 0.007). Provisional LR patients with ETV6-RUNX1 or favorable DNA index ≥ 1.16 and PB-MRD <0.01% had 5-year EFS 96.4±2.6% (n=75) compared to 82.6±8.2% for other provisional LR with PB-MRD <0.01% but lacking these favorable features (n=26) (p= 0.021).Provisional LR patients with ETV6-RUNX1 or favorable DNA index ≥ 1.16 and PB-MRD

Conclusions: Early induction PB-MRD by flow-cytometry constitutes an early prognostic index for children with B-Precursor ALL and can help in identification of a subgroup of patients provisionally classified as low-risk ALL with either favorable DNA index or ETV6-RUNX1 having an excellent outcome that can be cured with limited therapy.

P055

Minimal residual disease kinetics in Philadelphia-chromosome negative adult acute B-lymphoblastic leukemia

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Background: Patients diagnosed with adult acute lymphoblastic leukemia (ALL) at centers within the Czech Leukemia Study Group - for Life (CELL) who are fit for intensive therapy are treated according to a joint pediatric-inspired protocol. Patients with high-risk (HR) features and a suitable donor proceed to allogeneic stem cell transplant (SCT). Minimal residual disease (MRD) is monitored by polymerase chain reaction (PCR) at several time points throughout the whole study.

Aims: MRD has become a widely accepted risk factor influencing survival and treatment decisions. We wanted to investigate whether the kinetics of the MRD response also plays any role in patient outcomes.

Methods: All patients with Ph-negative B-ALL diagnosed between 2006 and 2017 who were treated according to a joint "ALL CELL 2012 junior" protocol were included into this retrospective analysis. The data obtained were analyzed for descriptive statistics, hematologic and molecular remission, allogeneic transplant and relapse rates, and survival.

Results: Between 2006 and 2017, a total number of 114 consecutive patients were diagnosed with Ph-negative B-ALL. A complete remission (CR) was achieved in 108 (94.7%) patients. Sufficient MRD data are available in 85 (74.6%) patients. Of these, seventy-three (85.9%) patients achieved a complete molecular remission (CMR) at any time during the treatment. The proportion of patients in CMR tends to increase with ongoing treatment. An allogeneic SCT was performed in 45 (39.5%) patients. Forty (37.0%) patients experienced a relapse in a median time of 11.6 months. Median progression-free (PFS) and overall survival (OS) in the whole group were 34.9 and 79.6 months, respectively. Five-year PFS and OS were 46% and 53%, respectively.

We have not found any influence of age, sex, leukocyte count, immunophenotype, molecular genetics or karyotype on the survival. The only major risk factor that remained to significantly affect the survival was the MRD response. The highest survival benefit of an allogeneic SCT can be seen in the HR and MRD positive subgroups, while the benefit in the standard risk (SR) and MRD negative subgroups is insignificant.

Interestingly, the time point of achieving the CMR is even more important as patients with early and sustained MRD negativity (starting day 26 or 46) do better than those who become negative late (week 11 and later), or have a poor MRD response (just temporarily or never at all). This kinetic MRD model helps to distinguish between low and high risk of relapse (and hence a potential benefit of a SCT) more adequately taking into account the complex MRD response in a particular patient,

not only the MRD level at a single time point.

Summary/Conclusion: In this retrospective analysis of adult Ph-negative B-ALL patients we report very high CR and CMR rates. According to our data, the level of minimal residual disease was proven to be the most important factor affecting the survival. Moreover, with respect to all the limitations of a retrospective setting, we can conclude that achieving MRD negativity in the earliest treatment phases is a critical factor for a sustained remission. Whether any novel drug or procedure, incorporated to the very initial treatment phase to decrease the MRD more rapidly, could change the fate of the slow responding patients will have to be further evaluated, preferably in a prospective manner.

Supported by Czech Leukemia Study Group – for Life (CELL).

P056

Outcome of Minimal Residual Disease Adapted Therapy in Children with B-precursor Acute Lymphoblastic Leukemia

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Background: Several studies have shown that minimal residual disease (MRD) measured during treatment is the single most important prognostic indicator in pediatric acute lymphoblastic leukemia (ALL).

Aim: To apply personalized treatment based on MRD to improve outcome of pediatric B-precursor ALL patients by identifying those who can be successfully treated with low intensity and less toxic regimens and others who require more intensive therapy to increase their chance of cure.

Patients and Methods: From July 2007 to October 2011, 675 newly diagnosed B-precursor ALL patients aged 1-18 years were treated at Children Cancer Hospital Egypt with MRD response directed treatment adopted from SJCRH ALL Total Study XV.

Results: Based on initial presentation and response to therapy measured by flow cytometry MRD in bone marrow (BM) on day-15 and end of induction (day-42), 389 (57.6%), 230 (34%) and 56 (8.3%) of the patients were finally classified and treated as low (LR), intermediate (IR) and high risk (HR); respectively. The 5-year relapse free survival (RFS) for LR, IR and HR were 91.6% ± 1.5%, 83% ± 2.7% and 64.8% ± 7.4%; respectively ($p < 0.001$), while the 5-year event free survival (EFS) were 83.5% ± 1.9%, 71% ± 3% and 47.3% ± 6.7%; respectively ($p<0.001$). The median follow up for patients alive in continuous complete remission is 7 years (range, 5 - 9.4 years). Based on BM-MRD response, 76 (16%) of the provisionally LR patients who had poor early response or MRD 0.01-<1% on day-42 were upgraded and treated on IR arm. They had 5-year RFS of 85.2% ± 4.4% which was statistically not significant compared to RFS of other provisionally LR patients who had good MRD response and treated on LR arm ($p=0.166$). On the other hand, the 25 patients with MRD ⩾1% on day-42 or MRD⩾0.1% on week 7 who were upgraded from LR or IR to HR group had poor outcome having 5-year RFS of 62.2% ± 11.3%. Of the 389 LR patients, 36 (9.3%) had age ≥10 years or leukocyte count (WBC) ≥50x109/L at presentation but treated on

LR arm based on presence of ETV6-RUNX1 or favorable DNA index ≥ 1.16 and on their MRD response (MRD day-15 <1% and day-42 <0.01%). Their 5-year RFS was 93.8% ± 4.2% which is comparable to RFS of 91.3% ± 1.6% for other LR patients with favorable age and WBC (p=0.48). LR patients with NCI-SR and MRD <0.01% on day-15 as well as having favorable genetic features (ETV6-RUNX1 or favorable DNA index ≥ 1.16) had 5-year RFS of 98.3% ± 1.7%, compared to 83.3% ± 5.8% for other LR patients with the same response but lacking these favorable genetic features (p=0.002).

Conclusions: MRD directed therapy allowed children with LR ALL to achieve good outcome with modest therapy and to be spared more intensive and toxic treatment. Also, it increased the chance of cure for provisionally LR patients who were upgraded to IR based on their MRD response. Moreover, therapy was successfully de-escalated based on favorable genetic features and negative end of induction MRD irrespective of age and WBC at presentation.

Having negative MRD (<0.01%) on day-15 and on day-42 combined with ETV6-RUNX1 or favorable DNA index can further identify a subgroup of patients (~ 19% of LR ALL) with 98% RFS at 5 years in whom further de-escalation of therapy can be considered especially in developing countries to help reducing therapy related morbidities and mortality.

P057

Concordance of minimal residual disease measured by three methods in Ph-positive B-ALL adults in Russian study Ph+RALL-2012

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National Research Center for Hematology, RUSSIAN FEDERATION

Background: Measurement of minimal residual disease (MRD) in B-cell acute lymphoblastic leukemia (B-ALL) is integrated in treatment protocols due to it prognostic significance. In Ph+B-ALL *BCR-ABL1* transcripts are the preferential molecular targets for MRD assessment. However it is known that *BCR-ABL1* may persist not only in leukemic cells but in myeloid and other cells [1]. In this case MRD determined by other methods may clarify the real status of the disease.

Aims: To evaluate MRD in Ph+B-ALL patients by three methods: reverse transcription PCR (RT-PCR) for *BCR-ABL1*, multicolor flow cytometry (MFC) and PCR with patient-specific primers for clonal rearrangements of *Ig/TCR* genes.

Methods: From Dec 2011 to Feb 2017 23 Ph+B-ALL patients were treated according to Ph+RALL-2012. The main principle of Ph+RALL-2012 is non-intensive treatment but non-interruptive with simultaneously use of tyrosine kinase inhibitors. This study included 7 patients (m=4, f=3, median age=31). MRD was determined at +36, +70 and +133 days. *BCR-ABL1* transcripts were evaluated by RT-PCR with sensitivity of at least 0.01%. Real-time quantitative PCR with patient-specific primers for clonal rearrangements of *Ig/TCR* genes was carried out according to guidelines published by (2007). MRD by MFC was analyzed by 6-color flow cytometer BD FACSCanto II with minimal sensitivity of 0.01%.

Results: On day +36 the MRD by MFC was determined in all 7 patients, *BCR-ABL1* was

measured for 5 patients and PCR with patient specific primers was applied for 3 patients. 5 patients had MRD detected by MFC (MFC^+) and detectable level of BCR-ABL1 transcripts ($RT\text{-}PCR^+$). 3 of them had MRD measured by PCR with patient specific primers (PCR^+).

On day +70, the MRD was examined in 7 patients by MFC and RT-PCR. Only one patient was $RT\text{-}PCR^-$ and MFC^-. Among 6 $RT\text{-}PCR^+$ patients, MFC^- was in 3. MRD by *Ig/TCR* PCR was investigated in 4 patients: 2 were PCR^+ and 2 were PCR^-. It is interesting that only 1 result coincided with both RT-PCR and MFC ($RT\text{-}PCR^+MFC^+PCR^+$), 1 was $RT\text{-}PCR^+PCR^+$ but MFC^-, 1 was $RT\text{-}PCR^+MFC^+$ but PCR^- and 1 was $MFC\text{-}PCR^-$ but $RT\text{-}PCR^+$.

On day +133, MRD by MFC and RT-PCR was examined in 5 patients. Only 1 was $RT\text{-}PCR^-$, and 3 were MFC^-. MRD by *Ig/TCR* PCR was examined in 3 patients. Only one had concordant results: $RT\text{-}PCR^+MFC^+PCR^+$. In 1 MRD was PCR^-, but $RT\text{-}PCR^+MFC^+$, and 1 was $RT\text{-}PCR^+$, but MFC^-PCR^-.

The parallel study of MRD by MFC and RT-PCR was performed in 17 samples. The concordance of the results was in 12 cases (70.6%), and 5 cases were $RT\text{-}PCR^+MFC^-$. The regression coefficient between quantitative results R^2 was 0.994, but the proportion of tumor cells determined by RT-PCR was 4 times higher than by MFC method (fig. 1a).

The parallel study of MRD by MFC and PCR with patient-specific primers was performed in 10 samples. The concordance of the results was in 7 (70%) cases. Discordance included: 2 cases MFC^+PCR^-, and 1 MFC^-PCR^+. The R^2 was 0.719 (fig. 1b).

Conclusion: On 36 day, the greatest concordance of MRD results was observed, probably due to low clearance of tumor cells. However, on 70 and 133 days, negative results were often observed using MFC and/or *Ig/TCR* PCR methods while *BCR-ABL1* transcripts persisted. This can serve as proof that transcript might rather persist in other cells than in blasts or RT-PCR may have higher sensitivity then other methods. This conclusion may improve the predictive ability of the MRD tests in the prospective studies.

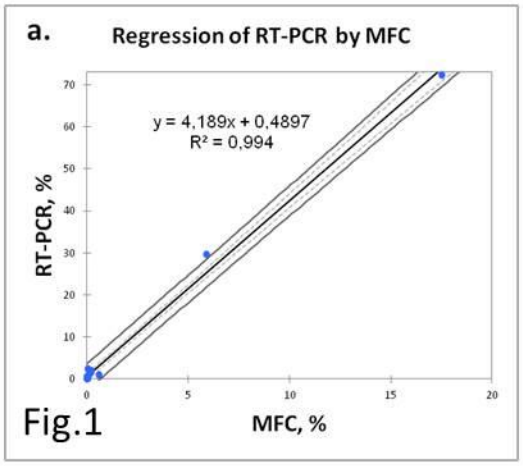
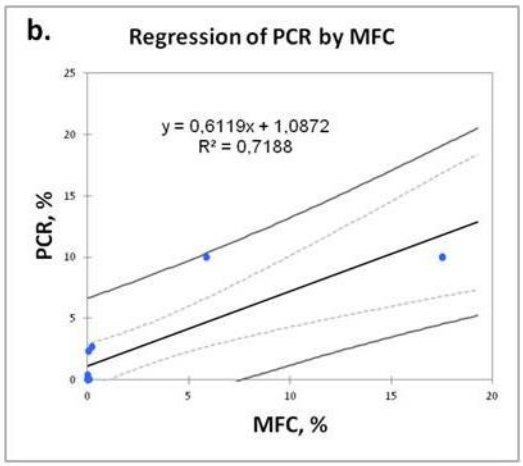


Fig.1



P058

Minimal residual disease (MRD) in adult Ph-negative acute lymphoblastic leukemia (ALL) patients treated in Russian multicenter study "RALL-2016"

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Background: The persistence of MRD in ALL is an independent adverse factor, and many studies have shown the prognostic significance of the quantitative evaluation of MRD. All patients included in the RALL-2016 protocol are provided with a centralized testing for MRD on 70, 133 and 190 days of the protocol. The main principle of the "RALL-2016" is non-intensive but non-interruptive treatment.

Aims: To estimate the presence of MRD in B-ALL and T-ALL patients on days +70, +133 and +190 of RALL-2016 protocol.

Methods: MRD was studied from December 2016 to January 2018 in 34 ALL patients, in whom clinical and hematological remission was confirmed by +70 day. 21 B-ALL patient (median age 35 years (24-54), m:f=10:11) and 13 T-ALL patients (median age 36 years (22-53), m:f=10:3) were included in the study. Abnormal karyorype was found in 9 patients (hyperploidy in 4, complex karyotype in 2, hypoploidy in 1, t(4;11) in 1, t(1;19) in 1).

The MRD testing was performed in bone marrow using 6-color flow cytometry (FACSCanto II) with minimal sensitivity of 0,01%.

The monoclonal antibodies panel for MRD testing in B-ALL included anti-CD19, CD10, CD34, CD20, CD45, CD38, CD58, CD45 antibodies; in T-ALL: anti-CD7, CD3cyt, CD3, CD5, CD4, CD8, CD5, CD45, CD99, CD2, CD1a.

Results: MRD was detected in 10 (47.6%) out of 21 B-ALL patients (MRD-positive) on +70 day. MRD was not detected in 12 (92.3%) out of 13 T-ALL patients (MRD-negative) on day 70. According to the data provided by Ravandi et al (2016) among CR B-ALL patients treated by hyperCVAD, MRD-negativity by flow cytometry was achieved in 166 (63.8%) out of 260. MRD-negativity in GMALL (Brüggemann M, 2006) by PCR before consolidation was achieved in 21 (58%) out of 36 patients. The proportion of MRD-negative patients in the end of induction did not differ between RALL-2016, hyperCVAD and GMALL.

MRD testing was performed in 16 B-ALL patients and 10 T-ALL patients on +133 day. All T-ALL patients were MRD-negative. 6 (37.5%) out of 16 B-ALL patients were MRD-positive. MRD testing was performed in 11 B-ALL patients and in 7 T-ALL patients on day 190. All of the T-ALL patients and 8 (72.7%) out of 11 B-ALL patients were MRD-negative. The proportion of MRD-positive patients was reliably higher in B-ALL patients than T-ALL on days 70 and 133 ($p=0.016$, $p=0.027$, respectively).

There were no significant differences between MRD-positive and MRD-negative B-ALL patients groups depending on age (39.0 ± 3.2 vs 34.3 ± 2.9 years, $p=0.27$), initial leukocytosis above 30×10^9/L (10% vs 9%, $p=0.944$), complete remission after second induction phase (10% vs 9%, $p=0.944$) and karyotype anomalies.

Quantitative value of MRD in B-ALL significantly lowered on +133 day comparing to +70 day ($p=0,008$) and did not change on +190 day comparing to +133 day (fig.1).

Conclusion: Despite low intensity of the RALL-2016 the proportion of MRD-negativity results did not differ between non-intensive Russian protocol, GMALL protocol and much more intensive hyperCVAD in ALL.

MRD was detected more often in B-ALL patients than T-ALL patients on +70 and +133 days of RALL-2016. Tumor clone decline was identified in B-ALL MRD-positive patients on +133 comparing to day +70 day. Quantitative value of MRD was not changed on +190 comparing to +130 day in cases of MRD persistence in B-ALL. It's too early to evaluate the long-term results regarding MRD-values on certain days.

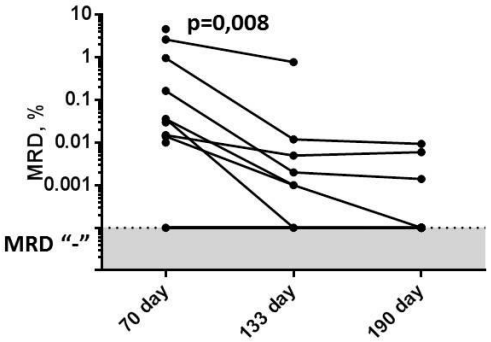

Fig. 1. Dynamics of MRD-values in B-ALL

ONLINE PUBLICATION ONLY

PB059

Effectiveness and safety of blinatumomab in relapsed and minimal residual disease positive B-cell acute lymphoblastic leukemia: a single center experience.

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Background: The prognosis of patients (pts) with relapsed/refractory acute B-cell lymphoblastic leukemia (R/R B-ALL) is very poor. Also minimal residual disease positivity (MRD+) is a high-risk condition, being the strongest predictor of relapse in ALL. Blinatumomab, a bispecific T- cell engaging antibody targeting CD19 on B-cell lymphoblasts, is an encouraging option for both R/R and MRD+ B-ALL.[1-2]

Aims: To report on efficacy and safety of blinatumomab, obtained either through compassionate or expanded access use, in 10 adult pts with MRD+ or R/R B-ALL treated at a single Institution.

Methods: MRD was determined by PCR through BCR-ABL or MLL fusion transcript quantitative analysis or Ig rearrangement, after induction (TP1, week4) and 2nd consolidation (TP2, week10). Molecular persistence at a level of 10-4 or reappearance of MRD during follow-up defined MRD+ALL.

Results: From Jan 2014 to Jan 2017, blinatumomab was administered to 10 pts (M/F = 1/1, median age 37 years, range 17-68), stratified by risk as follows: 1 standard-risk, 4 high-risk (abnormal karyotype[3] 2, resistance to induction 1, TP2 MRD+ 1), 5 very high-risk (Ph+ 3, MLL rearrangement 2). First-line therapy was given according to protocols NILG-GIMEMA1913 (5 pts), NILG 10/07– standard arm (3 pts)[4] and Ph+NILG (3 pts)[5]. Two pts with Ph+ALL received as second and third-line treatment dasatinib (2 pts) and ponatinib (1 pt); 1 pt aged >65 had received prior autologous stem cell transplant (ASCT).

Blinatumumab was administered to 3 pts with R/R disease at the dose of 9 mcg/d x 7

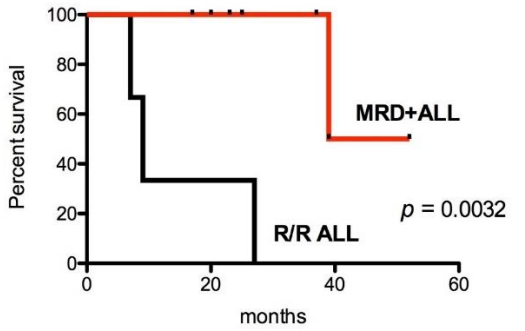
days and 28 mcg/d x 21 days[6]; 7 pts MRD+ at TP2 received 15 mcg/m2/d over 28 days2. Two pts were hospitalized for the whole treatment, 8 completed the cycle as outpatients. Median number of cycles was 1 (range 1-2). Overall, toxicity was observed in 5/10 pts (50%): grade 1-2 (G1-2, CTCAE) fever with chills was the most frequent adverse event (5/5 pts); neurological events consisted of G2 headache in 2 pts and G2 tremor in 1 pt; G3 erythema (1 pt) and G4 muscle pain (1 pt) were also observed but resolved completely after transient drug withdrawal.

Six pts (60%) obtained a complete molecular response (mCR): 1/3 with R/R ALL (33%), 5/7 with MRD+ALL (71%); 5/6 pts obtained mCR after 1 cycle. Two pts with R/R disease died short after the 1st cycle (1 progressive disease, 1 cerebral hemorrhage during aplasia). Two MRD+pts who did not achieve mCR obtained mCR after allogeneic stem cell transplant (HSCT): 1 pt is in mCR and 1 in molecular relapse 13 and 10 months (mo) after HSCT, respectively.

All 6 molecular responders received HSCT. GVHD developed in 4 pts (1 acute, 1 acute+chronic, 2 chronic). Four pts are alive in mCR (median time from HSCT 23 mo), 2 died of disease relapse and GVHD, 15 and 3 mo after HSCT, respectively). Median overall survival (OS) was 39 mo. For molecular responders, molecular leukemia free survival (mLFS) was not reached at 47 months. According to disease status before blinatumumab, MRD+ pts survived significantly longer than R/R pts (45.5 vs 9 months, p 0.0032) (Fig.1).

Conclusion: In our study, blinatumumab used in the setting of MRD+ALL yielded a high rate of mCR, in line with results of larger studies[7], allowing to maintain a prolonged continuous remission as a bridge to HSCT. Blinatumumab proved safe also in elderly ALL, 40% of pts being >55 years old, and did not worsen toxicity related to HSCT procedure.

Figure 1 - Overall survival R/R vs MRD+ ALL



PB060

Immunophenotypic Comparison between Reactive Bone Marrow B-Lymphocyte Precursor (Hematogones) and B-Neoplastic Lymphoblast Leukaemia Using Cd 34, Cd 123

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Abstract

Background: Flow cytometric study found that lymphoblasts of B acute lymphoblastic leukemia exhibited multiple aberrant antigens by which they can be distinguished from hematogones. These antigens are CD34 and CD123.

Objective: To determine the immunophenotypic pattern of CD34 and CD123 expression in hematogone of reactive bone marrow and in neoplastic lymphoblast in B-acute lymphoblastic leukemia (ALL) patients and to evaluate the impact of that pattern in the residual disease detection after chemotherapy.

Methods: This is a case control study to determine the expression of CD34 and CD123 in 30 patients newly diagnosed with B-ALL. Re-assessment was done for 20 patients of them after 4-6 weeks of chemotherapy; in addition to 10 patients with reactive bone marrow to assess hematogones.

Results: In (93.4%) of the newly diagnosed B-ALL cases, leukemic blasts expressed both CD34 and CD123, Conversely, in (6.6%) cases, neither antigen was expressed. In hematogones; the immature hematogones (dim CD45, CD34 +) did not express CD123 while the mature hematogones (moderate CD45+, CD34-) expressed CD123. The strategy of concordant and discordant patterns of CD34/CD123 expression on B-ALL blasts and hematogones respectively in post chemotherapy patients remain stable.

Conclusion: The distinct pattern of CD34 and CD123 expression on hematogones (discordant) and B-ALL blasts (concordant) is useful in correctly classifying immature B cells as residual leukemic blasts or hematogones in the bone marrow of patients treated for B-ALL.

PB061

Cluster of differentiation 97 as a biomarker for detection of Minimal Residual Disease in common Acute Lymphoblastic Leukemia

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Background: Acute lymphoblastic leukemia (ALL) is a biologically heterogeneous disorder. Clinical parameters, immunophenotype, cytogenetic and minimal residual disease (MRD) are among currently used factors in risk stratification and therapy determination of ALL patients. Minimal residual disease (MRD) is gaining importance nowadays both for therapy efficacy, follow up and relapse risk estimation. recent studies have high lightened potential markers that may improve the sensitivity of MRD detection by flowcytometry.CD97 is one of these markers which show over expression in pediatric ALL. In this study we aimed to asses the value of CD97 as biomarker for detection of MRD in pediatric ALL.

Methods: This cohort study was conducted on thirty newly diagnosed patients with B-lineage ALL. they were 16 males and 14 females with mean age of 8,38±4.21 and a range from(1 -18) year. 20 patients were low risk group and 10 patients were high risk group treated according to modified CCG 1991. a panel of monoclonal antibodies was used with special emphasis on CD10,CD19,CD34 andCD97at diagnosis and at day 14 post induction of chemotherapy for detection of MRD**.**

Results: Three patients(10%) presented with total leucocytic counts(TLC) ≥50 x103/mm3 while twenty seven patients(90%) had TLC < 50 x103/mm3. Mean multiparameter flow cytometry of CD19/CD97, CD34/CD97 and CD10/CD97 at day 0 was 57.15±21.74, 57.73±21.20 and 57.87±20.77 while at day 14 was 6.09±2.50, 10.67±8.89 and 5.97±2.44 respectively p value<0.001. CD97 was expressed in 81.5% of patients at diagnosis and wasn't detected at day 14 p value <0.001.one patient had blast counts >5% by light microscopy while twenty nine patients had MRD>0.1 by multiparameter flow cytometry at day 14 p valu<0.001.

Conclusion: CD97 can be used for MRD-tracing in pediatric ALL.

Targeted Immunotherapy: Planned trials of National Study Groups

POSTER PRESENTATION

P062

Blinatumomab combined with Tyrosine Kinase Inhibitors in Relapsed/Refractory Acute Lymphoblastic Leukemia -- clinical effectiveness and lymphocytes subpopulations dinamics

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Background: IKZF1 deletions, FLT3-ITD and BCR-ABL are the potential targets for tyrosine kinase inhibitors (TKI). Combining blinatumomab, a bispecific anti-CD3/CD19 construct, with TKI is the promising treatment approach in relapsed/refractory acute lymphoblastic leukemia (R/R ALL) with aforementioned molecular entities.

Aims: To avoid myelotoxicity in heavily pretreated with chemotherapy high risk ALL patients and to assess key lymphocyte subpopulations behavior during blinatumomab treatment.

Methods: From October 2015 to November 2017 we treated 11 consecutive patients (pts) with R/R ALL (CD19 positive). Median follow up is 15 months (10-27). All pts were treated with blinatumomab + TKI combination. BCR-ABL-positive ALL was diagnosed in 8 pts, IKZF1 deletions were detected in 2 pts, FLT3-ITD - in 1 pt. ABL mutation T315I was detected in 2 BCR-ABL-positive ALL. Blinatumomab 28 mcg/day continuous infusion was administered as 4-5 cycles. Each cycle consisted of 28 days, interval between cycles – 14 days. 9 pts were treated with dasatinib, 1 – sorafenib, 1 – ponatinib. Dasatinib was substituted for nillotinib in 2 pts and for bosutinib in 1 pt due to toxicity. All TKI were administered continuously (with temporary suspension due to toxicity in 1 pt on sorafenib). 2 IKZF1 deleted ALL pts received concomitant ATRA treatment. T-helper, T cytotoxic, T-regulatory and NK peripheral blood lymphocytes subpopulations were measured weekly by flow cytometry during blinatumomab treatment .

Results: No myelotoxicity observed. 2 pts has neurological toxicity 1 - 2 grade on blinatumomab (headaches in 1 pt, ulnar neuropathy in 1 pt). 9 pts received intravenous human normal immunoglobulin replacement because of significant decrease of IgG. There was 1 hand-foot syndrome on sorafenib. Diarrhea on dasatinib resolved on nilotinib replasement in 2 pt and on bosutinib in 1 pt. Face edema and hyperemia on dasatinib resolved on nilotinib replacement in 1 pt. CMV colitis was diagnosed in 3 pts with intestinal ulcer bleeding in 1 of them. T-helper, T-regulatory, T-cytotoxic and NK peripheral blood lymphocytes subpopulations were decreased during 1st blinatumomab cycle. From 2nd to 4th cycles T-cytotoxic and NK returned into normal range while T-helper and T-regulatory remained lowered. During 1st blinatumomab cycle 10 pts achieved complete remission (CR) and 1 pt had progressive disease. On subsequent cycles 9 pts achieved molecular remission (MolCR), one pt – cytogenetic remission (CyCR). In 9 pts allo-SCT was performed and 1 pt in MolCR received auto-SCT. 1 pt with CyCR achieved

MolCR after allo-SCT. TKIs restarted 3 – 4 weeks after transplant. 1 pt had molecular relapse 8 months after auto-BMT and is on blinatumomab retreatment. 1 pt awaiting allo-BMT has overt hematological relapse and repeated MolR was achieved on bortezomib-based chemotherapy.

Conclusions: The combination of blinatumomab and TKI/TKI+ATRA has high MolCR rate and low toxicity profile. Low T-helper level at the start of treatment and T-cytotoxic an NK subpopulations restoring during blinatumomab treatment correlated with clinical effectiveness.

ONLINE PUBLICATION ONLY

PB063

Estimation of Peripheral Blood Transforming Growth Factor Beta 1 Level in patients (pts) with Acute Lymphoblastic Leukemia (ALL) and its correlation with the prognostics indexes of disease

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Background. Tansforming growth factor beta 1 (TGFβ1) is a potent inhibitor of hematopoietic cells proliferation. The number of works on changes in TGFβ1 concentration in case of ALL is insufficient and their results are often contradictory. Some studies reported the possible diagnostic and prognostic use of blood TGFβ1 level in newly diagnosed acute leukemias (AL) and myelodysplastic syndromes [1].

Aims: to study TGFβ1 concentration in plasma of pts with ALL, its production by the primary culture of peripheral blood mononuclear cells (PBMC) (mixture of blasts and lymphocytes) and to estimate the the connection with the prognostic indexes of ALL.

Methods. The study includes 44 pts with acute leukemia (AL): 23 pts were diagnosed with ALL and 21 pts - with acute myeloid leukemia (AML). The TGFβ1 concentration was determined by bioassay using cells line CCL64, mink lung epithelial cells. The control group consisted of 15 healthy persons.

Results. In the pts with AL, TGFβ1 concentration in plasma was 4,854±0,136 ng/ml, which is more than 2 times as much as that in the control group 2,110±0,380 ng/ml; p< 100 g/l manifested statistically unconfirmed high TGFβ1 concentration (6,033 ± 0,149 ng/ml) compared to the pts whose Hb was > 100 g/l (3,721 ± 0,233 ng/ml); p < 0,05. The content of TGFβ1 in pts with thrombocytopenia (< 100x109/l) was higher than that in the pts with the normal number of platelets (6,053 ± 0,189 ng/ml and 4,930 ± 0,274 ng/ml, respectively; p < 0,05). TGFβ1 concentration in plasma was the lowest in the pts with leukopenia (number of leukocytes < 4x109/l) vs normal number of leukocytes (3,796±0,253 and 7,090±0,229 ng/ml). In ALL pts we established indirect correlation between absolute blast cell number in peripheral blood (PB) and TGFβ1 level (r = - 0,380). The same correlation in AML had lower expression (r = - 0,152). In pts with AL, TGFβ1 production by PBMC was lower as the corresponding estimate in the control group and reached 6,200± 0,072 ng/ml vs 7,85 ± 0,80. PBMC of pts with ALL produce more TGFβ1 than that of pts with AML (6,112 ±0,130 and 4,242±0,056 ng/ml, respectively). TGFβ1 production by PBMC in case of AL pts with more significant BM infiltration (> 75%

blasts) was approximately 1,5 times as lower as corresponding estimate in pts with lower (< 75%) percent of blasts in BM (3,497± 0,110 ng/ml vs *5*,200± 0,040 ng/ml; p < 0,02). TGFβ1 production by PBMC of the pts with low Hb level was two times higher than that of pts without symptoms of anemia. In the ALL pts, we observed weak negative correlation between absolute blast cell number in peripheral blood and TGFβ1 production by PBMC (r = –0,135). That correlation was characterized as middle grade negative in AML pts (r = -0,500).

Summary/Conclusion. Serum TGFβ1 level is elevated in AL pts and has a favorable prognostic value. TGFβ1 production by ALL is quite higher than its production by AML, and besides TGFβ1 could be considered inhibiting growth factor for in AML and it stimulate lymphoid blasts proliferation in ALL. Increased concentration TGFβ1 in plasma and diminishing its production by PBMC in AL pts are accompanied by higher number of blast cells in BM and PB, anemia, thrombocytopenia, what makes a possib

POSTER PRESENTATION

P064

Adolescents and Adults with T-lymphoblastic lymphoma treated on a pediatric ALL-like protocol have improved outcomes which are predicted by early PET response.

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Background and objectives
The management of T- lymphoblastic lymphoma (T-LbL) in adults poses uncertainties, including optimal chemotherapy regimen, need for mediastinal radiotherapy and the benefit of stem cell transplant. Lack of defined prognostic indicators contributes to the management dilemma. This retrospective case series investigated the efficacy of the pediatric BFM-90 regimen in older teenage and adult patients and evaluated the role of early response assessment by 18-FDG positron emission tomography with CT (PET-CT) in predicting outcomes.

Design and Methods: Sequential patients aged15 years or older with T-LbL diagnosed at Tata Memorial Hospital, Mumbai were studied. Treatment-naïve patients treated between January 2010 till November 2016 were included. Patients with bone marrow disease involvement were excluded. Patients were evaluated for early response by interim PET-CT post-induction (PET-CT scans were done either after Phase I or II) and monitored for toxicity and long-term outcomes. We looked at the long-term survival outcomes of these patients treated in a uniform manner and the value of PET CT as a prognostic tool.

Results: 38 patients (Median age-23 years, range-15-44years) were treated, out of whom 23 were males. Serum LDH levels were elevated (≥ Upper limit normal) in 32 patients. Baseline serum albumin levels were ≤ 4g/dL in 16. 33 patients had a bulky disease. Cerebrospinal fluid was involved in 1 patient. Baseline PET CT scans were done in 15(40%) patients. In the remaining patients, either it was not possible to perform a PET-CT scan due to the presence of dyspnea on lying down or were not performed if they had already got a contrast-enhanced CT scans. All the patients received modified BFM-90 protocol (High dose Methotrexate at 3g/m2). Mediastinal RT was given to 6 patients and 19 patients received cranial irradiation (Prior to 2013) 25 patients underwent a PET-CT after phase I and 19 patients (Includes 7 patients who had a PR on the PET-CT that were done after phase I) underwent beyond the phase II of induction. At the end of phase I, 18/25 had a complete response (CR) and 7/25 had a partial response (PR). On the PET CT scans done beyond the phase II, 15/19 had a CR and 4/19 had a PR. Overall, 33 (86%) patients had a CR, 4(10%) had a PR and in one the response evaluation was not done. 5 patients had disease progression and there were 8 deaths (5-Disease-related, and 3-Toxicity related). With a median follow up of 45 months, the 5-year OS is 78% and the 5-year DFS is 85%. The survival was better in those who attained a CR as compared to those with a PR on PET CT scan (Log-rank test).

Interpretation and conclusions: This study demonstrates the feasibility and efficacy of using the BFM-90 approach in adolescents and adults with T-LbL. We also found a potential role of PET-CT scan as a prognostic tool that needs to be verified in a prospective manner.

ONLINE PUBLICATION ONLY

PB065

T-cell lymphoblastic lymphoma: Pearls and pitfalls in haematopathology approach.

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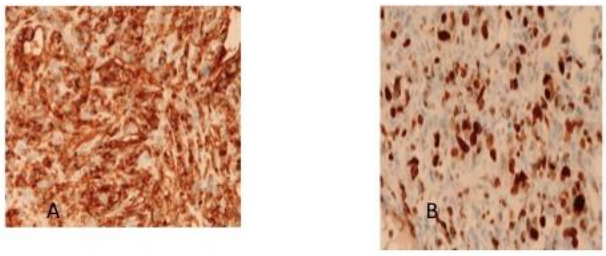
Precursor T lymphoblastic leukemia (T-ALL)/ T lymphoblastic lymphoma (T-LBL) are neoplasm of lymphoblasts committed to the T-cell lineage, typically composed of small to medium-sized blast cells with scant cytoplasm, with features of moderately condensed to dispersed chromatin and inconspicuous nucleoli, involving bone marrow and peripheral blood (T-ALL) or presenting with primary involvement of nodal and extranodal sites (T-LBL).

The T-LBL are a diagnostic challenge and the initial step implies the correlation of the HE sections, clinical features, and molecular diagnosis. Since 95% of T-LBL is immunoreactive for TdT immunohistochemical demonstration of TdT is "diagnostic for lymphoblastic lymphomas of both B-cell neoplasm and T-LBL". Immunophenotype is essential in the workup as Lymphoblasts express cytoplasmic CD3 and other T-lineage makers as well as one or more markers of immaturity, including CD34, CD1a, and TDT The latter is normally expressed in T- (and B-) cell precursors.

We presented the histopathology results of three patients and discuss the additional immunostaining: the experienced histopathologists are needed to correctly classify this aggressive disease and then we will discuss the pearls and pitfalls.



An example of tumor cells which shows a positive CD45, CD45RO, CD34 (A), CD99, p53 (F) and K-i67 (labeling approximately 60%), but TdT negative (B).

POSTER PRESENTATIONS

P066

Patient and Therapy-related Factors Affecting the Toxicity of Pegylated-Asparaginase for the Treatment of Adult Acute Lymphoblastic Leukemia

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Background: The application of full pediatric or pediatric inspired regimens in the treatment of adult acute lymphoblastic leukemia (ALL) has led to a significant improvement in patients outcome. Concerns about the feasibility of intensive therapies and the use of pegilated L-Asparaginase (PEG-ASP) in adult patients have emerged. Some patient-related risk factors as high BMI or hepatic steatosis have been identified as risk factors, but few data are available on the role of concomitant drugs contributing to PEG-ASP toxicity.

Aims: To evaluate the incidence of PEG-ASP related adverse event in a cohort of adult ALL patients in order to identify factors contributing to toxicity.

Methods: Since 2013, 23 adult ALL patients received PEG-ASP in our institution. Median age was 44 yrs (19-76); 14 pts were treated in frontline setting (9 according to a full pediatric protocol), 9 pts received therapy for relapsed/refractory disease. We analyzed each course which included PEG-ASP administration as an independent event, accounting 45 episodes. Patients' features (age, sex, BMI, disease status) and concomitant therapies were analyzed as factors potentially affecting PEG-ASP toxicity. The incidence of major thrombotic/bleeding complications and grade III-IV hepatic or pancreatic toxicity was analyzed. Management of PEG-ASP related complications were performed according to guidelines published by Stock et al.

Results: No grade III-IV pancreatic, thrombotic or hemorrhagic adverse event were recorded. Five pts experienced grade III hepatotoxy; 3 pts experienced grade IV hepatotoxicity**,** unexplained severe weight gain and painful epathomegaly, a clinical picture resembling sinusoidal occlusive disease. Ultrasonography showed the onset of acute liver steatosis. All 3 pts had received concomitant therapy with idarubicin (IDA), vincristine (VCR) and vancomycin.

In univariate analysis the incidence of grade III/IV hepatic toxicity was significantly higher when concomitant chemotherapy with at least 2 mg/sqm cumulative dose of VCR (p 0.044, HR 4.75) or at least 1 mg/sqm cumulative dose of IDA (p 0.046, HR 1.45) were administered. Steroids therapy had a borderline increase in toxic risk (p 0.068, HR 1.688). No increase in toxicity was observed with any dosing of daunorubicin, cyclophosphamide, cytarabine, methotrexate, and 6-mercaptopurine. Among antimicrobial therapies, vancomycin administration increased the incidence of grade III-IV hepatotoxicity (p 0.02, HR 1.863). No significant increase in the risk of toxicity was observed with carbapenems and azoles.

Older age (>45 yrs), receiving PEG-ASP with active leukemia or BMI >25 were not related with an increased incidence of grade III-IV hepatotoxicity. Notably, none of the patients undergoing full pediatric induction (who received the highest doses of PEG-

ASP), regardless of age, experienced grade III/IV hepatotoxicity.

A multivariate logistic regression analysis disclosed that concomitant administration of IDA, VCR or vancomycin were independent predictors of grade III/IV hepatotoxicity (p 0.004, 0.027 and 0.042).

Conclusion: The toxicity profile of PEG-ASP in adult patients is manageable. However, several drugs and their timing of administration may play a crucial role contributing to PEG-ASP hepatotoxicity. Anthracyclines with shorter half-life, i.e. daunorubicin, should be used. VCR administration if initial toxicity is present should be avoided or postponed. Attention should be paid when antibiotic therapy is required.

P067

Treating Adult Acute Lymphoblastic Leukemia in Brazil - An increased early-mortality using a GMALL-based regimen

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BACKGROUND: Acute Lymphoblastic Leukemia(ALL) in adults is usually treated with multidrug regimens, which have been improving since the 70's[1]. Very few data on adult ALL is available in developing countries and even though differences in epidemiology and biological issues have been reported in Latin America, results of chemotherapy and survival in adults are quite limited[2–5].

AIMS: To review the characteristics and results of adapted-GMALL 07/2003 (aGMALL)[6-8] to treat adult ALL at *Hospital das Clínicas/Instituto do Câncer de São Paulo*. Complete response (CR), relapse rates and Overall Survival (OS) were the main outcomes.

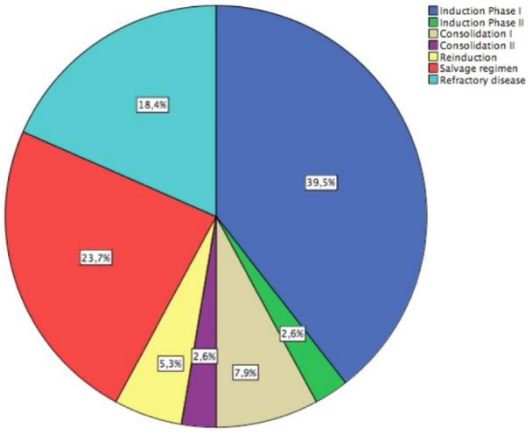
METHODS: A retrospective chart review of all patients over 15 years treated for ALL with aGMALL from 2009 to 2015. This study was approved by the local Research Ethics Committee.

RESULTS: Fifty-nine patients with newly-diagnosed ALL were treated and included in this analysis(Table 1). There were 10 deaths in the first induction phase, preventing early evaluation of response in these patients. All these early deaths occurred due to febrile neutropenia, resulting in an early mortality of 17%. The median age was 35 years(range, 16-71) and 54% were female. Philadelphia chromosome was positive in 45% of B-cell ALL patients.

	Total (n=59)	B (n=45)	T (n=14)
Age in years, median (range)	35 (16-71)	33 (16-61)	36,5 (16-71)
WBC in cells/mm3, median (range)	12,6x1000 (0,36-566,5x1000)	9,0x1000 (0,36-372,9x1000)	30,8x1000 (0,86-566,5x1000)
CNS infiltration (%)	29	32	21
High-risk stratification (%)	68	64	79
	Not applicable	I(7) II (25) III (12) IV (0) Unclassifiable (1)	I(3) II(1) III(4) IV(6)

Overall, 45 of 59 patients(76%) achieved CR on aGMALL. Age lower than 35 years and low risk cytogenetics were significant predictors to achievement of CR (p=0,005 and 0,029, respectively), among other factors (WBC, CNS involvement, phenotype or Philadelphia-status). Refractory disease was noticed in 4 patients (6,8%). A relapse rate of 35%(16 patients) was found. None of clinical factors addressed in this analysis were statistically significant to relapse. Among patients who achieved remission, few patients (15 patients, 25%). underwent allogeneic stem-cell transplantation (SCT). Among these 15 patients referred to SCT, 8 died from complications related to the procedure and the other 3 died from relapse. The median OS of the patients in the whole

group was 17 months. After a median follow-up of 6 years (range, 5,2-6,7), long-term survival was achieved by only 15,3% of subjects. In univariate analyses, there were no differences in survival when adjusted for sex, phenotype, risk stratification or performing SCT. For non-SCT patients (n=34), excluding those who died early, most deaths were due to bacterial infections – 61,4% (presumed or documented by blood culture). Just 8(21%) subjects among non-SCT patients were in CR at time of death. Indeed, most deaths were in refractory patients, during salvage regimen or from disease progression(figure 1).



CONCLUSION: In the studied population, a high rate of CR with aGMALL protocol was found, but with a significant reduction in OS, related to the high mortality rates due to toxicity, especially in the subsequent phases of regimen. There was also an increase in relapse rate, related to internal peculiarities, such as unavailability of autologous SCT, unexpected and prolonged interruptions of chemotherapy and delay to performing allogeneic SCT in high-risk patients. Adequate protocols for current treatment of ALL, especially in young patients, are needed in developing countries. High toxicity rates are encountered, and the difficulties for laboratory assessment, risk stratification and cooperative regimens are challenges to obtain satisfactory results with intensified chemotherapy, which has been proved being essential for this group[9].

P068

Clinical and Radiological Characteristics of Methotrexate Related Neurotoxicity in Pediatric Patients with Acute Lymphoblastic Leukemia

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Background: Methtotrexate (MTX) is a backbone for treatment of pediatric ALL. Common side effects include myelosuppression, hepatotoxicity and mucositis especially with the systemic high dose MTX. Intrathecal methotrexate is crucial for CNS prophylaxis, but it is not devoid of adverse reactions as well.

Aims: To report the clinical presentations, radiological characteristics and prognosis of methotrexate-related encephalopathy in Omani children with Acute Lymphoblastic Leukemia (ALL).

Methods: A retrospective cohort study of all children diagnosed with ALL who developed encephalopathy while being on chemotherapy during the period January 2005 through December 2017. Demographic data, clinical presentation, diagnosis and treatment are discussed.

Results: Out of 191 pediatric patients with ALL, 9 children (4 males and 5 females) developed transient encephalopathy after intrathecal methotrexate (IT MTX) during treatment with UKALL, constituting 4.7% of the total number of patients. Eight patients were above 10 years of age, and 3 patients

were Trisomy 21, accounting for 33.3 % (3/9) of leukemic children with Down syndrome during the study period. Six children had one episode, and 3 patients had got recurrence (2 of them were trisomy 21). Most patients presented during consolidation phase (7/9 = 77.8 %). Presenting features were ranging from transient focal seizures to cranial nerve involvement, impaired sensorium and even quadriplegia. All patients recovered completely within 2 to 5 days after symptomatic treatment. All children had normal coagulation and thrombophilia studies. Five out of the 9 patients subsequently received intrathecal cytarabine instead of MTX without any neurotoxicity, recurrence or subsequent CNS disease. The other 4 patients were re-challenged with IT MTX, and 3 of them had a recurrence. Eight children with MTX-induced toxicity had MRI findings of white matter changes. Overall, 6 out of the 9 children are in complete remission, two are still on maintenance chemotherapy while one passed away after a subsequent hematological relapse.

Conclusion: Risk factors of MTX-induced encephalopathy in pediatric ALL patients include age above 10 years, short interval between subsequent doses, trisomy 21 and re-challenge with IT MTX. Diffusion weighted MRI is the best available diagnostic tool. Treatment is largely supportive and the condition is usually self-limited. Further studies are needed to define other risk factors and the role of leucovorin rescue as a possible preventive strategy.

POSTER PRESENTATIONS

P069

Long-term follow-up of a largest series of consecutive patients with acute lymphoblastic leukemia treated with HOVON-70 protocol in a single institution reveals outcomes comparable to other pediatric-inspired protocols

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Background: HOVON 70 is a chemotherapy protocol first evaluated in a multicentric prospective, non-randomized trial by the Dutch-Belgian HOVON study group. In 54 acute lymphoblastic leukemia (ALL) adult patients up to 40 years of age it was confirmed both feasible and effective, with estimated 2-year overall survival (OS) of 72% and event-free survival (EFS) of 66% at 2 years. However, to this date, no other data evaluating the outcomes of this protocol in ALL patients are available.

Methods: From 2005, all adult ALL patients up to 40 years of age were treated with HOVON 70 in the University Hospital Centre Zagreb. As per study protocol, allogeneic stem cell transplantation (allo-SCT) was done in all patients who had a HLA-identical related donor and all high-risk patients. According to protocol, patients were considered high-risk if they had white blood cell count greater than 30x109/L for B-ALL or 100x109/L for T-ALL, bcr-abl or MLL translocation or did not achieve complete remission (CR) after induction therapy.

Aims: This study aimed to retrospectively evaluate the feasibility and toxicity of HOVON 70 in our patients and to provide long-term results of this protocol.

Results: Untill 2016, 37 male and 20 female ALL patients were treated in our institution according to HOVON 70. We analyzed outcomes of 51 patient with available follow-up. The median age at diagnosis was 26 years (range 18-40), and 14 patients (27%) were considered as high-risk. CR after induction therapy was achieved in 46 patients (90%). Thirty-eight patients (74%) completed therapy according to protocol with maintenance therapy or allo-SCT. Among the remaining patients, 2 patients died during induction or consolidation and in 11 patients therapy was discontinued due to toxicity or refractory disease. Asparaginase toxicity was acceptable with thromboembolic events in 8 patients (16%), acute pancreatitis in 2 patients (4%) and severe hepatotoxicity in one patient (2%). Most serious side-effect of high-dose methotrexate was severe central neurological toxicity in five patients (10%), with no acute renal failures requiring hemodialysis or severe mucositis requiring total parenteral nutrition. Myeloablative allo-SCT was performed in 26 patients (17 related and 9 unrelated donors) with a transplant-related mortality (TRM) of 19%, mostly due to acute graft-versus-host disease (GVHD). Chronic GVHD occured in 15% of patients. Finally, OS was 77% (95% CI 60-85), while EFS was 71% (95% CI 55-79) in our series at 2 years. At 5 years, OS estimated 60% (95% CI 48-77), and EFS 59% (95% CI 46-75), significantly higher in standard risk patients (71%, 95% CI 57-88) compared to high risk

patients (29%, 95% CI 12-71) (p=0.04). Two patients died during induction (intracranial bleeding, septic shock), one patient during consolidation (severe neurotoxicity), TRM was the cause of death in 5 patients, while the remaining 10 patients died from relapse. With a median follow-up of 67 months for living patients (range 12-135), we observed two secondary breast cancers as late effects.

Conclusion: We report the largest consecutive series of patients treated with HOVON 70 in a single institution. Our results are in concordance with the original HOVON trial, showing similar feasibility, toxicity, rates of CR, OS and LFS at 2 years. Moreover, we are the first to report long-term outcomes of this protocol, which seem comparable to different pediatric-inspired protocols from other international study groups.

P070

Results of the Russian Acute Lymphoblastic Leukemia study- RALL-2009 - provided a basis for further de-escalation of consolidation phase in a new RALL-2016 study with centralized MRD-monitoring and randomization for auto-HSCT with non-myeloablative conditioning for adult Ph-negative ALL patients

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Background: MRD-tailored therapy based on pediatric-inspired intensification is a back-bone of the majority of the European study groups in adult ALL. Up to 50-70% of patients from the high risk group defined by specific molecular markers and MRD persistence are being now transplanted from allogeneic donors. These approaches together with the incorporation of some new drugs (Nelarabine, PEG-asparaginase, blinotumomab) provide ~50-55% 5-years overall survival (OS) in a total 16-55y ALL cohort. The aim of the RALL-study group was to create the protocol that could be easily reproduced in any regional center, to reduce toxicity with the preserving high efficacy.

Materials and patients: The Russian multicenter ALL-2009 trial defined as non-intensive but non-interruptive approach was conducted since Apr 2009 till Dec 2016 and included 330 Ph-negative ALL pts with a median age 28 y (15-55) (BCP-ALL-195 pts, T-ALL-125, biphenotypic-4, unknown-6). Only 7% of pts were transplanted in 1CR from allogeneic donors. Late intensification with autologous HSCT after non-myeloablative BEAM conditioning with following maintenance was proposed for all T-ALL. So 42 of 104 CR T-ALL pts were transplanted at a median time of 6 months of CR (land-mark for chemotherapy group). Allo-HSCT was indicated only in t(4;11) and CR pts after induction failure. MRD monitoring was not performed.

Results and discussion: The induction death rate was rather high in RALL-2009 – 8,8%(n=29), refractory ALL was registered in 16 pts(4,8%), CR was achieved in 285 pts(86,4%). Death in CR occurred in 6%, of some pts after Methotrexate 1,5 g/m2 1 day plus dexa 3 days). Regardless of non-intensive approach the total death rate reached ~15%. OS and disease-free survival (DFS) at 7-years constituted 54,7% and 57% for BCP-ALL and 61% and 68% for T-ALL, respectively. In a land mark analysis, DFS in T-ALL pts, who underwent autoHSCT (n=42) and did not (chemotherapy only, n=46), differed substantially in favor of autoHSCT: 91% vs 56% (p=0,003). Among 21 relapses in T-ALL pts 8 occurred very early, less than 6 mo of CR, and 3 were isolated CNS relapses.

Taking in consideration the major pitfalls of RALL-2009 (high CR death rate, early CNS relapses in T-ALL, selection bias in autoHSCT vs chemotherapy comparison, absence of MRD monitoring) we developed a new RALL-2016 protocol. One day high-dose MTX and high-dose ARA-C blocks were eliminated and substituted by 2 months of non-interruptive therapy, L-asparaginase was scheduled for 1 year of treatment instead of 2,5y, 15 intrathecal injections were increased up to 21 mostly while consolidation phase, CR T-ALL pts were brought to randomization after the informed consent: autoHSCT vs no autoHSCT, with the similar further maintenance. All primary bone samples are collected and tested for cytogenetics and molecular markers, all included pts are MRD monitored by flow cytometry in a centralized lab. The RALL-2016 study started in Jan 2017.

Within 1 year of RALL-2016 study 44 adult Ph-negative ALL pts from 5 centers were included: median age 36 y (22-53) (BCP-ALL-26(59%) pts, T-ALL-17(39%), biphenotypic-1(2%)). Induction results are evaluable in 37 pts: 32(86,5%) achieved CR, 4 died during induction(10,8%), 1(2,7%) was resistant. 12 patients with T-ALL after CR achievement were randomized for chemotherapy or autoHSCT: 5 and 7 pts, respectively. So far 6 of 7 T-ALL pts were transplanted at a median time of 6 months of CR. Long-term results will come in some year

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P071

Allogeneic stem cell transplantation for acute lymphoblastic leukemia in Slovak republic

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During the years 2011-2017 we performed 57 allogeneic hematopoietic stem cell transplantation (HSCT) for 53 patients at Department of Hematology and Transfusion Medicine, University Hospital in Bratislava. The median age was 30 years (15-58), male to female ratio was 1.65 and B-ALL to T-ALL ratio 1.9. The majority of patients were treated with GMALL protocol and 81% of patients underwent allogeneic HSCT in first complete remission. We used mainly myeloablative conditioning (TBI plus cyclophosphamide) in 95% of HSCT. Retransplant occured in 4 cases for relaps of the disease. Median time from diagnosis to allogeneic HSCT was 6 months (4-51). In Slovak republic we are still mising molecular examination for minimal residual disease and therefore to every fit patient with ALL is offered allogeneic HSCT. Allogeneic HSCT underwent 8 patients in standard risk (SR), 26 patients in high risk (HR), 16 patients in very high risk (VHR) and 3 patients in unknown risk (missing data). We performed 35 HSCT from matched unrelated donor (HLA 10/10 – 17, HLA 9/10 – 16, HLA 8/10 – 2), 21 HSCT from HLA-identical sibling and 1 HSCT from haploidentical donor.

We achieved acceptable transplant-related mortality, in 1 year after HSCT 22%. 5-year overall survival rate was 55%. 5-year survival rate in SR was 100%, in VHR 58% and in HR 39%.

POSTER PRESENTATIONS

P072

The fetal target cell for MLL rearranged leukaemia: implications for the origins of infant ALL

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Background: Infant acute lymphoblastic leukaemia (iALL) originates *in utero*[1] and despite best efforts to optimise treatment, remains an aggressive disease with dismal prognosis[2]. *MLL* gene rearrangement is often sufficient to cause leukaemic transformation without additional genetic abnormalities in the majority of cases[3]. The fetal target cell for development of iALL is poorly understood. We have previously identified a novel population of PreProB progenitors (CD34+CD19+CD10-) in second trimester fetal liver (FL)[4] that is further expanded in fetal bone marrow (FBM)[5], and co-exists with adult-type CD34+CD19+CD10+ ProB progenitors. We hypothesise that PreProB progenitors are transient fetal B progenitors that proliferate in a developmental stage and site-specific manner and are likely to be the target for MLL rearrangements in a defined prenatal time window.

Aims: We set out to characterise the novel PreProB progenitors, with a view to understanding the origins of infant ALL.

Methods: FBM haematopoetic stem and progenitor cells (HSPC) were characterised using multiparameter flow cytometry; differentiation and clonogenic assays; flow cytometry-based cell cycle assays; and PCR-based methods for IgH rearrangement detection. Gene expression profiling included transcriptomic analysis performed by bulk RNASequencing of flow-sorted HSPC as well as single cell RQ-PCR of the same populations using a 96-gene panel. The gene expression profile of FBM HSPC was compared to that of iALL samples.

Results: All stages of B cell development were demonstrable in human fetal BM, with PreProB progenitors present at a significantly higher frequency compared to other tissues. They were most abundant in late first trimester/ early second trimester FBM and were functionally and transcriptomically distinct from ProB progenitors. While both PreProB and ProB progenitors gave rise to B cells but not myeloid, T or NK cells *in vitro;* PreProB progenitors could generate ProB progenitors but not *vice versa*. Single cell gene expression analysis demonstrated a differentiation trajectory from HSC to mature B cells with PreProB progenitors falling between lymphoid primed multipotent progenitors (LMPP) and ProB. PreProB progenitors had no or incomplete IgH rearrangements and their chromatin accessibility pattern was distinct from ProB progenitors and B cells. Comparison of FBM progenitor populations with iALL samples showed that PreProB

progenitors closely matched iALL blasts immunophenotypically, and that their transcriptomic profile was most similar to iALL blasts with *MLL* rearrangements.

Conclusions: Detailed immunophenotypic, functional and molecular studies allow us to characterise a fetal PreProB progenitor population distinct from adult type ProB/ PreB progenitors. Gene expression data, *in vitro* assays and the lack of complete IgH rearrangements place PreProB progenitors upstream of ProB progenitors in the B cell developmental hierarchy. This population rapidly proliferates in FBM during a specific developmental window, and based on transcriptomic and chromatin accessibility data, may be a substrate for leukaemia initiating hits in iALL.

P073

TP53 somatic mutations as early leukemogenic events in acute lymphoblastic leukemia

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Introduction: Alterations of the tumor suppressor gene *TP53* are observed in 15-20% of patients with acute lymphoid leukemia (ALL) and are associated with resistance to standard treatment regimens and inferior survival. *TP53* mutations (*TP53*^{mt}) have been reported as an early leukemogenic event in patients with acute myeloid leukemia. In ALL however, only limited data from our case study are available, where we reported that *TP53*^{mt} may also be a pre-leukemic event in ALL.[1] To validate this finding, we retrospectively analyzed the presence of the *TP53* somatic mutations during treatment in the group of 43 adult ALL patients harbouring *TP53*^{mt} at the diagnosis and compared the *TP53* mutation burden to kinetics of classical IG/TR minimal residual disease (MRD).

Methods: *TP53*^{mt} allele-burden was analysed in bone marrow and peripheral blood samples of 43 ALL patients. The patients received induction/consolidation treatment according to the GMALL 07/03 and GMALL register protocol. For each patient *TP53* mutations were analysed in at least one diagnostic/MRD-positive (n=54) and one MRD-negative sample before the allotransplantation, if available (n=41). *TP53*^{mt} was validated by Sanger sequencing and quantified using molecular barcoding (unique molecular identifier-UMI) by next generation sequencing (Assay sensitivity: $5x10^{-3}$) and allele-specific ddPCR. MRD was monitored using real-time quantitative PCR of clonal immune gene rearrangements (Assay sensitivity: $1x10^{-5}$) and multiparameter flow cytometry.

Results: In our series of 43 *TP53*mt ALL patients (B-ALL 90.7%, T-ALL 9.3%) MRD analysis revealed quantifiable IG/TR MRD persistence in 7 patients (16.3%), complete MRD remission in 30 patients (69.7%), six and low level MRD-level MRD persistence in 6 patients (14.0%). In 9 patients (20.9%) the *TP53* mutation was detected at levels between 2.0 and 17.4% (median 8.1%) despite IG/TR MRD negativity, pointing to the presence of *TP53*^{mt} in a pre-leukemic

compartment. Interestingly, one patient who relapsed after two years with a fully unrelated IG/TR rearrangement profile harbored the same *TP53* mutation as was detected in the diagnostic sample, pointing to the involvement of the *TP53*^{mt} in the ALL reoccurrence. In group of 7 MRD-persisters, *TP53* mutations were present in 4 patient samples. Out of discordant samples MRD-load was below the *TP53*-assay sensitivity level in 2 patients and one MRD-positive follow up sample remained negative for the *TP53*^{mt} despite the 1.9% tumor load. Further analysis, including the analysis of *TP53*^{mt} in sorted progenitor and mature cell compartments of MRD-negative samples and the correlation of clinical outcome with the observed mutation pattern are ongoing.

Conclusion: Hereby, we validate our initial findings and show that a considerable number of adult patients with *TP53*^{mt} ALL carry a *TP53* mutation in pre-leukemic cells, causing clonal expansions in different hematopoietic compartments and thus persisting in MRD negative follow-up samples. Considering *TP53* somatic mutations as MRD-marker requires careful interpretation, as it can also persist in pre-leukemic cells and not necessarily reflects the ALL tumor load. Understanding the prognostic value of this mutation pattern requires further analysis in a larger patient cohort.

P074

In vivo blocking of NG2 antigen mobilizes MLLr B-ALL cells to PB, making blasts more sensitive to VXL treatment leading to a delayed relapse of treated mice

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Background: MLL-rearranged (MLLr) infant B-cell acute lymphoblastic leukemia (iMLLr-B-ALL) has a dismal prognosis and is associated with a pro-B/mixed phenotype, therapy refractoriness and frequent central nervous system (CNS) disease/relapse. Neuron-glial antigen 2 (NG2) is specifically expressed in MLLr leukemias and is clinically used because of its predictive value for MLLr acute leukemias. However, its role in MLL-mediated leukemogenesis remains elusive. We previously reported in an Interfant patient cohort that high expression of NG2 associates with lower EFS, higher WBC and more frequent CNS disease/relapse. Furthermore, we showed that NG2 is a malleable marker not enriching for leukemia-initiating cells. Importantly, NG2 expression was highly upregulated in blasts infiltrating extramedullar hematopoietic sites and the CNS

Aim: We suggest that NG2 may be a potential therapeutic target to reduce the risk of CNS disease/relapse.

Methods: For *in vivo* experiments, blasts from ALL patients were transplanted intravenously in NSG mice. Leukemic mice (≥ 0.5% blasts in peripheral blood) were treated with Vincristine, Dexamethasone and L-asparaginase (VXL). For chemoresistance, NG2+ and NG2- sorted cells were incubated for 48 hours with VXL and viability was measure by Annexin V.

Results: Here we demonstrate that *in vivo* blocking of NG2 promotes the exit of MLLr blasts from BM to PB, suggesting a role for

the extracellular domain of NG2 in retaining MLLr blasts within the BM likely through survival-promoting interactions with the BM stroma. *In vivo* co-treatment with both NG2 inhibitors and VXL (Vincristine, Prednisone and L-Asp) results in 3-fold decreased levels of minimal residual disease. Importantly, follow-up of mice in (near)-complete remission evidenced that co-administration of NG2 inhibitors with VXL significantly delayed mice relapse. Finally, preliminary studies suggest that NG2+ blasts are more resistant than NG2- blast to GC. In addition, NG2 promoter has binding sites for GC receptors, and treatment with dexamethasone results in increased levels of NG2, likely reflecting a NG2-mediated mechanism to escape GC toxicity.

Conclusion: Collectively, our data propose NG2 antigen as a therapeutic target in MLLr-B-ALL using either antibody-based or CAR T-cell-based immunotherapy.

P075

Reducing dexamethasone concentration by combination with JAK or XPO-1 inhibitors for the treatment of acute lymphoblastic leukemia.

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Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer, with 85% of ALL being of B-cell origin and 15% of T-cell origin[1,2]. Survival of ALL patients has significantly improved by the use of intensified chemotherapy, improved supportive care, and precise risk group stratification. Despite the high survival rates for pediatric ALL, incurable relapses and toxic deaths remain major challenges. Moreover, treatment with multi-agent chemotherapy is associated with short- and long-term side-effects.

Recent insights into the biology of ALL have identified novel targets for therapy such as the JAK/STAT pathway and exportin-1 (XPO-1), for which small molecule inhibitors exist and are being evaluated in clinical trials for the treatment of other malignancies[3,4]. In this study, we tested combinations of the JAK inhibitor ruxolitinib or the XPO-1 inhibitor KPT-8602 in combination with dexamethasone, vincristine and doxorubicin. We hypothesized that such combination could reduce the dose of toxic agents and concomitantly reduce the side effects. If synergistic dose combinations were identified, we aimed to elucidate the molecular mechanisms underlying the synergy.

We have used B- and T-ALL cell lines to screen for synergistic interactions and identified a synergistic interaction between dexamethasone and KPT-8602 in several cell lines (independent of genotypes) and synergy between dexamethasone and ruxolitinib in the IL7R mutant cell line DND41. These drug combinations reduced proliferation and increased apoptosis in the sensitive cell lines. We further validated these findings using clinically relevant patient-derived xenograft (PDX) samples transduced with luciferase to monitor disease progression in a non-invasive way via bioluminescent imaging. The drug combinations showed synergistic effects on ex vivo cultured PDX samples, and reduced the growth of the PDX samples in vivo in

immune deficient mice significantly more than each drug alone.

Our data show that it is possible to reduce the dose of dexamethasone by combining it with targeted therapy such as JAK or XPO-1 inhibitors. Since these agents are already approved or in clinical trials it should be feasible to test such combinations soon in clinical trials for ALL.

P076

Predictive Outcome and translocation of BCR/ABL rearrangements by interphase fluorescence in situ Hybridization (FISH) in CML and ALL

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Objective: To compare the signal patterns of dual color dual fusion BCR/ABL probe (D-FISH) in the fluorescence in situ hybridization (FISH) detection for CML and ALL, and to explore their diagnostic and prognostic value

Introduction: BCR/ABL fusion gene usually as a result of Philadelphia(Ph) translocation between chromosomes 9 and 22 in Chronic Myeloid leukemia (CML) as well as Acute Leukemia(ALL). This rearrangement results in the formation a chimeric BCR/ABL fusion gene on the derivative chromosome 22. Fluorescence in-situ hybridization (FISH) analysis using dual color BCR/ABL translocation probes allows the visualization of BCR/ABL rearrangements in both interphase and metaphase cell, and the presence of the BCR/ABL fusion gene on chromosomes 22 have been reported in substantial subset of these patients.

Method: The incidence of both classical and variable BCR/ABL gene rearrangement was determined in 860 patients suspected of CML and ALL using dual fusion fluorescence in situ hybridization (DF-IFSH) probes

Result: This study investigated 860 patients of CML and ALL enrolled between January 2016 and September 2016 at the Aga Khan University Hospital .Out of 860 patients 775(90%) were diagnosed as CML and 85 cases (10%) were diagnosed ALL. About 659 cases (76%) of both CML and ALL patients displayed the classical DF-FISH signal pattern and 201(24%) of CML and ALL shown variable DF-FISH signal pattern. In variable DF-FISH signal various different pattern were analyzed. In these variable patterns 1F1G1R is 31%, 1F2G1R is 27%, 1F2G2R is 26% and 1F1G2R is 14% were observed in both CML (22%) and ALL (2%). There is also are rare combination of classical and variable which is around 2 % seen in CML.

Conclusion: The classical pattern were typically seen in CML but also reported in ALL. The variable pattern there is equal proportion of 1F1G1R, 1F2G1R, and 1F2G2R but in comparable 1F1G2R is less. Patients having genetic alteration with loss of 9q and 22 q sequences may be associated with poor prognosis and the time to disease progression Glivec treatment shorter. Hence establishment of signal pattern with FISH is important as atypical patterns may have clinical diagnostic and prognostic implications

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P077

Novel targeted therapies for resistant childhood acute lymphoblastic leukemia

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Background: During recent decade, the event-free survival of childhood acute lymphoblastic leukemia (ALL) has improved up to 80%, but there are still subgroups with a dismal prognosis. This potentiates the need to develop novel therapeutic compounds, with a low toxicity profile. We, therefore, aimed to develop and characterize novel precision compounds, which target oncogene stabilization by HSP90 and by the proteasome-HDAC6 axis.

Aims: We developed an HSP90 inhibitor which is active as a pan-leukemia inhibitor against LSCs without the induction of any HSR and a dual proteasome-HDAC6 hybrid inhibitor, with sensitivity in BCR-ABL1+ TKI resistant BCP-ALL.

Methods: The specificity of aminoxyrone was evaluated by microscale thermophoresis (MST), cell-based luciferase refolding assay, 2D NMR spectroscopy, circular dichroism (CD) spectroscopy, analytical ultracentrifugation and molecular dynamics simulations.

Autodock 4.2 and co-crystal structure were performed to predict the binding mode of RTS-V into the β5/β6 active site of 20S yeast proteasome (PDB ID: 3MG8) and HDAC6 (PDB ID: 5EDU).

Results: HSP90 act as molecular chaperone and is highly expressed in several therapy-resistant leukemia subtypes thereby ensuring correct protein folding of several oncogenic proteins such as BCR-ABL1, FLT3-ITD and AKT. Therefore, targeting HSP90 could be a promising option in the treatment of therapy-refractory leukemia. Majority of available HSP90 inhibitors target the N-terminal domain thereby induce a protective mechanism called heat shock response (HSR), which potentially weakens the cytotoxic effect of HSP90 inhibitors and induce toxicity. We have developed first in class HSP90 inhibitor 'aminoxyrone' through structure-based molecular design and chemical synthesis which specifically targets C-terminal dimerization of HSP90. Aminoxyrone is effective in preclinical TKI (2nd and 3rd generation) resistant cell line models *in vitro* and *in vivo*, induces apoptosis in primary BCR-ABL1+ BCP-ALL and in Ph-like BCP-ALL patient-derived LSCs, without inducing any HSR.

We furthermore developed a novel dual proteasome-HDAC6 hybrid inhibitor 'RTS-V'. Aggresomes are used by many malignant cells as an alternative route to degrade proteins that accumulate after proteasome inhibition. However, this mechanism depends on HDAC6 to transport ubiquitinated proteins by microtubules. Thus, inhibition of the proteasome and HDAC6 results in synergistic anticancer activity by induction of apoptosis in cancer cells. We found out that RTS-V specifically blocks chymotrypsin-like proteasome activity and at the same time causes preferential inhibition of HDAC6. The screening of RTS-V in more than 20 cell lines (including TKI-resistant BCP-ALL) and in primary relapse BCP-ALL samples revealed >20-fold higher specificity of RTS-V against leukemic cells as opposed to healthy PBMNCs. Besides that, we could show that in selected leukemic cell lines RTS-V specifically inhibits cell proliferation, induces apoptosis, accumulates cells in S phase, induces early differentiation and inhibits colony formation. Our next aim would be to test its efficacy in *in vivo* studies.

Conclusion: Taken together, 'aminoxyrone' and 'RTS-V' represents a promising starting point for future efforts towards the development of novel targeted inhibitors to overcome drug resistance and reduce toxicity, especially for the treatment of relapsed/refractory ALL.

P078

Clinicopathologic Effect of DNMT3A Mutation in Adult T-Cell Acute Lymphoblastic Leukemia

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Background: DNA extraction, amplification with sequencing analysis using the 310 ABI genetic analyzer for detection of a mutation (R882H) in 64 patients with T-cell acute lymphoblastic leukemia (T-ALL) at diagnosis. DNMT3A is frequently mutated among T-ALL patients and has been associated with a poor prognosis. These findings could help in risk stratification and treatment choice for patients with T-ALL.

Aim: The present study aimed to determine the frequencies and clinicopathologic effect of a DNMT3A [DNA (cytosine-5)-methyltransferase 3A] mutation in patients with adult T-cell acute lymphoblastic leukemia (T-ALL).

Methods: A total of 64 patients with T-ALL who had been admitted to Mansoura University Oncology Center were included in the present study. For all patients, DNA extraction and amplification with sequencing analysis using the 310 ABI genetic analyzer for detection of a mutation (R882H).

Results: The DNMT3A mutation (R882H) was found in 12 of the 64 patients (18.8%). The DNMT3A mutation was frequently detected in the older age group and was associated with high leukocytic counts, a high bone marrow blast cell percentage, and the frequent presence of extramedullary disease. However, it was not associated with the hemoglobin level, red blood cell count, or platelet count. The patients with mutant T-ALL had a low tendency to achieve remission after induction. These patients had significantly shorter overall survival and shorter disease-free survival compared with those with wild-type T-ALL (P = 0 .037 and P = 0.006, respectively).

Summary and Conclusion: DNMT3A is frequently mutated in T-ALL and is associated with distinct clinicopathologic entities and a poor prognosis. These findings could help in risk stratification and treatment decisions for patients with T-ALL.

P079

LONG TERM OUTCOME OF 138 ALO TRANSPLANS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA ON A SINGLE TRANSPLANT CENTER IN SPAIN

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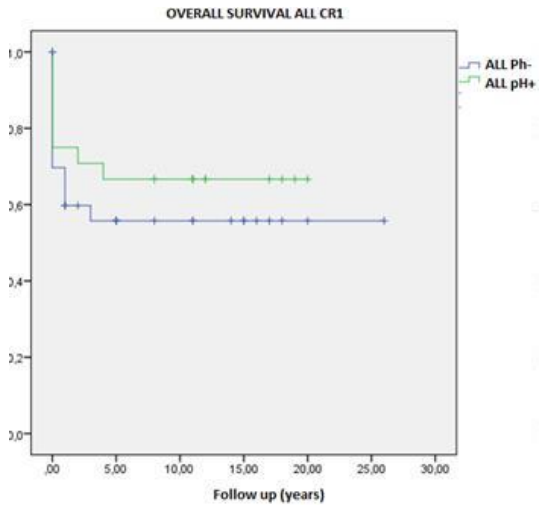
Background: Leukemia relapse remains the main cause of failure for long term survival in adult ALL, despite high rates of first complete remission (CR1) after induction and consolidation with risk adapted modern chemotherapy schemes. Further consolidation with allogeneic hematopoietic stem cell transplantation (HSCT) offers the best chance of cure for high-risk and relapsed patients. Long term follow of large patient cohorts should be evaluated for better definition of alo transplant indication and timing.

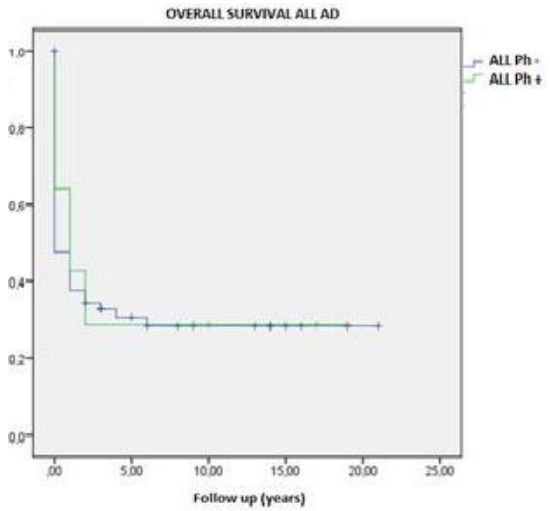
Aims: The primary endpoint was disease-free survival (DFS) and overall survival (OS). Secondary endpoints were acute graft vs. host disease (aGvHD) and chronic GvHD (cGvHD) incidence, relapse incidence (RI), and non-relapse mortality (NRM).

Methods: Between 1990 and 2016, 138 HSCT were performed in 128 patients with ALL in our hospital (10 double alloHSCT; 7,2%). Median age was 28 years (12-65). 76% of patients were younger than 40 y. 27,5% of cases (n=38) were Ph+ALL. Disease status before transplant was distributed as follows: 42% CR1, 60,5% (n=23) Ph+ALL and 35% (n=35) Ph-ALL. 58% had advanced disease (AD = ≥RC2, MRD+, or active disease), 39,5% (n=15) Ph+ALL and 65% (n=65) Ph-ALL. Bone marrow (BM) progenitors were initially the preferred HSCT source (65,2%), but changed to peripheral blood (PB) after 2001. Antithymocyte globulin was administered with conditioning in case of HLA disparity (n=13; 9,4%). Patients, disease, and HSCT characteristics are shown in table 1.

CHARACTERISTIC	RESULTS
Median age (years)	28 (12-65)
40 years	105 (76%)/33 (24%)
Gender (male/female)	77 (55,8%) / 61 (44,2%)
Ph+ / Ph- ALL	38 (27,5%) / 100 (72,5%)
B ALL/ T ALL/ biphenotypic AL	113 (81,9%)/18 (13%) / 7 (5,1%)
Years of HSCT: 90-2000/01-08/09-16	61 (44,2%) /57 (41,3%) / 20 (14,5%)
HSCT source: BM/PB/CB	90 (65,2%)/46 (33,3%)/2 (14,5%)
Conditioning regimen: CyTBI/CyBu/other	108 (78,1%) / 17 (11,7%) /14 (10,2%)

Results: With a median follow up of 11 years (21 days-26 years), the whole cohort disease free survival (DFS) an overall survival (OS) rates were 37,7% and 42,8%, respectively. OS and DFS for patients who underwent HSCT in CR1 were 66,7% and 66,6% ,for Ph+ALL and 57,6% and 51,4% for Ph-ALL. DFS and OS in AD were similar in Ph+ and Ph- ALL: 28,6 and 13,3% and 30,8 and 24,6 %, respectively. Global incidence of acute graft versus host disease (aGvHD) was 32,6% (n=45) and 29% for chronic GVHD (n=40). Twenty six cases (18,8%) developed extensive cGvHD. Global relapse rate was 36,2% (n=50) occurring at a median time of 6 months after transplant. Late relapse occurred very rarely after 3 years post HSCT (n=3). The incidence of early non-relapse mortality (NRM <100 days) was 18,8%(n=26). Causes of death included pneumonia or respiratory failure (n=11; 36,7%), sinusoidal obstructive syndrome (SOS) (n=4; 13,3%), septic shock (n=3; 10%) and acute GvHD (n=2; 6,7%).





Focusing on the cases with available minimal residual disease (MRD) information by flow cytometry (FCM) before HSCT in Ph- ALL , there were 6 relapses in the 13 positive cases (MRD ≥0,01%) whereas there was no relapse in the 12 negative (MRD <0,01%) cases (46% vs. 0%).

In the 10 patients with a 2nd HSCT (n=10), only 1 case was a Ph+ALL, three had a complex caryotype (30%) and 4 had relapsed with extramedular disease. Two patients remain alive. The main cause of death was relapse (n=5; 50%) followed by SOS (n=3; 30%). MRD before HSCT was available in 4 four cases, being all of them positive (1 molecular MRD + in Ph+ALL and 3 FCM ≥ 0,01%).

Conclusions: Our series shows a quite high, over 65%, long term DFS, for both Ph+ and Ph- ALL in patients who underwent HSCT in CR1, demostrating the high curability of alo transplant when applied after first line chemotherapy. However it also shows that a fair proportion, 25-30%, of advance disease patients could also be cure by 25-30%.

P080

LIGANDS FOR INHIBITORY AND ACTIVATING NK CELL RECEPTORS IN CHILDHOOD B- AND T-ALL

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Background: Natural killer (NK) cells are lymphocytes of the innate immune system specialized in the recognition and lysis of tumours and virus-infected cells. Function of NK cells is finely tuned by the balance of signals delivered from inhibitory (iRec) and activating (aRec) receptors that recognize their ligands on target cells. The level of HLA class I (HLA-I) molecules expressed by the leukemic cells is one of the most important factors influencing NK-mediated lysis of paediatric blast cells. Consistent with the "missing self" hypothesis, down regulation of HLA class I can render tumour cells valid targets for NK cell mediated lysis. Efficient lysis requires interaction of additional activating receptors such as DNAM-1 (CD226) and NGK2D with their ligands on tumour cells such as Nectin-2 (CD112) and Poliovirus receptor (CD155) for DNAM-1, and Major histocompatibility complex class I-related Chain molecules A/B (MICA/B) and UL1b-binding protein (ULBPs) for NKG2D. It has been described that HLA-I down-regulation is more frequent in myeloid than in lymphoblastic leukaemias and

that expression of CD112 and CD155 is consistent in myeloid leukaemias but MICA/B and ULBPs were either absent or weakly expressed.

Aims: To evaluate the role of inhibitory and activating NK-cell/tumour interactions on childhood acute lymphoblastic leukaemia (ALL) at diagnosis.

Patients and methods: The expression of ligands for inhibitory (total HLA class-I and HLA-C) and for activating (CD112, CD155, ULBP1 and MICA/B) receptors was evaluated in 45 paediatric patients at diagnosis (36 B-ALL and 9 T-ALL) by flow cytometry on a FACSCanto, both on bone marrow tumour cells and on residual lymphocyte as a control of basal expression. Percentage and mean fluorescence intensity (MFI) was evaluated for each receptor.

Results: Higher expression of CD112 as percentage (65.9% vs. 6.9%, $p<0.01$) and as MFI (1696 vs. 287, $p<0.01$) was detected for B-ALL blast than for normal lymphocytes. Higher number of CD155+ cells was observed in T-ALL blast than in normal lymphocytes (31.5% vs. 13.49%, $p<0.05$). No differences in the expression of MICA/B and ULBP were observed between B or T ALL blast and normal lymphocytes. No differences in the expression of HLA-I (MFI: 25439 vs. 19539) and slight reduction in HLA-C (MFI: 10909 vs. 12865) was detected for B ALL blast compared to normal lymphocytes. Remarkably, lower expression of both HLA-I (MFI: 7817 vs. 18114, $p<0.05$) and HLA-C (MFI: 4124 vs. 10010, $p<0.05$) were observed in T-ALL blasts than in normal lymphocytes.

Conclusion: Differential expression of ligands for NK cells activating and inhibitory receptors in B- and T-ALL childhood blast could condition NK cell antitumor response and should be taken in consideration in NK immunotherapy protocols.

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P081

A retrospective cohort study of adolescent and young adult patients (15-25years) with acute lymphoblastic leukemia (ALL) treated on modified BFM 90 ALL protocol and the prognostic value of minimal residual disease assessment.

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Background: Adolescent and young adult patients with ALL remain an underrepresented cohort in clinical trials. Their outcomes are inferior to those seen in the pediatric population. This age group poses unique challenges with respect to disease biology, social issues, non-compliance and poor tolerance to chemotherapy. Even then it has been seen that intense pediatric protocols produce better results compared to the adult regimens. The feasibility and efficacy need to be tested in a real-world setting wherein <5% patients are insured and <10% patients undergo allogeneic stem cell transplant. We have analyzed the survival outcomes of patients treated at our center and looked at the prognostic value of bone marrow minimal residual disease (MRD) assessment.

Material and methods: This is a retrospective cohort study of newly diagnosed adolescent and young adult ALL patients (15-25years) treated at Tata Memorial hospital

from January 2011 until December 2017. We see 300 new cases of ALL registered annually, treatment is given as an outpatient for the most part, except for the high dose methotrexate phase. Close to 90% of our patients are supported through various funding agencies. Patients treated with the modified BFM90 regimen were included. Epidemiological details, treatment details, toxicities, responses including bone marrow minimal residual disease status were recorded. Descriptive statistics were used to summarise the epidemiological data, responses, and toxicities. Bone marrow responses were defined using the CALGB criteria. MRD assessment was done using multi-color flow cytometry. MRD assessment was done at the end of phase I of induction and in those who were MRD positive defined as ≥ 0.01% it was repeated either after the phase II or consolidation phase. Survival was calculated using Kaplan-Meier method. Overall survival was calculated from the date of registration till last follow up or death due to my cause. Patients who were lost to follow up with residual disease were censored as an event. Relapse-free survival was estimated from the date of registration till relapse or death due to any cause. Survival outcomes based on the post-induction MRD status and the last available MRD were compared using log-rank test.

Results: A total of 381 patients were treated during this period. Baseline features are summarised in table 1. There were 16(4%) induction deaths. At the end of induction-330(86%)patients were in remission,18(5%) were not in remission and 33(9%) were not evaluable. MRD status post phase I induction- Negative in 186(48%), positive in 107(28%) and not evaluated in 88(24%). On subsequent MRD assessment- Negative in 222(58%), positive in 75 (20%) and not evaluated in 84(22%). Cranial irradiation (12.4 Gy-Prophylactic and 18 Gy- Therapeutic) was administered to 258 (68%) patients. 40 patients (10%) underwent allogeneic stem cell transplant. Toxicities that could be retrieved from medical records included- Cortical venous thrombosis-24 (6%), culture positive infections- 100 (26%) and suspicious fungal nodules in 113 (29%) patients.There was a total of 45 (12%) Deaths out of which 24 were due to disease and 21 due to toxicities. There were 57(15%) relapses. The median follow-up of the cohort is 25 months. The projected 4-year RFS is 70% and 4-year OS is 63%. There was a difference in survival outcomes based on the MRD.

Conclusions: AYA ALL treated on a pediatric protocol produce excellent results even in the real world setting.

Variable	Number
Age, years- Median(Range)	18(15-25)
Gender Males n (%) Females n (%)	300(79) 81(21)
Hyperleucocytosis, n (%)	40(12)
Cerebrospinal fluid Uninvolved n (%) Involved n (%) Not done n (%)	346(91) 27(7) 8(2)
Cytogenetics by FISH, n (%) Negative n (%) Not done n (%) Ph positive n (%) Trisomies n (%) MLL translocation n	152(40) 45(11) 36(09) 39(10) 04(01)
Subtype T ALL n (%) B ALL n (%)	151(40) 230(60)

P082

Survival of Acute Lymphoblastic Leukemia: Experience of a Single Center in Colombia

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Background: Acute lymphoblastic leukemia (ALL) is a heterogeneous group of hematologic disorders characterized by malignant transformation and proliferation of

lymphoid progenitor cells (from B-precursor or T-precursor lineage) in bone marrow, periphery blood, and extramedullary sites [1]. The incidence of ALL has a bimodal distribution; the first peak incidence occurs between two and five years of age, and the second peak occurs around 50 years of age. ALL is the most common type of cancer in children in the United States and the second most common acute leukemia in adults. Survival of ALL has increased as a result of advances in management efficacy as well as risk stratification to determine the most appropriate treatment regimen. The aim of the present study is to assess survival rate for patients with diagnosis of ALL in a single center in Colombia, South America.

Methods: A retrospective cohort study was conducted at a tertiary referral center in Colombia on 73 patients who were diagnosed with ALL between July 2013 and November 2017. All patients were managed based on PETHEMA protocol for ALL. The Kaplan-Meier method was used to assess overall survival and relapse-free survival rates at one year.

Results: All patients were older than 18 years with a mean age of 39 years (range 18-80 years). Thirty-nine were men and 34 were women. The most common type of ALL was B-precursor lineage (86.4%), followed by T-precursor lineage (6.8%), and undetermined for lack of information in 6.8% of the patients. Of all the patients, 80.82% were classified as high risk. Subclassifying ALL by immunophenotype, the most common type was pre-B (46.57%), followed by B common (28.76%) and pro-B (8.21%). Fifty-five (76.38%) patients underwent cytogenetic studies for BCR/ABL detection; BCR/ABL was identified in five patients. Twenty-three (31.5%) patients received allogenic hematopoietic stem cell transplantation.

Overall survival was 61.5% (IC 48.7-72) at one year, and 22.9% (IC 10.5-38.1) at three years. Overall survival for those who received allogenic HSCT was 90.5% (IC 67-97.5) at one year and 63.3% (IC 29-84.4) at three years, and for those who received chemotherapy 46.7% (IC 31.6-60.5) at one year and 4.9% (IC 0.4-19.3) at three years. Relapse-free survival for those who received HSCT was 60.6% (IC 31.9-80.3) at one year and 48.5% (IC 19-73) at three years, and for those who were managed with chemotherapy was 51.1% (IC 26.4-71.3) at one year and 38.3% (IC 12.9-63.9) at three years.

There were 20 (47.61%) deaths due to either relapse or disease progression. Mortality related to treatment was low (12.32%). Fourteen patients were lost to follow-up.

Conclusions: Overall and relapse-free survival of ALL was very similar similar to those reported in the existing literature. The most common cause of death in ALL patients was primary disease, and treatment-related mortality was low.

ONLINE PUBLICATION ONLY

PB083

BURKITT LYMPHOMA: SINGLE INSTITUTION EXPERIENCE WITH BURKIMAB-13 SPANISH PROTOCOL

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Background: Burkitt lymphoma (BL) is a rare, highly aggressive B cell non-Hodgkin lymphoma characterized by the translocation and deregulation of the *MYC* gene on chromosome 8. Incidence in Europe is estimated in 2.2 cases per million persons per year. Treatment consists of intensive and short duration chemotherapy regimens including cyclophosphamide, methotrexate and cytarabine such as BURKIMAB-13, current Spanish protocol for the treatment of this disease.

Aims: Describe the incidence and characteristics of BL patients in our region and the results of BURKIMAB-13 Spanish protocol in our institution.

Methods: Between February and December 2017, six adult cases of BL have been diagnosed in our Hospital. We perform a retrospective study to describe the clinical features, incidence and outcomes of these patients.

The diagnostic techniques used included immunohistochemistry, cytomorphology, multi-parametric flow cytometry (MPFC) and FISH for the *MYC* translocation detection. *BCL2* and *BCL6* translocations were performed when the morphology and/or MPFC were atypical. Burkitt leukemia is defined by bone marrow involvement ≥ 20%.

Lumbar puncture was performed in all patients at the start of therapy to test central nervous system (CNS) involvement.

All patients were treated with the BURKIMAB-13 protocol, the protocol distinguish two arms: for patients >55 and < 55 years old and consists of the administration of 6 cycles of chemotherapy for the stages II (with bulky disease), III and IV.

Results: During the year 2017 have been diagnosed in our institution 6 cases of BL, that represents a crude incidence of BL in our health area of 12,7 cases per million persons per year.

In *table 1* are shown the patient characteristics. There were 4 male and 2 female with a median age at time of diagnosis of 55 years. Two patients were HIV seropositive, an 4 (66,66%) have CNS involvement at diagnosis. All patients had high levels of LDH and were a stage IV of Ann Arbor. The Burkitt leukaemia variant were present in 66,66% of patients.

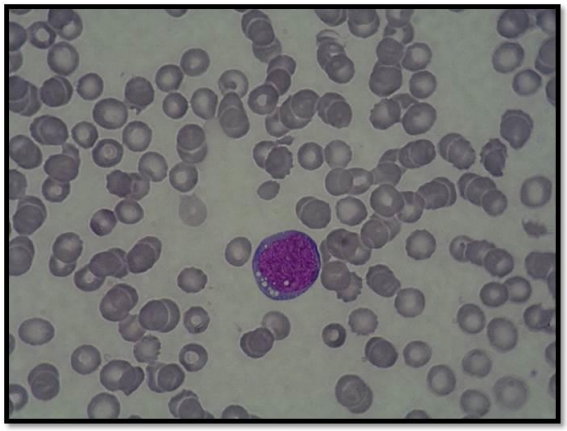
All patients had haematological and infectious toxicities grade 3-4 during the treatment BURKIMAB-13 protocol (figure 1), but any patient died because of infection complication.

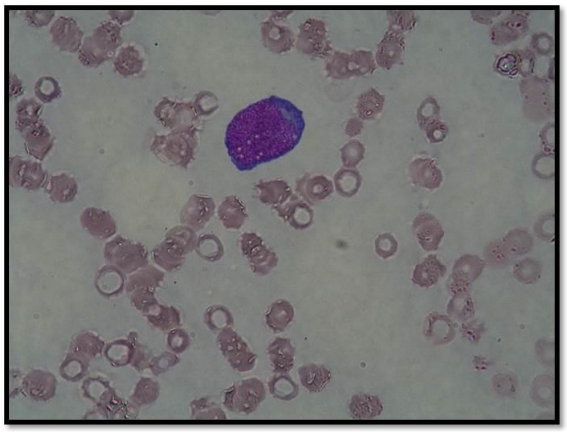
With a median follow up of 5 months (range, 0-11), 4 patients are alive, 3 in complete remission (50%) and one not valuable. The 2 patients HIV positive progressed and died. No dead in remission occurs.

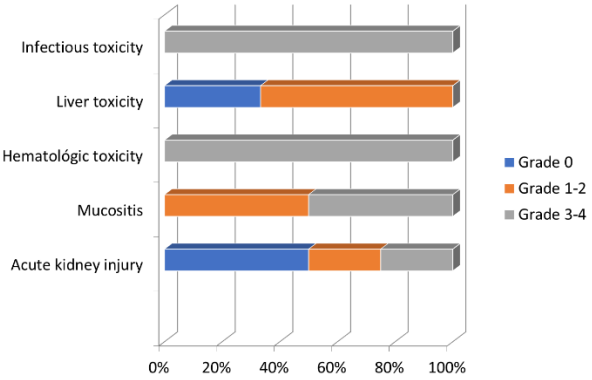
Summary: The BL is an aggressive and rare disease and haematologists have to be prepared to deal with it urgently and effectively. The unusual increase of incidence in our region be alert us in this pathology.

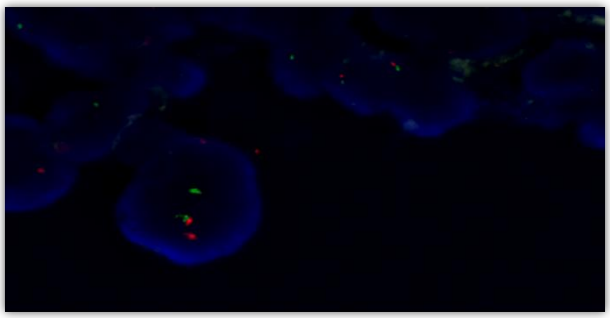
We have an optimal experience with our preliminary results and toxicities of BURKIMAB-13 Spanish protocol.

Patient Characteristics (n=6)	
Gender - no. (%) Male / Female	4 (66.66) / 2 (33.33)
Median Age - yr. (range)	55 (33-75)
Diagnosis - no. (%) Burkitt lymphoma / Burkitt leukaemia variant	2 (33.33) / 4 (66.66)
ECOG ≥ 2 - no. (%)	3 (50)
HIV - no. (%)	2 (33.33)
LDH - median (range)	4084 UI (914- 10.880)
Central nervous system involvement - no. (%)	4 (66.66)
Ann Arbor stage IV - no. (%)	6 (100)
Bone marrow involvement - no. (%)	5 (83.33)
Treatment - no. (%) BURKIMAB-13 >55. / BURKIMAB-13 <55.	3 (50) 7 3 (50)
Months of follow up - no. Median (range)	5 (0-11)
Outcomes - no (%) Complete remission / Progression / Death in remission / Death / Not valuable	3 (50) / 2 (33.33) / 0 (0) / 2 (33.33) / 1 (16.67)









PB084

Silent Cytomegalovirus Infection in Kids with Acute Lymphoblastic Leukemia: Is There a Way to Suspect and Save Resources?

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Background: Cytomegalovirus (CMV) infection may present with silent course in immune-compromised acute lymphocytic leukemia (ALL) kids (1). Only few studies traced the incidence and manifestation of CMV in ALL patients (1)(3)and concluded better outcomes in cases treated for associated CMV infection. Limited availability of CMV diagnostic tests lead to missed CMV infection treatment with subsequent flaring of the infection on starting chemotherapy resulting in high mortality (1)(2).

Aim: Our aim was to study the incidence and clinical features of CMV infection in children with newly diagnosed ALL to set a group of clinical and laboratory criteria which can support the diagnosis of CMV infection in health facilities where performing a CMV Polymerase Chain Reaction (PCR) technique for each case is limited.

Method: We ran an observational prospective study between January 2017 and July 2017 to investigate 31 newly diagnosed ALL kids at the Oncology Unit in Mansoura University Children's Hospital, Egypt to detect CMV silent infection. The diagnosis of CMV

infection was performed by measurement of serum anti-CMV specific immunoglobulin M (IgM), immunoglobulin G (IgG) titers and Polymerase Chain Reaction (PCR) method in the blood.

Results: The median age at diagnosis was 4 years (.3 years -13.5years). Males constitute 74.2% of the total cohort. CMV infection proved in 22%. The most common physical examination findings frequently observed in PCR positive cases were fever & hepatosplenomegaly (HSM) (P= 0.000). The mainstream laboratory variables tested were neutrophil count (mean= 1.3; SD= .44 in positive cases & 3.5, SD= 1.9 in negative cases), lymphocytic count (mean was 32 in positive cases & 22 in negative cases).

Conclusion: The presence of fever, HSM, low neutropenic count and high lymphocytic count together in an ALL pediatric case should raise the suspicion of an oncologist for a silent CMV infection to perform the required diagnostic tests for early detection of CMV infection and initiation of its targeted therapy promoting better outcome. This study needs to be conducted on a larger number of patients and in multi centers

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PB085

Treatment of Pulmonary Mucormycosis with Liposomal Amphotericin B and Posaconazole Followed by a Pneumonectomy in Relapse Acute Lymphoblastic Leukaemia after Allogeneic Stem Cell Transplantation

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Background: Infections are the most important cause of morbidity and mortality in hematopoietic stem cell transplant patients. Pulmonary mucormycosis associated with haematological malignancy is a rare but important invasive fungal infection and usually fatal. We present a case of allogeneic stem cell transplantation (ACST) with ALL diagnosis and recurrence in + 28th month and developing pulmonary mucormycosis after rescue treatment.

Case: A sixteen-year-old woman admitted to our clinic in December 2013 with weakness and abdominal pain. White bloodcell-count: 160x109/l, neutrophils: 0.49x109/l, a platelet count of 2.6 x1010/l, hemoglobin level: 113 g/l. Bone marrow biopsy was compatible with B-ALL (moderate risk).Hoelzer Phase I-II treatment were initiated, resulting in complete remission. She underwent allogeneic haematopoietic stem cell transplantation (AHSCT) from an HLA full-matched donor after a reduced-intensity conditioning (RIC) regimen of Flu180Bu12.8ATGf10. Cyclosporine A and methotrexate were given for prophylaxis of acute graft versus-host disease (GVHD). The total CD34-positive cells infused were 6 × 106 /kg. Neutrophil-platelet engraftment was observed on day 20 after transplantation. GVHD prophylaxis was performed with CsA and MMF. There were no early or late GVHD complications. The patient was followed up. In September 2016 (+28th months), nausea and vomiting were detected and creatine increase was detected. Urinary USG: Increased bilateral renal size and increased parenchymal thickness. The renal biopsy result: extramedullary ALL involvement. Concurrent bone marrow biopsy B-ALL. The patient was diagnosed with recurrent ALL and salvage therapy FLAG + DLI given. Control bone marrow biopsy remission. After salvage therapy when the patient complained of dyspnea and coughing, pulmonary CT was

performed: the left pleural effusion and collapsed near the lower lung lobe of the left lung was reported. Voriconazole 3 mg/kg treatment given. Lesion biopsy and bronchial lavage was performedunder bronchoscopy. Biopsy was resulted mucormycosis. Posaconazole 800 mg/day and Ambizome (5-10 mg / kg) treatment were started as antifungal therapy. Control pulmonary CT scan at 1 week of antifungal therapy; an increase in lesion size, progression was detected. Left pneumonectomy was performed because the patient did not respond to antifungal therapy. After pneumonectomy, bone marrow biopsy was remission. 2nd (AHSCT) from an HLA full-matcheddonorafterFlu (30mg/m2 for3 days) in June 2017.The total CD34-positive cells infused were 6.5 × 106 /kg. The patient was treated with posaconazole 800 mg/day and ambizome (3 mg / kg) in the course of AHSCT. Neutrophil engraftment was observed on day 16. GVHD prophylaxis was done with CsA. On control pulmonary CT at 60 days post AHSCT: no new lesion was detected. The patient is on the 90th day after transplantation and the complete remission is followed.

Conclusion: In the treatment of acute leukemia, fungal infections are often a major problem. Pulmonary mucormycosis is a rare but important invasive fungal pneumonitis. Amphotericin B is the gold standard antifungal agent used against mucormycosis. Oral posaconazole is also recommended in the treatment of mucormycosis. Surgical treatment, such as pulmonary wedge resection, lobectomy, and pneumonectomy combined with medical treatment. Early surgical treatment should be considered in cases with pulmonary mucormycosis.

PB086

Retrospective analysis of large cell B Lymphoma treated with Rituxumab combined with chemotherapy compared to chemotherapy alone.

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Introduction: Large-cell B lymphoma B (LCBL) is a heterogeneous group with 30 to 58% of non-Hodgkin's lymphoma (NHL). Its therapeutic management requires a multidisciplinary approach to improve the prognosis. The objective of this work is to report the therapeutic results according to the administration or not of the rituximab.

Material and methods: This is a retrospective study involving 22 cases of LCBL treated in the department of medical oncology at Habib Bourguiba Hospital in Sfax between 2013 and 2016. Nodal, digestive and mediastinal LCBL were treated according to NHL protocol. Bone, cutaneous and testicular lymphomas had received further treatment with radiotherapy. Brain lymphomas were treated according to the primary brain lymphoma protocol (by high-dose MTX). The median follow-up was 2.5 years.

Results: LCBL accounted for 55% of all NHL. This was extra-ganglionic LCBL in 77% (17 cases): digestive in 22% (5 cases), osseous in 13% (3 cases), mediastinal in 13% (3 cases), cerebral in 9% (2 cases), nasopharngeal in 9% (2 cases), cutaneous in 4.5% and testicular in 4.5%. There was a male predominance (59%). The average age was 52 years old. Thirteen patients (59%) had a good PS (0-1) with localized stage (stage I /

II). LDH level was normal in 11 patients. All LBGCs expressed CD20 (+). Fifteen patients had rituximab (68.2%). A complete response (CR) was found in 45.5%. It was 53% in patients who received rituximab (GR1) against 28.5% in the absence of rituximab (GR2). The mean OS was 13.4 months. It was 16.8 months and 6.29 months in case of GR1 and GR2 respectively. The one year OS was 79.5%. It was 93.3% in the GR1 against 40% in the GR2 (p = 0.025). The 1-year DFS was 65.8%. It was 75.8% in case of GR1 against 35.7% in case of GR2 (p = 0.05).

Conclusion: OS in our series was similar to literature data (79.5% vs. 79.7% at 1 year). The combination of Rituximab (anti CD 20) with chemotherapy significantly improved overall survival (93.3% vs. 40% at 1 year). Hence it is not allowed to treat a LCBL without rituximab.

AUTHOR INDEX