The 13th Biennial Childhood Leukemia and Lymphoma Symposium (CLLS 2023)

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The 13th Biennial Childhood Leukemia and Lymphoma Symposium

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Jazz Pharmaceuticals

7 May

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16:55 **Eva Fronkova (CZ)**
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18:10 - 18:30 **Adjourn**

NOVARTIS

Oral and invited speakers presentations

IL 1 - Options in the future therapy of childhood AML, MDS and JMML

Michel Zwaan[1]

1 *Prinses Maxima Centrum, Trial and Data Centrum, Utrecht, Netherlands*

Pediatric AML and MDS/JMML are myeloid diseases that, despite improvements in outcome, require either intensive chemotherapy, with HSCT in selected cases (AML), or some bridging chemotherapy and HSCT in case of MDS and specific subtypes of JMML. For pediatric AML, with survival rates of approximately 50% at relapse, almost no approvals occurred in children, despite the approval of many new drugs in the adult space. There are however some discrepancies between Europe and the US, as FDA approvals exist for Gemtuzumab ozogamicin (relapsed AML) and Vyxeos liposomal (for MDS and therapy-related AML) for use in children. For newly diagnosed JMML azacitidine was approved by FDA based on the AZA-JMML-001 study.

After the Pediatric Strategy Forum on AML in 2019, and with support from the Leukemia Lymphoma Society and COG and the European AML study groups, a large international project was launched to study new drugs for pediatric AML, and to set new standards of care for 1st and 2nd relapse of AML. This project is referred to as the PedAL project, and is implemented in Europe by ITCC and the upfront AML groups collaborating in the EuPAL foundation, and in the AML-BFM SG. The programs aims not only at studying the safety and activity of the drugs, but aims at obtaining regulatory approval where applicable. Studies may be performed in the context of regulatory commitments of the market authorization holders, such as a PIP or PSP. Scientific advice from EMA was obtained on several topics and resulted in generating a complex clinical trial protocol with subtrials (study ITCC-101).

In this presentation, we will address several new compounds that will be studies either via PedAL or otherwise, including venetoclax, menin inhibitors, FLT3 inhibitors, CD47 inhibition, FOL1R targeting, and CD123 directed compounds. We will also briefly touch upon new developments in immunotherapy in AML, especially regarding an NK-cell engager, which will soon recruit children 12 years of age and older in the first in men phase 1 study, consistent with the FAIR (fostering age inclusive research) principle to drop the age whenever feasible. Of interest, the project also has resulted in a QA/QC network for MRD assessment in pediatric AML, and in a project to define ELN criteria for pediatric AML.

After the successful introduction of new molecules for ALL over the past decade, the aforementioned drugs may alter the therapeutic armamentarium for pediatric AML, and hopefully contribute to improved outcome with a better risk/benefit balance for children with newly diagnosed and relapsed AML.

IL 2 - How to envision next-generation childhood ALL trials in 2030

Kjeld Schmiegelow[1]

1 *Chair of Pediatric Oncology- Rigshospitalet, Departm. of pediatric hematology/oncology, Copenhagen, Denmark*

Treatment of childhood ALL now achieves cure in more than 90% of patients, primarily based on better use of the anticancer agents that has been around for decades.

The tracks to the future have been laid down through extensive and integrated basic science and clinical research programs that covers the whole disease trajectory from diagnosis to cure, including (a) etiology; (b) tumor biology and dissemination; (c) the germline-tumor genomics continuum; (d) novel treatment strategies, including therapeutic drug monitoring (TDM), precision medicine, immunotherapy, and alternative transplantation options; (e) supportive care; and (f) acute and late somatic and psychosocial toxicities. However, more than 50% of deaths are not due to resistant leukemia, but rather severe treatment-related toxicity, and the majority of survivors are burdened by serious toxicities. Current main goals are improvement in risk-adapted treatment stratification based on (i) genetics, (ii) MRD monitoring in bone-marrow and CNS, and (iii) proneness to serious toxicities, in order to allow more individualized treatment. As immunotherapy and precision drugs emerge, future therapy for the lowest risk patients may become chemo-free.

A large proportion of ALL cases are initiated prenatally, and development from a preleukemic state to overt ALL probably reflecting interactions between genetic predisposition, random development of preleukemic cells, and immune system maturation that moderate survival and further mutations in preleukemic cells. Potentially, ALL could in the future become a preventable disease.

For those diagnosed with ALL:

1. diagnostics can be improved by mapping both germline and tumor DNA, as well as CNS dissemination by flow-cytometry.
2. TDM and pharmacogenomics of conventional chemotherapy could lead to a more rational use of antileukemic agents.
3. MRD monitoring of leukemic cells in CSF and the velocity of their elimination may guide intensity of CNS targeted therapy.
4. novel treatment options include both immunotherapy (some of which are included in first line therapy) and the multiple small molecules that in contrast to conventional chemotherapy target specific biological pathways (=precision drugs).
5. toxicities remain an unsolved challenge. In addition to bacterial and fungal infections, more than 50% of all patients develop serious organ toxicities. Many of these experience truncation of one or more of their planned antileukemic therapies (most often asparaginase), which may significantly increase their risk of relapse.
6. the goal of treatment is not just “cure”, but rather toxicity-free survival, and there is a need for global, consensus-based strategies for systematic integration of the most unacceptable toxicities in the outcome evaluation (Severe Toxicity-Free Survival, STFS) in addition to the traditional event-free and overall survival.

As ALL paved the way for better treatment of cancer in general, it may in the coming decade provide novel strategies for individualized and immune-based therapy to provide cure for more at a lower cost to patients.

IL 3 - Diagnostic stratification of childhood ALL: What we need to know and what we want to know

Oskar Haas[1]

1 *St. Anna Children's Hospital, Central Laboratory, Wien, Austria*

The prognostic and therapeutic stratification of childhood ALL is based on the phenotypic and genotypic definition of specific disease entities and their respective treatment-dependent dynamic and clinical behavior. The amazing progress of sequencing technologies within the last decades led to the recognition of already more than 20 different sub-types, an achievement that has primarily been accomplished by the retrospective analysis of a huge number of cases. Despite this success, however, it is surprising that the stratification demands of virtually all treatment studies are still based on rather crude classification systems, whose attributes can still be easily clarified with well-established "old-fashioned" laboratory methods. Although the implementation of more sophisticated genome-wide sequencing approaches into the routine diagnostic work-up of childhood leukemias is therefore not yet an imminent necessity, it is of course nevertheless highly desirable to do so. Such an undertaking is, however, especially for laboratories with a low sample through-put a big challenge and difficult to achieve in a manner that is not only cost-efficient but that also enables the delivery of the results within the required time frame.

In common parlance, the term "diagnostics" refers to "what we need to know", whereas "research" is perceived as something that "we want to know". Yet, as I will allude to in my presentation, the boundaries of these seemingly clear concepts are extremely vague and fluid. The vast amount of information that is nowadays at our disposal, makes it virtually impossible to draw a precise border between these two lines of thought. One of the big challenges is therefore, to meaningful integrate results that derive from large-scale genomic screening approaches into the daily diagnostic work-up of childhood leukemias. The question of what counts as diagnostic necessity is exclusively regulated by the demands of the respective treatment protocol. At present, all these categories can still be easily identified and delineated with well-established methods, such as DNA-index measurements, cytogenetics, FISH, (multiplex) RT-PCR, MLPA, arrays and/or gene expression profiling. Since all of them have their advantages and disadvantages, different labs apply several of these techniques in fitting combinations to guarantee that they can securely identify all relevant abnormalities. Although this approach will mostly suffice the intended purpose, it delivers also frequently incidental, ambiguous, or difficult to interpret results. The most outstanding examples that illustrate this latter point regard the difficulty to appropriately sub-divide aneuploid leukemias in a clinically meaningful manner and to make sense of the heterogeneous "IKZF1^{plus}" and "Ph-like" sub-groups. Despite being deceptively well-defined, the former by a certain constellation of deletions and the latter by a particular gene expression profile, they both conceal a variety of distinct genetic sub-forms that may, as for instance those with a tyrosine activating gene fusion, require an individually adapted type of therapy. A further detailed elucidation of any unclear cases will then, even when performed for clinically indispensable purposes, formally fall into the field of research, which consequently raises the question who will cover the ensuing costs. Thus, the current practice to formalistically detach the diagnostic from research work is rather impracticable and impedes any vital development in the diagnostic field. Since the ensuing consequences of this differentiation extend far beyond the involved laboratories, they also concern the treating physicians and, not least, also those who conceive and survey the respective treatment studies.

IL 4 - How to approach r/r mature B-cell neoplasms

Amos Burke[1]

1 CUHFT- Addenbrookes Hospital, Paediatric Haematology- Oncology and Palliative Care, Cambridge, United Kingdom

Whilst frontline therapy for mature B-cell non-Hodgkin lymphoma is highly successful, with over 90% survival expected, relapsed and refractory mature B cell non-Hodgkin lymphoma (r/r B-NHL) has a very poor prognosis and the vast majority of children and adolescents will die despite attempts at salvage. r/r B-NHL is rare and international studies are needed to make advances in this area. A large number of new agents are in clinical practice or development for adult r/r lymphoma but few of these have been evaluated in childhood and adolescent r/r B-NHL and individual trials of all possible agents are not possible due to the limited numbers of patients who might be available to participate in such trials.

In this session, current approaches to r/r B-NHL will be reviewed. The issue of prioritisation of the many possible agents to be investigated in childhood and adolescent r/r B-NHL will be discussed and trials in development will be identified.

IL 5 - How to envision CAR-T cell therapies in primary and first relapse of ALL

Claudia Rössig[1]

1 *University Children's Hospital Muenster, Pediatric Hematology and Oncology, Muenster, Germany*

Major innovations have been introduced into the treatment of B cell acute lymphoblastic leukemia (ALL) based on selective targeting of B-lineage markers. CAR T cells act by an entirely different mode of action than cytotoxic chemotherapy. They have shown remarkable activity to induce molecular remissions in patients with (multiple) relapses and with refractory disease in both children and adults and can be curative as stand-alone therapy in a proportion of pediatric patients with relapsed or refractory disease.

Academic researchers within the large established international study groups now aim to establish the value of CAR T cell therapy within current treatment algorithms, to prevent rather than treat refractory disease. In addition to further increasing the proportion of event-free survival, advanced treatment algorithms aim to improve the quality of life of the survivors. In this respect, safe replacement of allogeneic hematopoietic stem cell transplantation (HSCT) with its organ toxicities and risks of debilitating graft-versus-host disease and secondary malignancies is a key goal. This may be achieved in part by more effective first-line therapies, reducing the numbers of patients with high-risk relapses requiring HSCT for cure. Furthermore, CAR T cell therapy could be effective to replace HSCT in at least a proportion of patients with current transplant indications. A key challenge in the prospective evaluation of the value of CAR T cells in national and international multicenter trials remains reliable and affordable access to these individualized gene therapy products fulfilling the highest quality standards, even across international borders. Due to the high complexity and cost of CAR T cell therapy and the late effect of sustained B cell depletion requiring potentially lifelong immunoglobulin substitution, superiority over current therapies will have to be clearly demonstrated. Future challenges are the development of superior products with longer durability of T cell control and measures to prevent antigen escape, as well as innovations with equivalent potential also for T-lineage leukemias.

Acute myeloid leukemia & JMML & MDS

O 01 - Deciphering the role of Nidogen-1 in pediatric acute myeloid leukemia

Jolien Vanhooren[1,2,3], Barbara Depreter[4], Larissa Deneweth[2,3], Martijn de Jong[2,3], Wouter Sleeckx[2,5], Mattias Hofmans[2,5,6], Steven Goosens[2,5], Barbara De Moerloose[1,2,3], Lammens Tim[1,2,3]

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2 *Cancer Research Institute Ghent, crig, Ghent, Belgium*
3 *Ghent University Hospital, Pediatric Hematology-Oncology and Stem Cell Transplantation, Ghent, Belgium*
4 *Vrije Universiteit Brussel, Department of Laboratory Hematology, Brussels, Belgium*
5 *Ghent University, Diagnostic Sciences, Ghent, Belgium*
6 *Ghent University Hospital, Laboratory Medicine, Ghent, Belgium*

The outcome for children with acute myeloid leukemia (AML) has improved significantly over the past decades. Nevertheless, 30–40% of the young patients still relapse, and less than half will be cured long-term, but with an increased burden of side effects due to high cumulative doses of chemotherapy and for some patients a hematopoietic stem cell transplantation. The high relapse rates have been shown to be attributed in part by the leukemic stem cells (LSC). Therefore, there is an urgent need of novel therapeutic strategies to improve the survival and quality of life of the pediatric AML patients.

We recently explored mRNA (n = 27071) expression in pediatric AML (pedAML) subpopulations (LSC and leukemic blast) and their normal counterparts (hematopoietic stem cell (HSC) and myeloblast, respectively). Nidogen-1 (NID1) was found to be overexpressed in both leukemic subpopulations, while absent/low expressed in the HSC and myeloblasts. These observations were validated in public datasets and in different hematological cell lines (n = 32) using RT-qPCR, which showed that the expression was highly specific for AML (pedAML and adult AML). The TARGET database (n = 354 patients) showed a significant association of NID1 expression with RUNX1::ETO and KMT2A-rearranged clinical subtypes of which the latter association was also found in a PDX model generated within our team. Gene Ontology enrichment analysis on $NID1_{high}$ (n = 35) and $NID1_{low}$ (n = 35) patients showed involvement in biological processes such as leukocyte migration, immune response and signal transduction. Moreover, a NID1 knockdown model (shRNA-mediated) was generated in the AML cell line OCI-AML5 and the knockdown, validated by RNA sequencing, revealed that NID1 downregulation caused differential expression of genes involved in AML progression, chemosensitivity and genes associated with the RUNX1::ETO and KMT2A-rearranged genotypes, which are in line with our previous observations.

Altogether, these results highlight the identification of NID1 as a novel gene involved in the pathogenesis of pedAML. Substantiating our findings, NID1 overexpression has recently been demonstrated in several solid tumors (breast cancer, salivary gland cancer, glioma) to confer the metastatic potential and thus aggressiveness of these cancers. Functional studies to further unravel the role and function of NID1 in pedAML are ongoing and we will explore NID1-oriented therapeutic opportunities to improve personalized treatment options for these children.

O 02 - Tracking ROS levels to hunt leukemia stem cells in pediatric acute myeloid leukemia

Maddalena Benetton[1], Ambra Da Ros[1], Giulia Borella[1], Giorgia Longo[1], Alberto Peloso[1], Silvia Bresolin[1], Claudia Tregnago[1], Franco Locatelli[2], Martina Pigazzi[1]

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2 *Bambino Gesù Children's Hospital- Catholic University of the Sacred Heart, Department of Pediatric Hematology and Oncology, Rome, Italy*

Background:

Standard chemotherapy targets actively proliferating cells and induces complete remission in most of children with acute myeloid leukemia (AML), however relapse still represents an unsolved problem. Leukemic stem cells (LSCs) have been proposed as the therapy-resistant *reservoir* of cells responsible for disease recurrence and the big challenge is to identify novel treatment strategies aimed at killing them. However, the gold standard CD34+/CD38- gating strategy to identify LSCs is debated, and no unique marker has been detected yet.

Objectives:

We aim to find novel LSCs characteristics and dissect mechanisms of chemoresistance to be targeted to completely eradicate residual disease and avoid AML re-emergence.

Methods:

We isolated the lowest (namely ROS low, RL) and the highest (namely ROS high, RH) 20% of blasts by their reactive oxygen species (ROS) content (by CellROX probe) from primary pediatric AML bone marrow samples at diagnosis since LSCs have been associated to a peculiar metabolism and characterized them.

Results:

We first tested key stemness features and demonstrated that RL cells displayed a higher clonogenic capacity with respect to RH fraction ($p = 0.006$), which is maintained at second plating ($p = 0.013$). Engraftment experiments did not reveal different capacity in recreating leukemia in NSG mice, but only AML from RL cells were able to induce leukemia in second and third mouse recipients, while RH cells exhausted their engraftment capacity at first implant. To further corroborate the hypothesis that RL cells represent LSCs, we took advantage of our three-dimensional (3D) long-term culture model of AML cells together with mesenchymal stromal cells (MSCs) and after RL and RH seeding, we implanted *in vivo* in NSG mice to allow vascularization for three months. Then, by histological analysis of the 3D, we observed that RL cells located more distant from blood vessels than RH ($p < 0.0001$). We considered RL an enriched LSCs fraction. Thus, we performed RNAseq on RL and RH ($n = 5$), identifying 417 differentially expressed genes belonging to pathways related to cell proliferation, metabolism and adhesion. To confirm *in silico* results, we performed cell cycle analysis revealing that RL cells are relatively dormant, being mostly in G0/G1 cell cycle phase (85%), and proliferate more slowly (at day 3, 66% undivided cells in RL *versus* 20% in RH by CSFE, $p = 0.04$). Moreover, phalloidin staining at confocal microscopy revealed that, when adherent, a higher proportion of RL cells form stress fibers with respect to the RH population (49.3% *versus* 24.2%, $p = 0.05$), and that RL cells showed a stretched morphology ($p < 0.0001$), supporting a higher adhesion capacity. In parallel, to deepen into mitochondrial features, we demonstrated that RL cells are characterized by lower levels of mitochondrial membrane potential (TMRE, $p < 0.0001$), ROS (MitoSOX, $p = 0.003$) and mass (MitoTracker, $p = 0.01$) with respect to RH, suggesting that mitochondrial were affected. Low basal ATP production in RL cells with respect to RH ($p < 0.0001$) was documented. Finally, the mitochondrial inhibitor IACS-010759 resulted effective in inducing cell death in RL cells, but not in RH ($p = 0.02$), whereas RL cells were less sensitive to cytarabine ($p = 0.05$) exclusively when grown adherent or in the 3D model.

Conclusion:

In conclusion, this strategy allows to isolate a cell fraction enriched for LSCs from the AML bulk with distinct mitochondrial and adhesion features to be further considering in drug screening approaches. These experimental evidences suggest the use of 3D models as mandatory for the identification of predictive LSCs active drugs.

O 03 - Patient-derived xenograft models are the leading strategy to identify new agents for pediatric acute myeloid leukemia

Ambra Da Ros[1], Valentina Indio[2], Giulia Borella[1], Maddalena Benetton[1], Giorgia Longo[1], Claudia Tregnago[1], Silvia Bresolin[1], Andrea Pession[3], Franco Locatelli[4], Martina Pigazzi[1]

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Background:

Acute myeloid leukemia (AML) in children is a life-threatening disease with chemotherapy and hematopoietic stem cell transplantation still representing the standard of care. Nowadays, most of children affected from AML reached the complete remission, but 30% of them experienced a relapse with an urgent need to identify effective therapies to treat resistance and recurrence events. In these last years, genomic characterization improved the success rate in the identification of new molecularly targeted therapies. Nevertheless, despite promising preclinical data, less than 10% of novel candidate cancer drugs have been authorized for market, failing during Phase I or II of clinical trials. This latter phenomenon is due to a poor correlation of drug safety/efficacy in humans with respect to preclinical models. Thus, the optimization of preclinical tools to advance clinically relevant data have been pursued, with patient derived xenografts (PDXs) being considered the most realistic *in vivo* AML modeling.

Objectives:

This study aims to establish, characterize, and use pediatric AML-PDXs to accelerate the identification and evaluation of innovative medicines for AML.

Methods and Results. We inoculated primary AML samples in NSG mice and, when engrafted (namely P0), in two consequent mice recipients (namely P1 and P2-PDX), generating 26 AML-PDXs representatives of 14 different AML genetic subtypes. We characterized AML-PDXs for immunophenotype, genomic and transcriptomic profiling observing a strong similarity to the original AML. By whole-exome sequencing we detected a consistent number of variants in each model (ranging from 24 to 67) and we did not identify mutations recurrence among AML, confirming an high intra-tumoral heterogeneity. We tracked clonal evolution from patients' AML to P2-PDX recognizing „founder" clones characterized by an average of 25 variants (at least one in driver AML genes) which are maintained up to P2 at the same allelic frequency (50%), other small clones increasing the allelic frequencies in P2 and, in a restricted number of models, we observed patients' clones being lost in matched PDXs even if characterized by variants in driver genes (KRAS,MYCN,SETD2). By RNA-seq analysis we highlighted aberrantly expressed and druggable pathways allowing the selection of novel targeted compounds (IACS-010759, Asparaginase, Disulfiram, Gallein, Thioridazine, SNDX-5613, Trametinib). By exploiting a three-dimensional (3D) co-culture system *in vitro* (a biomimetic scaffold seeded with AML cells and mesenchymal stromal cells) we screened the efficacy of these targeted drugs, used alone or combined with Venetoclax at low dose for exploring their synergy in reducing AML proliferation. The two most efficacious combinations *in 3D*, Venetoclax+IACS-010759 and Venetoclax+Asparaginase ($p < 0.001$, $n = 4$), have been evaluated in 6 different AML-PDX models. Results showed that 3/6 models underwent remission after 4 weeks of treatment with Venetoclax, whereas 6/6 models treated with combinations had leukemia eradication at stop treatment. At day +15 after stop therapy, 6/6 models treated with Venetoclax showed an increased disease burden, whereas only one model treated with VEN+IACS, and one with VEN+ASPN, relapsed but 4/6 PDXs treated with the combos are still in remission after 45 days.

Conclusions:

The generation of a large panel of AML-xenograft models and relative omics data represent a concrete perspective for the identification of new variants and pathways involved in AML progression. The possibility to perform predictive drug screenings in PDXs will increase AML drugs with higher chance to advance in pediatric AML second line treatments.

O 04 - Challenges in CBL-mutated juvenile myelomonocytic leukaemia: To treat or not to treat?

Bianca-Andreea David[1], Serpil Alkan[2,3], Julie Harvengt[3], Barbara de Moerloose[4], Benoit Florkin[1]

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Background:

Patients with germline *CBL* pathogenic variants have syndromic features like growth and neurodevelopmental delays, facial dysmorphia, autoimmune manifestations, and a predisposition to develop juvenile myelomonocytic leukaemia (JMML) because of the loss of heterogeneity for the *CBL* gene in the leukemic cells. Due to its rarity and lack of markers that predict spontaneous resolution or progression to aggressive disease, the best follow-up and therapy approach is unknown at the time of diagnosis. The options could vary between *watch-and-wait* strategy to haematopoietic stem cell transplantation (HSCT).

Objective:

We describe the case of a child with a newly diagnosed *CBL*-mutated JMML and the therapeutic challenges encountered by the clinicians and the family.

Clinical case: A term born girl was referred to our hospital at the age of 17 months after multiple hospitalisations for recurrent infections and failure to thrive requiring tube feeding since the age of 6 months. Physical examination revealed two xanthogranulomas and two small *café-au-lait* spots and mild splenomegaly. The presence of leucocytosis, monocytosis, and mild thrombocytopenia prompted us to perform RASopathy panel for JMML and the c.1111T>C pathogenic variant (Class 4) (p.Tyr371His) on exon 8 of the *CBL* gene was identified both on somatic (bone marrow) and germinal level (fibroblast culture) confirming the diagnosis of *CBL*-mutated JMML.

Foetal Haemoglobin levels were normal for age and no other genetic anomalies were detected on somatic or germinal level. Exome sequencing for secondary mutations and genome-wide methylation profiling is pending. After multidisciplinary discussion, experts' opinion, and family approval, we chose the *watch-and-wait* approach. A follow-up schedule was made with monthly clinical evaluation and blood tests, and bone marrow aspirates every 6 months to look for secondary mutations or additional chromosomal alterations. No transfusion was required, and no severe complication was observed to date.

Discussion:

The milder manifestations encountered in some clinical presentations of *CBL*-mutated JMML could lead to diagnostic delays. Some patients with *CBL*-mutated JMML may have stable disease and even achieve spontaneous remission without treatment. Standard of care in most JMML patients relies on HSCT but in this case, hasty decision could lead to significant medical burden. The uncertainty regarding clinical evolution could be a trigger of anxiety both for physicians and family and alter the quality of life. Follow-up schedules, although largely dependent on the severity of the disease, could provide some reassurance and assure timely intervention if disease progression occurs.

Application to practice:

The *watch-and-wait* approach will spare some patients from unnecessary treatment and potential treatment-related toxicities. Further follow-up data and validation of the new classification of JMML based on the whole-exome sequencing and methylation profile is needed to identify which patients are the most appropriate candidates for *watch-and-wait* approach, and to better define the management schedule and the follow-up guidelines.

Keywords: JMML, c.1111T>C pathogenic variant, CBL gene, CBL syndrome, *watch-and-wait* strategy

Acute lymphoblastic leukemia (clinical topics)

O 05 - Improved outcome for acute lymphoblastic leukemia by prolonging therapy for IKZF1 deletion and decreasing therapy for ALL in children with ETV6::RUNX1, Down syndrome or prednisone poor response

Rob Pieters[1], *Hester de Groot-Kruseman*[1], *Marta Fiocco*[1]

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Background:

The ALL10 protocol improved outcome for children with ALL by stratifying and adapting therapy in minimal residual disease (MRD) defined standard risk (SR), medium risk (MR) and high risk (HR). IKZF1 deleted (IKZFdel) ALL in the largest MR group still showed poor outcome, in line with protocols worldwide, accounting for a high number of overall relapses. ALL10 showed high toxicity in Down syndrome (DS) and excellent outcome in ETV6::RUNX1 ALL. Poor prednisone responders (PPR) were treated as HR in ALL10.

Objectives:

In ALL11, we prolonged therapy for IKZFdel from 2 to 3 years to improve outcome. We aimed to reduce therapy for DS by omitting anthracyclines completely and omitting anthracyclines for ETV6::RUNX1 in intensification and for PPR by treatment as MR instead of HR without abrogating their outcome.

Methods:

819 ALL patients (aged 1–18 year) were enrolled on ALL11 and stratified as in ALL10. Results were compared to ALL10.

Results:

Five years overall survival (OS), event-free survival (EFS), cumulative risk of relapse (CIR) and death in complete remission (CID) on ALL11 were 94.2% (SE 0.9%), 89.0% (1.2), 8.2% (1.1), 2.3% (0.6) respectively.

Prolonged maintenance for IKZF1del MR improved the 5-year CIR by 2.2-fold (10.8% vs 23.4%; p = 0.035) and EFS (87.1% vs 72.3%; p = 0.019). Landmark analysis at 2 years from diagnosis showed a 2.9-fold reduction of CIR (25.6% to 8.8%; p = 0.008) and EFS improvement (74.4% to 91.2%; p = 0.007).

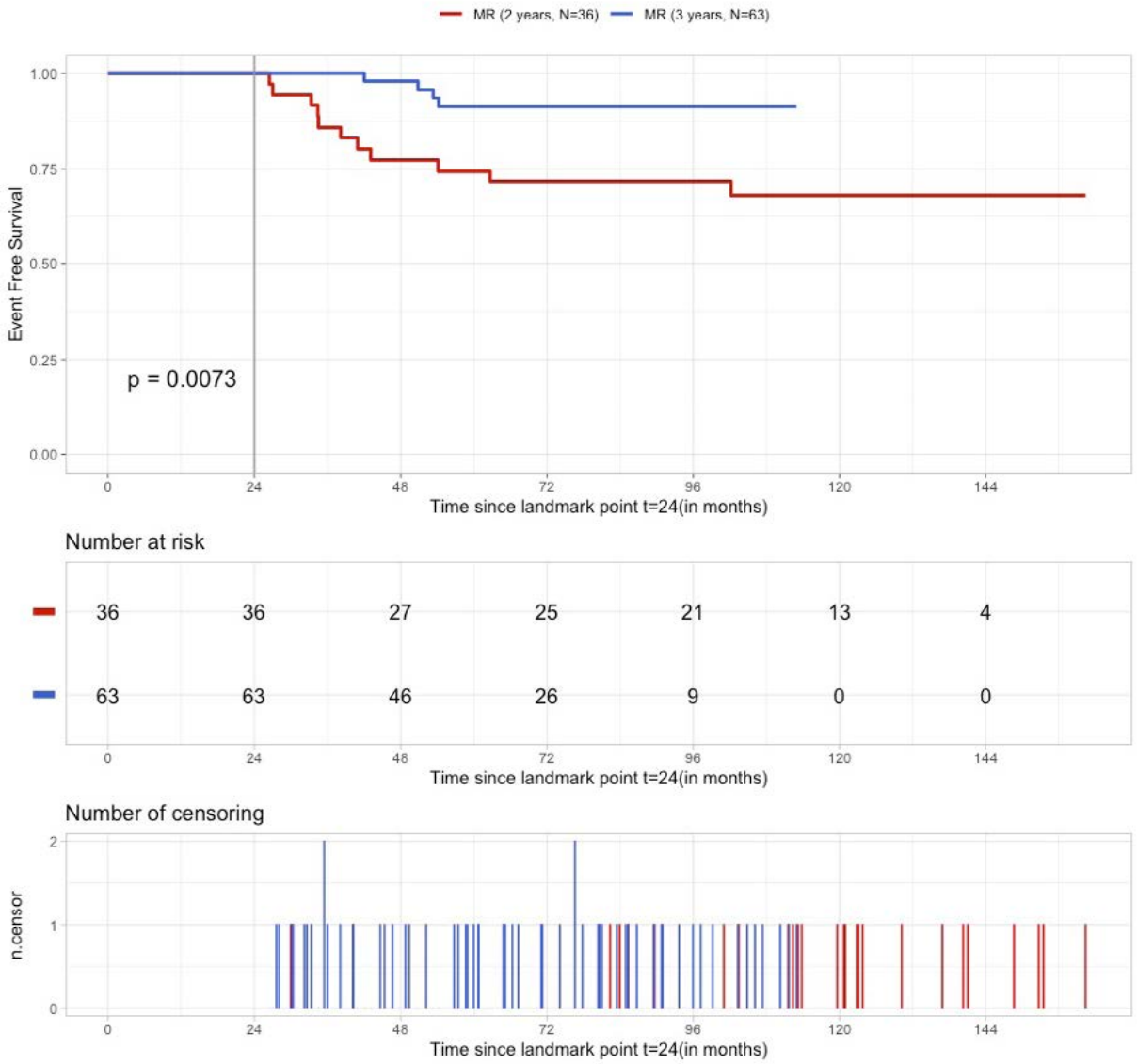
Reduced therapy did not abrogate the 5-years outcome for ETV6::RUNX1 (EFS 98.3%; OS 99.4%), DS (EFS 87.0%; OS 87.0%) and PPR (EFS 81.1%; OS 94.9%).

Conclusions:

Children with IKZF1del ALL benefit from prolonged maintenance therapy. Chemotherapy was successfully reduced for ETV6::RUNX1, DS and PPR ALL patients.

Trial registration number: EudraCT 2012–000067–25; NL3227 (clinicaltrialregister.nl).

Event Free Survival of IKZF1del in MR: 2 years vs 3 years treatment (landmark at 2 years).


MR (2 years, N=36)
MR (3 years, N=63)
Event Free Survival
1.00
0.75
0.50
0.25
0.00
p = 0.0073
0
24
48
72
96
120
144
Time since landmark point t=24(in months)
Number at risk
36
36
27
25
21
13
4
63
63
46
26
9
0
0
Time since landmark point t=24(in months)
Number of censoring
n.censor
2
1
0
Time since landmark point t=24(in months)

O 06 - HDAC6 ablation represses in vivo growth of BCR-ABL1 + leukemia cells by innate-immune response inducing RNase T2 expression

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Background:

Despite the success of tyrosine kinase inhibitors (TKIs), the clinical response in BCR-ABL1$^+$ B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is short-lived, with relapses being driven by mutations in the BCR-ABL1 kinase or activation of independent circuitries. The histone deacetylase (HDAC) family is an emerging drug target, which acts as an epigenetic eraser by catalyzing the decatylation of lysine residues. High expression of several HDAC isoforms and in particular HDAC6 is reported in different leukemia subtypes[1]. While other HDACs are mostly focused on histone proteins, HDAC6 acts on cytosolic proteins like a-tubulin, cortactin or heat shock protein 90 and organizes the formation and transport of aggresomes. Moreover, the unique structure of HDAC6 enables its highly selective targeting compared to other HDAC isoforms[2].

Objectives:

Despites its long list of important functions that involve core components of the cytoskeleton or protein machinery, HDAC6 inhibition by itself does not induce cytotoxicity in cancer cells [2,3]. Therefore, this study aims to decipher whether HDAC6 has any clinical relevance in the context of BCR-ABL+ leukemia, by investigating phenotypical changes induced upon its loss.

Methods:

We generated stable HDAC6 knock-out (KO) via CRISPR-Cas9 using BCR-ABL1+ cell lines and characterized their transcriptome, proteome, secretome and chromatid accessibility through multi-omics approaches. To corroborate our findings, we established a long-term treatment protocol with selective HDAC6 inhibitors and novel HDAC6-degraders as a validation tool of the genetic phenotype. Furthermore, changes in the differential sensitivity post HDAC6 loss were determined on a high throughput drug-screening (HTDS) platform involving commonly used chemotherapeutics and targeted small molecule Inhibitors.

Results:

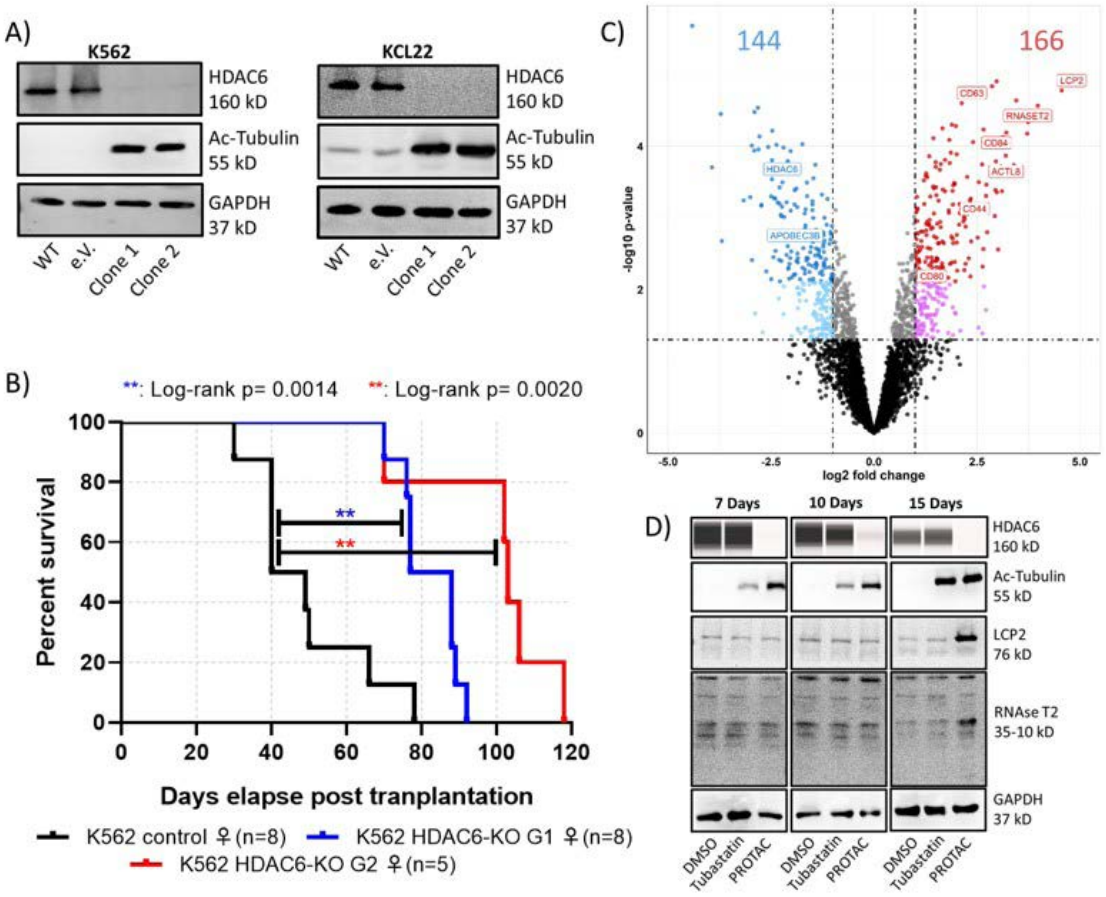
The HDAC6-KO was validated on DNA and protein level via Sanger sequencing and western blots (figure A). *In vitro*, we observed no changes in the proliferative and colony-forming abilities of HDAC6-KO cells compared to respective controls. In addition, no difference in the invasion of HDAC6-KO cells into (iPSCs derived) cerebral organoid model was noticed. However when the HDAC6-KO cells were injected into NSG mice, the median survival was doubled in relation to the control group (n = 5–8 per arm, median survival = 44 days vs 82 / 103 days, p = 0.0014 / 0.002, figure B). Our multi-omics findings showed highly increased levels of RNAse T2 (both intracellular and secreted) and LCP2 (FDR = 0.0086 / 0.0077, figure C). Both of these proteins are associated with increased immune infiltration[4] and especially RNAse T2 is known for its ability to polarize macrophages into pro-inflammatory anti-tumor M1 macrophages[5]. Interestingly the upregulation of RNAse T2 and LCP2 was reproducible by a long-term treatment over the course of 15 days with HDAC6-degrader, while the conventional HDAC6 inhibitor failed to induce the same effect (figure D). Using high troughput drug screening, we identified several drug classes with increased susceptibility toward HDAC6-KO cells than controls, including antimetabolites, kinase inhibitors (Axitinib) and NF-kB inhibitors.

Conclusion:

While conventional inhibition of HDAC6 has failed to induce cytotoxicity, long-term depletion of HDAC6 seems to induce a phenotype with therapeutically exploitable traits. Polarizing M2- into M1 macrophages offers an alternative strategy that relies on the innate immune system and less on the adaptive immune system. Combined with the ability to sensitize the cells to BCR-ABL1 inhibitors like Axitinib, our findings indicate a foundation for a novel treatment strategy for BCR-ABL1$^+$ leukemia, and could be valuable for other therapy refractory leukemia subtypes.

References:

1 PMID: **35918044**
2 PMID: **32803970**
3 PMID: **31511426**
4 PMID: **33911146**
5 PMID: **21189302**



O 07 - Prognostic relevance of DUX4 childhood and adolescent B-cell acute lymphoblastic leukemia – Results from Austrian ALL-BFM-studies

Dagmar Schinnerl[1], Marion Riebler[1], Sabrina Haslinger[1,2], Margarita Maurer-Granofszky[1,3], Stefan Köhrer[1,2], Karin Nebral[1,2], Michael N. Dworzak[1,3,4], Andishe Attarbaschi[1,4], Sabine Strehl[1], Austrian Berlin-Frankfurt-Münster BFM Study Group[1,4]

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During the last decade, comprehensive genome-wide analyses and integrative genetics and genomics facilitated the refinement of the subclassification of childhood B-cell acute lymphoblastic leukemia (B-ALL). One of the recently identified subtypes is *DUX4* B-ALL, which is characterized by *IGH::DUX4* rearrangements leading to the expression of truncated *DUX4* isoforms and a highly distinctive gene expression profile. Several studies have shown, that *DUX4* leukemia is associated with good outcome, however, the patients are often treated on high-risk protocols. Therefore, the aim of this study was to identify all *DUX4* patients enrolled in three consecutive Austrian Berlin-Frankfurt-Münster (BFM) ALL trials, to determine the prognostic relevance of the subtype and potential risk factors.

The patient cohort analyzed in this study consisted of B-other childhood B-ALL patients registered in the Austrian ALL-BFM 2000, 2009 and 2017 studies (06/1999–12/2022). Whole transcriptome sequencing (RNA-seq), CD371 cell surface marker expression and SNP array analysis for *ERG* deletion were employed to identify *DUX4* leukemia. RNA-seq data were used for gene expression profiling and mutation calling in selected hot spot regions. Demographic, clinical and outcome data were collected, and event-free (EFS) and overall survival (OS) were calculated using the Kaplan-Meier method with log-rank test.

In total we identified 69 patients (ALL-BFM 2000 n = 33; ALL-BFM 2009 n = 24; ALL-BFM 2017 n = 12) with *DUX4* B-ALL. The median age of the patients at diagnosis was 10.3±4.7 years (range 2.3–18.4 years) and there were 48% (33/69) females and 52% (36/69) males. Most of the patients were treated according to the medium (MRG) or high risk (HRG) arms of the respective protocol (standard (SRG) 8.7% (6/69), MRG 62.3% (43/69), HRG 29% (20/69)).

The median follow-up of all 69 *DUX4* patients was 7.5 years (range 0.2–17.4 years) and only 7.2% (5/69) experienced a relapse of whom 80% (4/5) died. One additional patient died early while still on therapy, thus in total 7.2% (5/69) of the *DUX4* patients died. The estimated 5-year and 10-year EFS of all *DUX4* patients was 91.5±3.7% and 89.1±4.3%, while the estimated 5-year and 10-year OS was 94.8±2.9% and 87.8±5.3%, respectively. Although other consortia have reported even higher survival rates (5-year EFS ~95%; 5-year OS 95–98%), this indicates that patients with the *DUX4* subtype have a very good outcome also when treated according to ALL-BFM treatment regimens.

However, our analysis also showed that certain coinciding mutations might be indicative of a worse outcome. Although the number of patients with a *TP53* mutation was low (5.8%, 4/69) those with mutations had a significantly worse outcome (50±25% vs to 94.3±3.2%, p = 0.001). A similar negative trend was observed for patients with the *IKZF1*^{plus} deletion profile (7.6%, 5/66; 60±21.9% vs to 93.9±3%, p = 0.0079).

Notably, while slow early response (SER; PCR-MRD day 33 ≥ $5x10^{-4}$ and any PCR-MRD positivity below $5x10^{-4}$ at day 78) to therapy, which is considered as high-risk factor, had no impact on EFS, all patients with PCR-MRD < 10^{-2} at day 15 (41.5%, 27/65) remained in first complete remission, whereas, those with PCR-MRD ≥ 10^{-2} (58.5%, 38/65) had a 5-year EFS-probability of 85.4±6% (p = 0.042).

In summary, we show that *DUX4* B-ALL is associated with a good prognosis when treated according to ALL-BFM protocols applying established risk stratification parameters. While *IKZF1*^{plus} is already implemented as risk stratifying parameter in the current AIEOP-BFM-ALL-2017 trial, prospective screening for *TP53*-mutations may identify additional patients who remain at risk of a poor outcome.

O 08 - AP-1 transcription factor complex members FOSB and FOS are linked with central nervous system (CNS) infiltration and inferior prognosis in childhood T-cell Acute Lymphoblastic Leukemia (T-ALL)

Lea Spory[1], Johannes Zimmermann[2], Michaela Vossen-Gajcy[1], Thomas Beder[3], Lorenz Bastian[3], Ameera Alsadeq[4], Martin Schrappe[1], Gunnar Cario[1], Denis M Schewe[5], Lennart Lenk[1]

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2 *University of Kiel, Zoological Institute- Department of Evolutionary Ecology and Genetics, Kiel, Germany*
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4 *Ulm University Medical Center, Institute of Immunology, Ulm, Germany*
5 *Magdeburg University Medical Center, Department of Pediatrics, Magdeburg, Germany*

Introduction:

Acute Lymphoblastic Leukemia (ALL) is the most common childhood malignancy. Due to its elevated propensity to invade the central nervous system (CNS) and insufficient strategies to treat relapsed disease, the T-cell phenotype (T-ALL) harbors an inferior prognosis. Because current detection methods of CNS leukemia are widely unsensitive, all patients irrespective of their CNS status receive prophylactic CNS-directed therapy, which is highly neurotoxic. Therefore, novel markers to reliably detect blasts in the CNS and to identify patients in need of intensified CNS-directed therapy are urgently needed, particularly in T-ALL.

Methods:

Comparative RNA-sequencing was performed with patient derived xenograft T-ALL blasts recovered from the bone marrow (BM) and the CNS of 6 different NSG-mice. *FOS* and *FOSB* mRNA were measured in diagnostic BM samples of 112 pediatric T-ALL patients and correlated with their CNS status and other clinical parameters. Moreover, machine learning algorithms involving multivariate regression models and considering clinical and molecular parameters were applied to identify marker panels for improved detection of CNS-leukemia.

Results:

The AP-1 pathway and AP-1 genes *FOS* and *FOSB* were significantly upregulated in blasts recovered from the CNS versus BM of NSG-mice. Accordingly, CNS+ patients exposed significantly elevated *FOSB*-mRNA levels as compared to CNS- patients ($p = 0.038$). Of note, 6/8 patients with CNS-relapse were FOS^{high} and $FOSB^{high}$ (mRNA levels above median) upon diagnosis. In multivariate analysis, FOSB in the fourth quartile was independently associated with a ~4-fold increased risk of initial CNS-positivity (odds ratio (OR) = 3.986, 95% confidence interval (CI) = 1.181–13.459, $p = 0.026$). Furthermore, high FOS levels were associated with prednisone poor response (OR = 2.543, 95% CI = 1.110, $p = 0.027$) and expression in the fourth quartile was linked with stratification to the high-risk group (according to ALL-BFM study criteria) (OR = 4.403, 95% CI = 1.341–14.464, $p = 0.015$). Additionally, high FOS and FOSB levels were independent predictors of death (OR = 3.384, 95% CI = 1.237–9.260, $p = 0.018$). Moreover, FOS^{high} and $FOSB^{high}$ patients showed significantly lower 5-year event free survival ($p = 0.031$ and $p = 0.011$, respectively) than FOS^{low} and $FOSB^{low}$ patients. Furthermore, employing machine learning algorithms, we identified a CNS-high risk marker panel that predicted CNS-positivity more reliably than clinical risk factors alone in our patient cohort. Of note, FOSB showed the most considerable influence on CNS positivity among all considered parameters.

Conclusion:

Here, we propose *FOSB* as a novel independent predictor of CNS leukemia. FOS and FOSB may be surrogate markers of inferior patient outcome with therapeutic potential in T-ALL requiring further prospective and mechanistic validation.

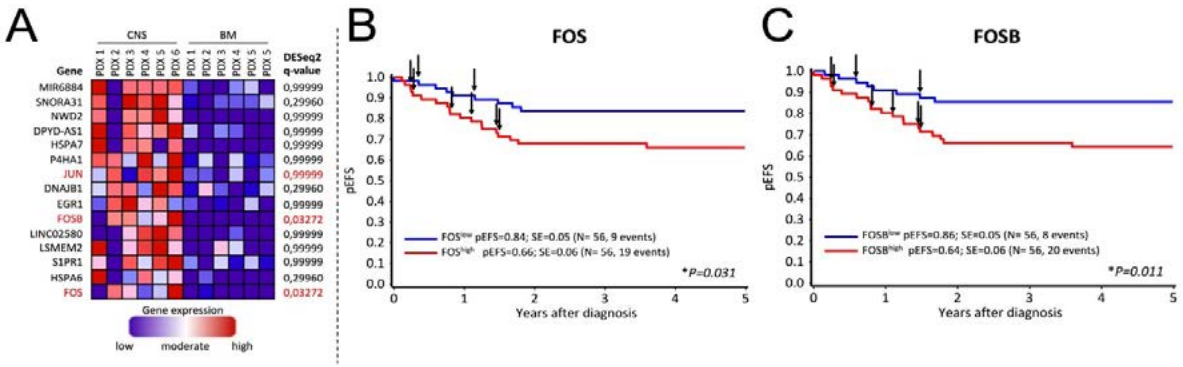

Figure 1: (A) Gene signature of the 15 most upregulated genes in CNS-derived blasts included AP-1 transcription factors JUN (q = 0.99999), FOS (q = 0.03272) and FOSB (q = 0.03272) (labelled red) as determined by gene set enrichment analysis (GSEA) compared to blasts from the BM of 6 T-ALL PDX-mice. Corresponding q-values were calculated by DESeq2 method. Significance set at q < 0.05. (B–C) Kaplan-Meier analysis showing reduced 5-year event-free survival of (B) FOS^{high} patients compared to FOS^{low} patients (probability of event-free survival (pEFS) = 66% vs. 84%; p = 0.031) and of (C) $FOSB^{high}$ vs. $FOSB^{low}$ patients (pEFS = 64% vs. 86%; p = 0.011). Classification into "high" or "low" expression is based on median mRNA expression of either marker. Black arrows indicate CNS relapses. Events included incomplete remission, relapse, second cancer or death from any cause. Log-rank statistics. **P* < 0.05. BM: bone marrow; SE: standard error.

Acute lymphoblastic leukemia (genetics / predisposition)

O 09 - IKZF1 deletions cause targetable resistance to cytarabine in acute lymphoblastic leukemia

Britt Vervoort[1], Miriam Butler[1], Kari Grunewald[1], Dorette Ingen Schenau[1], Trisha Tee[1], Vincent van der Velden[2], Roland Kuiper[3], Peter Hoogerbrugge[1], Laurens van der Meer[1], Frank van Leeuwen[1]

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2 *Erasmus MC, Immunology, Rotterdam, Netherlands*
3 *Princess Maxima Center for Pediatric Oncology, Kuiper group, Utrecht, Netherlands*

Background:

Deletions and mutations affecting lymphoid transcription factor *IKZF1* occur in about 10–15% of pediatric patients and up to 50% of adults with B cell precursor acute lymphoblastic leukemia (BCP-ALL) and predict a poor outcome. We showed previously that loss of IKZF1 function compromises the therapeutic efficacy of glucocorticoids (GCs). Here, we investigated whether loss of IKZF1 function also affects therapy response to other chemotherapeutic agents used in the treatment of ALL.

Results:

We used CRISPR-Cas9 to target the *IKZF1* locus in SEM pro-B ALL cells and evaluated the response to standard chemotherapy used in the treatment of ALL. We observed that *IKZF1*$^{-/-}$ SEM cells show a diminished response to the pyrimidine analogue cytarabine (AraC) with an 8-fold increase in IC^{50} compared to control cells. Using ex vivo cultures of patient derived xenografts (PDX), we observed that *IKZF1* deficient leukemic cells were significantly more resistant to AraC relative to cells wild type for *IKZF1*. To account for the effect of the different genetic backgrounds of patient cells, we used the thalidomide variant Iberdomide to target the IKZF1 protein for proteasomal degradation in PDX cells wildtype for *IKZF1.* Similar to genetic inactivation, drug-induced IKZF1 loss leads to AraC resistance in 7 out of 8 PDX samples. Furthermore, we compared AraC treatment response in mice transplanted with *IKZF1* wild-type leukemias (n = 5) with *IKZF1* deficient leukemias (n = 7). This confirmed that the *IKZF1* deficient tumors respond poorly to treatment.

Next, we interrogated available patient data to search for evidence of cytarabine resistance. In the induction phase of the Dutch ALL 10/11 protocols, a block containing only AraC is flanked by minimal residual disease (MRD) measurements. We used these measurements to calculate the reduction in tumor load and showed that the treatment response was attenuated in patients with *IKZF1* deleted ALL (n = 25) compared to patients with an ALL wildtype for IKZF1 (n = 35) (**p = 0.0065). Although during this phase patients are also treated with 6-mercaptopurine and cyclophosphamide, we attribute the difference in response to treatment to cytarabine resistance since we see no difference in response to 6-MP and cyclophosphamide in vitro.

Based on RNA sequencing of SEM cells, we hypothesize that the induction of oncogene Evi1 that was observed in IKZF1$^{-/-}$ cells, which was further substantiated by AraC treatment may contribute to resistance. This effect was confirmed in 4 PDX samples treated with Iberdomide. Although a link between IKZF1 and Evi1 in ALL has not been described before, studies in CML and AML suggest both a genetic and functional interaction.

To study whether resistance in response to IKZF1 loss can be reversed, we performed CRISPR/Cas9 screens to identify novel drug targets that can restore response to AraC therapy. Guide RNAs targeting the mediator kinases CDK8/19 as well as gRNAs targeting CSNK2, a kinase that activates both IKZF1 and Evi1, were selectively and strongly depleted during treatment, suggesting that these proteins modify response to cytarabine treatment. Indeed, small-molecule inhibitors targeting CDK8/19 or CSNK2 activity strongly synergized with AraC induced killing of leukemic cells.

We conclude that IKZF1 deficiency causes reversible resistance to cytarabine in BCP-ALL and we hypothesize that the implications of the potential interaction between IKZF1 and Evi1 may be extended to AML.

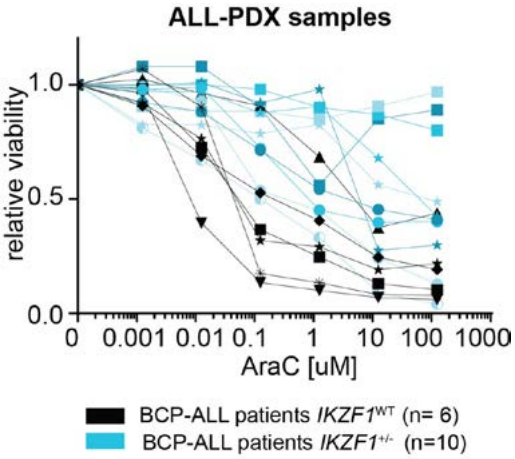

Figure: Dose response curves to AraC for PDX samples that are either wt (black) or carry a heterozygous deletion (blue) of the IKZF1 gene.

O 10 - The role of TP53 mutations in mutagenesis and selection dynamics under exposure of platinum-based compounds

Eline Bertrums[1], Jurrian de Kanter[1], Evangelia Antoniou[2], Dirk Reinhardt[2], Henrik Hasle[3], Mark Verheul[1], Marry van den Heuvel-Eibrink[1], C. Michel Zwaan[1], Bianca Goemans[1], Ruben van Boxtel[1]

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Introduction:

Over the past decades, childhood cancer survival has increased to around 80%. However, these children suffer from long-term adverse effects. Childhood cancer survivors have a five-fold increased risk of secondary cancers, amongst which therapy-related myeloid neoplasms (t-MN) are one of the most prevalent. Here, we aim to increase understanding of the origin and genetic evolution of pediatric t-MN.

Methods:

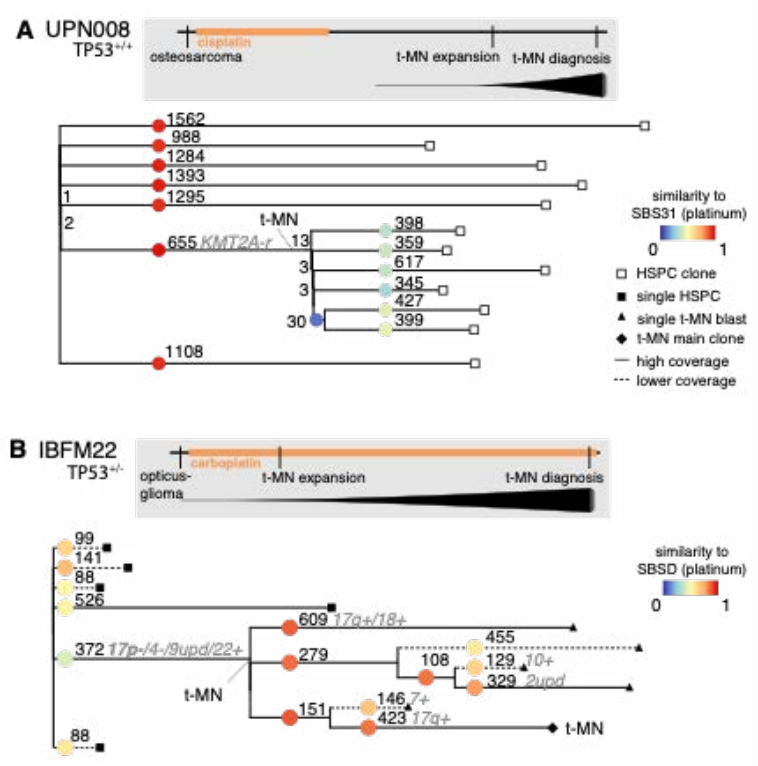
We studied mutation accumulation and clonal evolution of t-MN in 43 pediatric patients, which we were able to include via a collaboration with the International Berlin-Frankfurt-Munster (I-BFM) AML study group. Depending on available material, we performed whole genome sequencing (WGS) of bulk as well as single t-MN blasts and normal hematopoietic stem and progenitor cells (HSPCs). Single cell sequencing was performed by clonal expansion of HSPCs or directly after primary template-directed amplification (PTA) of the DNA of a cell.

Results:

The assessed patients were previously treated for a variety of first cancers, and the latency time between first diagnosis and t-MN varied from 1–12 years. Our data confirmed that pediatric t-MN is mainly driven by gene fusions, specifically *KMT2A* rearrangements (58%). t-MN blasts showed an increased mutation burden compared to a previously established baseline of mutation accumulation in healthy blood cells. In some patients, this increase was caused by distinct treatment-associated signatures. We identified platinum- and thiopurine-associated mutation patterns in patients treated with these respective drugs. Platinum-associated signatures included SBD31, SBS35 and the novel signature SBSD. SBSD was mainly identified in patient IBFM22, who was a patient with Li-Fraumeni syndrome (germline *TP53* mutation) treated with carboplatin for an opticus glioma. Besides these clear treatment-associated signatures, in other patients the increase in mutations could be attributed to signatures of processes that are also active during healthy life. This latter observation points towards an indirect mechanism of treatment-associated mutagenesis.

We investigated the clonal evolution of t-MN in patients with and without germline *TP53*-mutations, who were treated with platinum compounds, using retrospective lineage tracing. This analysis is based on the somatic mutations that are shared between different cells of the same patient. We found that platinum drugs prohibit the t-MN cell-of-origin to expand during exposure, in line with the induced DNA-crosslinking. Indeed, in most patients, we did not find any platinum-related signature in the unique mutations of the sequenced t-MN blasts, indicating that the t-MN clone started to expand after removal of the platinum exposure (UPN008, IBFM42; Figure 1A). We hypothesize that this cell-cycle arrest is *TP53*-dependent. Indeed, in patient IBFM22 the shared mutations did not show the platinum-related signatures, whereas the unique mutations in the single t-MN blasts did (Figure 1B), indicating that the t-MN blasts could expand during treatment. Similarly, in another LFS patient (UPN034) treated with cisplatin for an osteosarcoma, we found that HSPCs were able to divide multiple times during treatment.

Figure 1: Clonal evolution of t-MN in TP53-wildype and TP53-aberrant patients.



Conclusion:

t-MN in children is mostly caused by gene fusions, in specific *KMT2A* rearrangements. The increased mutation loads in t-MN blasts and treatment-exposed HSPCs were caused by both direct and indirect treatment-associated mutagenesis. Furthermore, aberrant *TP53* enables cell-division during platinum treatment, which is normally blocked by DNA-crosslinking. Therefore, t-MN development in LFS patient has more similarities to t-MN development in adults, with the founding cell already being present before therapy, and clonally expanding under selective pressure.

O 11 - Description of global cytogenomics features in childhood acute lymphoblastic leukemia by optical genome mapping

Elena García Sánchez[1], Jorge García[1], Ana Gómez[2], Reyes Giménez[2], David Ruano[2], Maitane Andion[2], Lorea Abad[2], Ana Castillo[2], Luis Madero[2], Manuel Ramírez[1]

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Background:

Childhood acute lymphoblastic leukemia (cALL) is the most common infant malignancy involving multiple genetic alterations, i.e., sequence and structural mutations. In the past few years, great advances in understanding the biology of cALL by cytogenetic and molecular techniques such as karyotyping, FISH and next generation sequencing have been achieved. Optical Genome Mapping (OGM) has recently arises as a novel technology capable of describing whole genome structural and copy number variations with a higher resolution than conventional cytogenetic techniques.

Aims:

To characterize global cytogenetic alterations in diagnostic samples of cALL by OGM.

Methods:

Cellular samples from diagnostic bone marrow aspirates of 97 patients with cALL were processed for OGM. In brief, the ultra-high molecular weight gDNA from cALL samples was extracted, labelled and loaded into chips to run in the Saphyr platform (Bionano Genomics). Collected data was analysed by Bionano Access 1.6 software. A binary matrix (presence/absence of each genomic alterations) was created with the chromosomal variants data, then clustering tests were performed to assess for clinical correspondence. In addition, all chromosomal variations were annotated to identify the genes involved. Furthermore, enrichment studies were performed to identify altered cellular pathways and a linkage test was carried out by the STRING interaction database.

Results:

We obtained a full concordance with previously diagnostic results obtained by conventional cytogenetic techniques and also found novel variants that could indicate common processes required for the development of ALL and may help in classification. CNVs losses and deletions represented almost 50% of all chromosomal alterations found. The number of alterations was diverse among patients ranging from 3 to 300 per sample. Clusters provided by K-Means algorithm based in chromosomal alterations were significantly correlated with lineage. The enrichment analysis identified recurrent involvement of known important pathways in leukaemia, such as JAK-STAT. Moreover, an abundant number of immune pathways were altered, particularly those in lost regions, suggesting that the loss of immune functions probably is key for leukaemia development. Network protein interaction using STRING database supported the involvement of the above mentioned pathways.

Conclusion:

The analysis of cytogenetic variations by OGM emerges as a powerful tool to improve the knowledge of the events that underlie cALL biology.

O 12 - Constitutional variants in patients developing second malignant neoplasms after therapy for pediatric acute lymphoblastic leukemia – A case-control study

Stefanie Verena Junk[1], Alisa Förster[2], Gunnar Schmidt[2], Martin Zimmermann[1], Anja Möricke[3], Gunnar Cario[3], Bernd Auber[2], Martin Schrappe[3], Christian Peter Kratz[1], Martin Stanulla[1]

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Background:

Today most of the children treated for acute lymphoblastic leukemia (ALL) can be cured by the application of intensive combination chemotherapy regimens. However, up to 10% develop a second malignant neoplasm (SMN) after undergoing ALL treatment with cure rates often being dismal. Thus, strategies are needed for an early identification of patients at risk for SMN development, an improved understanding of the underlying pathobiology and, ideally, the development of preventive actions. In this study, we analyzed genetic variation in 161 cancer predisposition genes to determine their potential association with SMN.

Patients and methods:

All patients were treated in multicenter trial AIEOP-BFM ALL 2000 for frontline treatment of pediatric ALL. Median follow-up for the entire patient group was 14.8 (4.6–19.2) years as of November, 2020. In a matched case-control approach, we analyzed germline DNA obtained from remission samples of 222 individuals (74 cases, 148 controls). Matching criteria were sex, age at diagnosis of ALL, immunophenotype and treatment. Candidate genes included genes, previously identified by us in previous projects plus genes recommended by Byrjalsen *et al* 2021. Pathogenicity was assessed according to current variant interpretation standards.

Results:

Overall 31 conspicuous findings were determined in 22 genes: 14 variants (9 likely pathogenic and 5 pathogenic) were detected in 14 (9%) control patients with ALL and 17 (6 likely pathogenic and 11 pathogenic) in 16 (23%) cases with ALL and subsequent SMN; for details see Table 1 and Figure 1. While some variants were found in patients from both groups (e.g., rs28909982 in *CHEK2*, rs113993993 in *SBDS* and rs764079291 in *NF1*), certain genes were exclusively altered in SMN patients (e.g. *ATM, TP53, A2ML1, MSH6 and PMS2*).

Among the SMN patients, (likely) pathogenic variants in one of the candidate genes were observed in 3 of 11 (27%) cases with brain tumors, 5 of 28 (18%) with other solid tumors and 8 of 35 (23%) with hematologic SMN. Three individuals with hematologic SMN carried variants in genes predisposing associated with Noonan syndrome and other rasopathies (*LZRT1* and *KRAS*). Two male patients, identified with astrocytoma at 9.2 and 9.7 years after diagnosis of ALL, carried deleterious variants in *ATM*. Two SMN patients carried variants in genes linked with constitutional mismatch repair deficiency: one patient with a mucoepidermoid carcinoma (*MSH6*) and one individual with an astrocytoma (*PMS2*); the later also carried an *ATM* variant. The *TP53* variant, rs121912651, was observed in a female patient developing a myelodysplastic syndrome at 8.8 years after diagnosis of ALL; rs121912651 was previously described in two B-cell ALL patients with SMN (Hof *et al* 2011, Qian *et al* 2018). Two observations, Pro470Valfs*3 (*PMS2*) and rs587776650 (*NBN*) were homozygous. All other variants were detected with a variant allele frequency of approximately 50% (i.e. heterozygous). Nonetheless, using remission bone marrow samples, we cannot completely exclude clonal hematopoiesis here.

Conclusions:

SMN patients in our study had a 2-fold higher frequency of unfavorable variants in genes associated with cancer predisposition. However, the majority of SMN patients (>70%) had no deleterious alterations in any of the included candidate genes. Our current results, consistent with our previous findings (Junk *et al* 2019), suggest that beyond high-risk variants, more common genetic variants are likely to contribute to SMN development, as well. Novel analytical approaches making collective use of genetic information may further enhance our understanding and profiling of SMN risk in ALL patients.

Table 1. Pathogenic and likely pathogenic variants determined in patients with acute lymphoblastic leukemia with or without second malignancies

Patients with ALL and subsequent SMN (n=74)					Patients with ALL (n=148)			
GRCh37 (hg19)[a]	Consequence	Variant ID	Class[b]	Gene[c]	GRCh37 (hg19)[a]	Consequence	Variant ID	Class[b]
11-108186099-G-T	NM_000051.4(ATM):c.6199-1G>T[d]	rs1591788932	4	*ATM*[c]				
11-108115654-C-T	NM_000051.4(ATM):c.802C>T (p.Gln268Ter)	rs557012154	5					
				RECQL4[1]	8-145738522-C-G	NM_004260.4(RECQL4):c.2464-1G>C	rs398124117	4
				CDH1[2]	16-68862112-A-T	NM_004360.5(CDH1):c.2200A>T (p.Arg734Ter)	-	4
				BRCA1[1]	17-41197784-G-A	NM_007294.4(BRCA1):c.5503C>T (p.Arg1835Ter)	rs41293465	5
13-32907428--A	NM_000059.4(BRCA2):c.1813dupA (p.Ile605Asnfs*11)	rs80359306	5	*BRCA2*[1]				
				FANCD2[1]	3-10130147-C-T	NM_033084.6(FANCD2):c.3481C>T (p.Gln1161Ter)	rs369022159	4
					3-10127560-C-T	NM_033084.6(FANCD2):c.3289C>T (p.Arg1097Ter)	-	4
17-7577539-G-A	NM_000546.6(TP53):c.742C>T (p.Arg248Trp)	rs121912651	5	*TP53*[2]				
22-29121326-T-C	NM_007194.4(CHEK2):c.349A>G (p.Arg117Gly)	rs28909982	4	*CHEK2*[2]	22-29121326-T-C	NM_007194.4(CHEK2):c.349A>G (p.Arg117Gly)	rs28909982	4
2-48030647--C	NM_000179.3(MSH6):c.3261dupC (p.Phe1088Leufs*5)	rs267608078	5	*MSH6*[1]				
7-6026988--AC	NM_000535.7(PMS2):c.1408delins47 p.(Pro470Valfs*3)[d,e]	-	5	*PMS2*[1]				
17-29528489-C-T	NM_000267.3(NF1):c.1246C>T (p.Arg416Ter)	rs764079291	5	*NF1*[1]	17-29528489-C-T	NM_000267.3(NF1):c.1246C>T (p.Arg416Ter)	rs764079291	5
				NBN[1]	8-90983445-GTTTT--	NM_002485.5(NBN):c.657_661delACAAA (p.Lys219Asnfs*16)[e]	rs587776650	5
12-9007428-G-A	NM_144670.6(A2ML1):c.2764+1G>A	rs773034576	4	*A2ML1*[1]				
12-25398285-C-G	NM_004985.5(KRAS):c.34G>C(p.Gly12Arg)	rs121913530	5	*KRAS*[1]				
22-21344765-G-A	NM_005767.4(LZTR1):c.742G>A (p.Gly248Arg)	rs869320686	5	*LZTR1*[1]	22-21350154-C-T	NM_005767.4(LZTR1):c.2062C>T (p.Arg688Cys)	rs587777178	4
22-21342297-AG--	NM_005767.4(LZTR1):c.401-2_401-1delAG	rs769200796	4					
				PTPN11[1]	12-112888157-A-G	NM_002834.5(PTPN11):c.173A>G (p.Asn58Ser)	rs751437780	4
16-3832811-G-A	NM_004380.3(CREBBP):c.1447C>T (p.Arg483Ter)	rs1555484797	5	*CREBBP*[1]				
7-66459273-T-A	NM_016038.4(SBDS):c.184A>T (p.Lys62Ter)	rs120074160	5	*SBDS*[1]	7-66459197-A-G	NM_016038.4(SBDS):c.258+2T>C	rs113993993	5
7-66459197-A-G	NM_016038.4(SBDS):c.258+2T>C	rs113993993	5		7-66459197-A-G	NM_016038.4(SBDS):c.258+2T>C	rs113993993	5
5-176638471-C-G	NM_022455.5(NSD1):c.3071C>G (p.Ser1024Ter)	-	4	*NSD1*[1]				
				ERCC2[1]	19-45868093-CACT--	NM_000400.4(ERCC2):c.594+2_594+5delTGAG	rs762309206	4
6-43578333-C-T	NM_006502.3(POLH):c.1117C>T (p.Gln373Ter)	rs121908564	4	*POLH*[1]				
				MPL[1]	1-43817975-G--	NM_005373.3(MPL):c.1653+1delG	rs755257605	4

Abbreviations: acute lymphoblastic leukemia (ALL); second malignant neoplasm (SMN); variant identifier (Variant ID). The shades represent the different SMN entities: brain tumors (blue, astrocytoma (n=3)[c], hematologic neoplasms (red, MDS (n=5), non-Hodgkin Lymphoma (n=2) and acute myeloid leukemia (n=1)) and other solid tumors (green, thyroid cancer (n=2), mucoepidermoid carcinoma (n=1), melanoma (n=1) and nerve sheath tumor (n=1)).
[a] Physical positions of the variants are according to the human genome assembly GRCh37 (hg19); [b] variant pathogenicity is given by commonly used scores: "likely pathogenic" classification is referred to as class 4 and "pathogenic" as class 5; [c] variants were classified according to the current ACMG classification guidelines or gene specific recommendations: [1] Richards, S. *et al.* 2015 (PMC4544753); [2] Lee, K. *et al* 2018 (PMC6188664), [3] Fortuno, C. *et al.* 2021 (PMC8374922); [4] Thompson, B.A. *et al* 2014 (PMC4294709, InSight, version 2018); [5] Wappenschmidt, B. *et al* 2020 (PMC7174002); [d] one SMN patient who developed an astrocytoma carried two deleterious variants; [e] these two variants were observed homozygous.

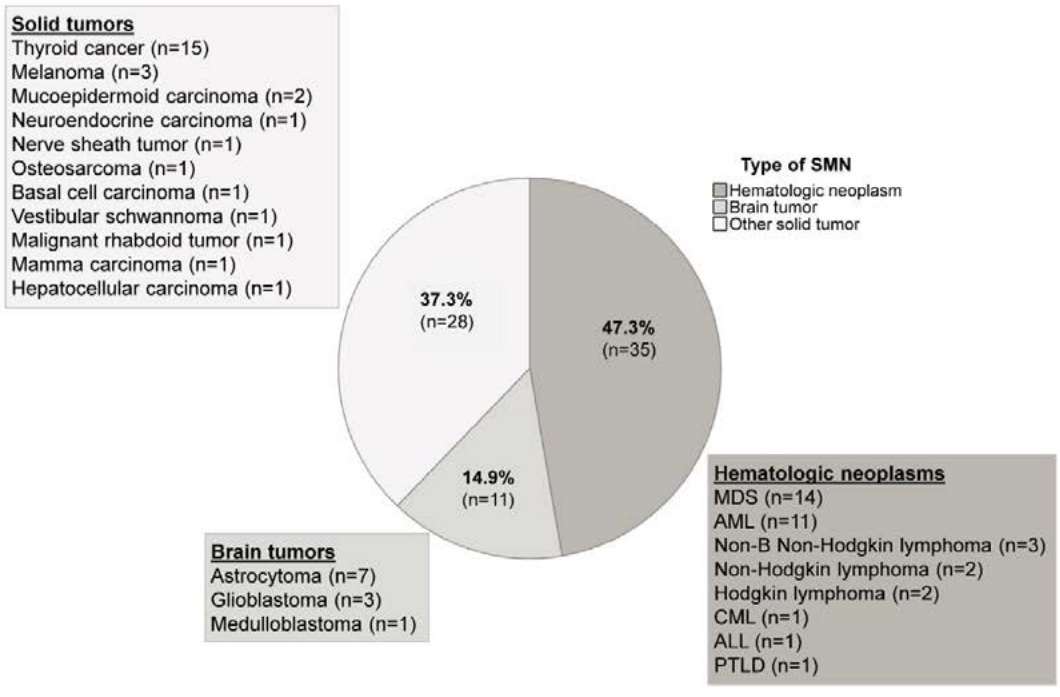

Figure 1. Frequency of second malignant neoplasms (SMN) identified in patients of the AIEOP-BFM ALL 2000 study population by entity.

Non-Hodgkin's lymphoma

O 13 - Molecular characterization of hematological malignancies in children with CMMRD

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Background:

Constitutional Mismatch Repair Deficiency (CMMRD) is a high-risk childhood cancer predisposition syndrome. In children with CMMRD, all cells have a defect in mismatch repair (MMR) due to biallelic germline pathogenic variants (gPV) in one of the MMR genes, *MLH1, MSH2, MSH6* and *PMS2*, resulting in accelerated accumulation of single base substitutions (SBS) and small insertions and deletions. As a result, these children often develop multiple primary malignancies during childhood. The tumor spectrum consists mostly of high-grade brain tumors, gastrointestinal (GI) tumors and hematological malignancies. While brain tumors and GI tumors in children with CMMRD have been extensively studied, the molecular characterization of hematological malignancies is relatively poor.

Objective:

To better understand the mechanisms underlying hematological malignancies in children with CMMRD, we study the mutational processes in this subgroup of our cohort.

Methods:

We included 17 patients with CMMRD and performed whole genome or whole exome sequencing on 41 tumors (13 hematological malignancies, 14 brain tumors and 14 GI tumors) and 17 matched normal samples. After somatic variant calling, we calculated the tumor mutational load (TML) and we determined the contributions of COSMIC reference single base substitution (SBS) signatures to the tumor mutational profiles.

Results:

Ten out of 17 children with CMMRD in our cohort developed one or more hematological malignancies. These children had biallelic gPVs in *MSH6* (n = 5) or *PMS2* (n = 5). Out of 13 hematological malignancies, nine were second primary malignancies or relapses and ten were T-cell malignancies. We found that hematological malignancies in children with CMMRD had a lower TML (median 17.4) than brain tumors (median 215.2) and GI tumors (median 24.9). Within the group of hematological malignancies, mutational signatures SBS26 and SBS1 were predominantly detected in malignancies of children with *PMS2* and *MSH6* deficiency, respectively, while SBS15 was found in both. SBS26 and SBS15 are known MMR-associated signatures, and although SBS1 is not, it has previously been identified in *MSH6*- and *MSH2*-deficient tumors. In several hematological malignancies, we identified additional mutational processes. For example, in a T-cell lymphoblastic lymphoma (T-LBL) in our cohort, we detected a high TML (150 mut/Mb) and contribution of polymerase proofreading insufficiency-associated signature SBS20, which are likely to be caused by a somatic *POLD1* mutation. In a T-LBL and a T-cell acute lymphoblastic leukemia (T-ALL) that two children developed after being treated for a high-grade glioma, we found strong contributions of signature SBS11, which could be directly associated with the Temozolomide treatment they received. Therefore, adding to the knowledge that Temozolomide is ineffective in treatment of MMR-deficient tumors, we now also see examples in which this therapy appears to have contributed to the development of second hematological malignancies in children with CMMRD.

Conclusion:

Here, we show that hematological malignancies in children with CMMRD are hypermutated (TML > 10Mut/Mb), but the TML is lower than in other types of malignancies in the context of CMMRD. Furthermore, the mutational profiles are influenced by the gene that is affected by gPVs, additional somatic mutations, and prior treatment for cancer. In-depth molecular characterization of tumors may help to guide individual treatment decisions in children with CMMRD.

O 14 - The genomic landscape of pediatric T-cell lymphoblastic lymphoma

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T-cell lymphoblastic lymphoma (T-LBL) and T-cell acute lymphoblastic leukemia (T-ALL) represent malignancies of immature T-cells. Given the similarities and differences between pediatric T-LBL and T-ALL, the question has been raised whether T-LBL and T-ALL represent two different diseases or different manifestations of the same disease. The genomic landscape of pediatric T-ALL has been extensively studied, revealing a spectrum of recurrently mutated driver genes and aberrant expression of oncogenes associated with T-cell development stages, which can divide T-ALL in four different subtypes (immature, TLX1/NKX2.1, TLX3, TALLMO). Due to the lower incidence of pediatric T-LBL and difficulties in obtaining diagnostic material, extensive research on T-LBL has not been performed and risk stratification in T-LBL lags behind. This study aims to characterize the genomic landscape of pediatric T-LBL, compared to the mutational spectrum, fusion genes and subtypes identified in pediatric T-ALL. We performed whole exome sequencing (WES) and RNA sequencing for a cohort of 44 and 26 T-LBL patients, respectively. WES revealed a median tumor mutational burden of 0.42 mutations per Mb. The most frequently mutated genes involved in our T-LBL cohort were *NOTCH1* (50%), *FBXW7* (16%), *RPL10* (14%), *PHF6* (11%) and *BCL11B* (11%); genes and frequencies comparable to T-ALL. In addition, gross chromosomal aberrations (>20Mb) were found in 39% of the patients in our cohort. Based on gene expression profiles, we show that the majority of T-LBL samples cluster together with one of the four known T-ALL subtypes. However, based on the selected genes, approximately a quarter of the T-LBL samples show a gene expression profile that appears to be different from the T-ALL subtypes. Furthermore, we detected *bonafide* in-frame fusions leading to a chimeric transcript in 27% of T-LBL patients which have not been reported in T-ALL, including two NOTCH1 activating fusions in *NOTCH1*/*FBXW7* mutation-negative patients and two fusions entailing *HOXA9*. We found no specific clustering of the fusion-positive T-LBL cases with subtypes, based on gene expression profiles. In conclusion, this study shows extensive overlap between the mutational profiles and gene expression profiles in T-LBL and T-ALL, indicating the similar biology between the two disease entities. However, a subset of T-LBL patients show notable differences in expression profiles and fusion genes. The meaning of this should be topic of future studies.

O 15 - Germline aberrations of DNA repair genes are frequently observed in selected children diagnosed with lymphomas

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Background:

The risk of pediatric hematological malignancies is particularly increased among patients with DNA repair disorders who usually carry biallelic constitutional aberrations in genes encoding key proteins involved in DNA replication and the cellular response to DNA damage. The presence of homozygous germline defects within DNA repair genes affects the prognosis of lymphoma and the risk of severe toxicities of oncological treatment.

Objectives:

We aimed to search for genetic predisposition to lymphoma in children showing the inherited cancer risk based on their phenotype, the course of lymphoma, and/or family history.

Methods:

The study group comprised 46 patients from 34 families diagnosed with lymphoma between 2018 and 2022 in centers of the Polish Pediatric Leukemia/Lymphoma Study Group, who met at least one of the following inclusion criteria: (1) patients with positive family history (at least two family members in the first or second-line affected by lymphoma, at least one <40 years old), (2) comorbidities, (3) at least 2 malignancies in past medical history and at least one was lymphoma, (4) rare subtypes of the lymphomas, and (5) excessive treatment toxicity. Pathogenic variants in the germinal DNA were identified using the whole-exome sequencing (WES) approach. We analyzed both affected and healthy family members, if available. The obtained variants within gene panel were filtered using algorithms based on variant frequency, gene function, pathogenicity, and their association with the phenotype.

Results:

Patients met the following criteria: rare subtypes of the lymphomas (13 families, 38%), positive family history (12 families, 35%), comorbidities (11 families, 32%), multiple malignancies (10 families, 29%), and excessive toxicity (2 families, 6%). We observed the following comorbidities: immunodeficiencies (8, 24%), hearing loss (2, 6%), and one case of polyposis, thrombocytopenia, vitiligo, autism, and dysmorphic features (1, 3%), respectively. Among patients with multiple malignancies, the most frequent were leukemias (4, 12%), and brain tumors (3, 9%), the remaining patients developed teratoma (n = 1), Ewing›s sarcoma (n = 1), thyroid cancer (n = 1), and two subsequent lymphomas (Hodgkin lymphoma and diffuse large B-cell lymphoma).

WES analysis was performed in 27 families. In 8 (30%) examined families pathogenic mutations were identified within DNA repair genes: 4 (15%) children were diagnosed with constitutional mismatch repair deficiency syndrome, 1 (3%) was diagnosed with ataxia–telangiectasia, and 1 (3%) was a carrier of heterozygous *PALB2* mutation. There was one family harboring *BRCA1* mutation and another one with concomitant variants within *BRCA1* and *MLH1*. Additionally, in 5 (19%) families we identified variants in the following genes which may potentially contribute to lymphoma development: *ADAR*, *CXCR4*, *LCK, NTRK3*, and *ANKRD26* (see **Table 1**). The heterozygous variant in *NTRK3* was located in the region encoding kinase domain, in the proximity of loci displaying recurrent somatic mutations in cancer, and which were associated with the overactivation of the Erk1/2, PLC-γ, AKT pathways.

Conclusions/Application to practice:

Germline aberrations of DNA repair genes frequently occur in children diagnosed with lymphomas, especially those with comorbidities and/or multiple malignancies

Figure 1: Clinical characteristics of patients diagnosed with lymphomas who carried pathogenic or likely pathogenic germline variants in cancer predisposition panel.

Family number	Age	Lymphoma type	Zygosity	Gene variants	Positive familial history	Comorbidities	Multiple malignancies	A rare subtype of the lymphoma/untypical course of lymphoma	Excessive toxicity
DNA repair disorders genes									
1	5 8	T-NHL T-NHL	homozygous	*PMS2*, NM_000535.7:c.1296delC; p.Asn432LysfsTer16	Two brothers: 1) T-NHL, 2)T-NHL, and glioblastoma	no	T-NHL, glioblastoma	no	no
2	4	T-LBL	compound heterozygous	*PMS2*, NM_000535.7:c.1939A>T, p.Lys647Ter with intragenic deletion (ex8-ex14)	no	ID	B-ALL, T-LBL	no	no
3	14	T-LBL	compound heterozygous	*PMS2*, NM_000535.5:c.1185delC, p.Met396TrpfsTer2 & c.1876G>C, p.Ala626Pro	no	no	T-LBL, CNS tumor*	T-LBL	no
4	7	T-LBL	compound heterozygous	*MSH6*, NM_000179.3:c.1135_1139del; p.Arg379Ter and NM_000179.2: c.2277_2293del; p.Glu760ProfsTer6	no	ID, polyposis	B-ALL, T-LBL, astrocytoma	no	no
5	6.5	LBCL***	compound heterozygous	*ATM*, NM_000051:c.434T>G; p.Leu145Arg and large deletion of ex40-ex64	no	ID	no	LBCL***	yes
6	10	HL	heterozygous	*PALB2*, NM_024675.4:c.110G>A; p.Arg37His	Father: HL	no	HL, Ewing's sarcoma	no	no
7	2	T-LBL	heterozygous	*BRCA1*, c.4035delA, Glu1346LysfsTer2	no	no	no	Multiple extramedullary involvement**	no
8	10	B-LBL	heterozygous heterozygous	*BRCA1*, NM_007300.3:c.3756_3759del, Ser1253Argfs*10 & *MLH1*, NM_000249.3:c.1360G>C, Gly454Arg	no	no	no	no	yes
Other genes									
10	11	BL	heterozygous	*ADAR*, NM_001111.5:c.577C>G; p.Pro193Ala	no	Hearing loss	no	no	no
11	N.D. N.D.	DLBCL HL	heterozygous	*CXCR4*, NM_003467.2:c.1012dup; p.Ser338PhefsTer6	Mother - DLBCL; Two brothers: 1) DLBCL, 2) HL	ID, hearing loss	no	no	no
12	12	Panniculitis-like T-NHL	heterozygous	*LCK*, NM_005356.5:c.1176C>G; p.Asn392Lys	no	no	no	Panniculitis-like T-NHL	no
13	13	DLBCL	heterozygous	*NTRK3*, NM_001012338.2:c.1745G>A; p.Arg582Gln	no	no	B-ALL, DLBCL	no	no
14	12.5	T-LBL	heterozygous	*ANKRD26*, NM_014915.2:c.4657G>T; p.Glu1553Ter	no	Thrombocytopenia	no	T-LBL	no

Legend: ALL – acute lymphoblastic leukemia, BL - Burkitt lymphoma, B-LBL - B-lymphoblastic lymphoma, DLBCL – diffuse large B-cell lymphoma, HL - Hodgkin's lymphoma, ID – Immunodeficiencies, LBCL*** - Large B-cell lymphoma with IRF4 rearrangements, NHL - Non-Hodgkin's lymphoma, T-LBL - T-lymphoblastic lymphoma, * - Diffuse pediatric - type grade glioma IDH-wild type and H3-wild type, ** - epicranial, kidneys, uterus, left ovary, salivary glands: left parotic, bilateral submandibular glands.

Cellular therapies in leukemia and lymphoma

O 16 - Clinical significance of low minimal residual disease (MRD) positivity in post-transplant acute lymphoblastic leukemia (ALL) monitoring

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Background:

Post-transplantation MRD monitoring is crucial for early decisions on the possible therapeutic interventions in patients with ALL. MRD testing via quantitative PCR (qPCR) using clone-specific immunoglobulin (Ig) and T-cell (TR) receptor gene rearrangements is currently the standardized and most widely used method for online post-transplant MRD monitoring. We have previously shown that MRD detection using Ig/TR monitoring via next-generation sequencing (NGS) is more specific than qPCR and is not burdened by false positives at the time of bone marrow regeneration (Kotrová, BMT 2017). However, Ig/TR monitoring is currently still the method of choice for most laboratories, due to its lower price and faster execution time with frequently repeated MRD measurements.

Objectives:

In this study, we investigated the clinical relevance of prospectively verifying positive non-quantifiable (PnQ) qPCR results with NGS-MRD.

Methods:

Sequential post-transplant MRD monitoring in pediatric and young adult ALL patients was performed at our facility for patients from 3 transplant centers in the Czech Republic and Slovakia. For qPCR-MRD monitoring, we used the standardized EuroMRD approach. MRD marker specificity was first evaluated by amplicon length analysis of the qPCR products (Fronkova, BMT 2008). In cases with corresponding length with the diagnostic sample or unavailable qPCR products for size comparison, the results were reported as positive according to the EuroMRD criteria, and further testing with NGS was performed using the EuroClonality-NGS working group protocols (Svaton, Blood 2023).

Results:

In total, we re-evaluated MRD using NGS in 32 patients. In 9 patients (28%), the results were confirmed as positive. Out of these 9 patients, 5 relapsed despite therapeutic efforts to avert relapse (median time to relapse: 2 months). All 4 patients positive by NGS who did not progress to relapse had immunosuppressive treatment (IST) reduced and one received 5 doses of donor lymphocyte infusions (DLI) in reaction to the qPCR and NGS positivity. One patient died of GvHD reactivation after IST withdrawal. Among the 22 patients determined as negative by NGS, only one relapse occurred (5 months after testing), despite the fact that only in 7 out of 22 NGS-negative patients therapy was intensified (IST reduction, DLI) on the basis of the qPCR result (including the patient who progressed to relapse, who also received 2 doses of DLI). In one patient (who did not relapse), NGS testing was evaluated as inconclusive due to low sensitivity.

Conclusions:

In our study, we confirmed that the NGS method is more specific for discerning low-positive MRD than qPCR, especially in the conditions of regenerating physiological lymphocytes, and thus more reliable for clinical decisions. Quantitative PCR remains the method of choice for repeated MRD measurements in most laboratories due to standardization and logistics. The combination of qPCR measurements and subsequent verification of low positive results via NGS appears to be the safest method for post-transplant MRD-guided clinical decision-making.

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O 17 - Impact of TP53 mutation on outcomes in pediatric and young adults (AYA) patients with relapsed/refractory B-cell acute lymphoblastic leukemia (R/R B-ALL) after CD19- CAR T-cell therapy

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Background:

Relapse after CAR T-cell therapy in pediatric and AYA patients with R/R B-ALL remain the leading cause of therapy failure and we are still not able to predict it. Prognostic factors of relapse are needed to identify those patients who might benefit from post CAR-T consolidation strategies. High-risk genetics has not been associated with higher relapse risk after CAR-T. However, TP53 mutation (TP53mut) was described as a risk factor for CD19 negative relapses in one series of patients. The aim of this study is to describe the outcomes of patients with TP53mut after CAR T-cell therapy.

Methods:

We analyzed 43 patients (pts) under 25 of age with R/R B-ALL treated with 4-1BB CD19 CAR T-cells and available biological material in a single center from Jan-16 to Jan-23. TP53 mutational status was identified by Sanger and next generation sequencing.

Results:

Forty three patients were infused (CTL019/Tisagenlecleucel: n = 38; ARI-0001-cells: n = 5). Patients with and without TP53mutation were comparable except for a female predominance in the TP53mut group (Table 1).

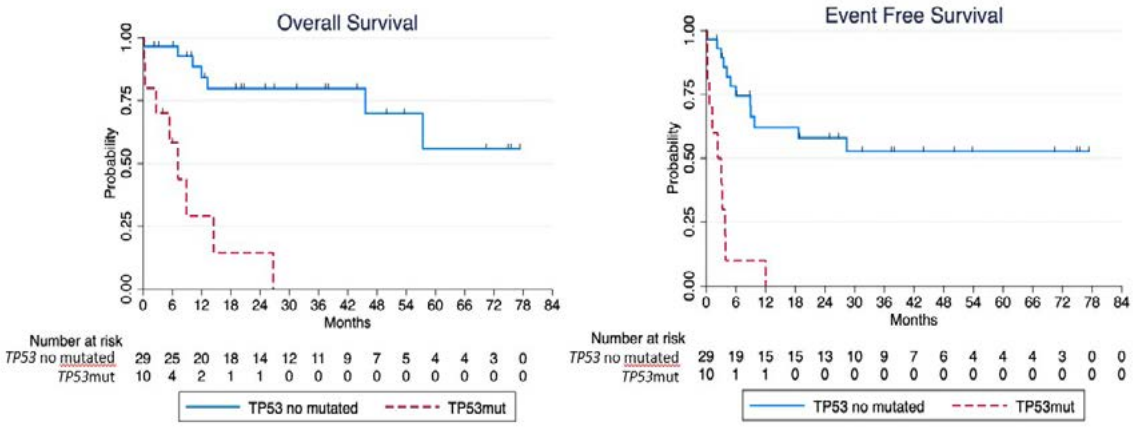
Among 11pts with TP53 mut, 1pt (9%) died from fulminant cytokine release syndrome (CRS) and 4pts (36%) were refractory (one made a lineage switch). Six patients (55%) achieved complete remission (CR). Five patients (83%) had CD19neg relapses in bone marrow (BM) and died from progressive disease (PD). One patient (17%) was bridged to hematopoietic stem cell transplant (HSCT) 1month after infusion and she was alive and in CR 1 month after HSCT.

Among the 32 TP53 non mutated patients, 2pt died due to fulminant CRS and 19pts (59%) are still alive and in CR. One patient received 1 cycle of Inotuzumab and 4pts received consolidation with HSCT post CAR-T: 2 for early B-cell recovery and 2 as per physician's decision. Eleven patients (34%) relapsed. One patient had CD19+ BM relapse and was bridged to HSCT after blinatumomab and died due HSCT related mortality at 10 months. Two patients had CD19+ extramedullary disease (CNS, testicle). Both died: 1pt died from PD and the other from toxicity after HSCT. Eight patients had CD19neg BM relapse and 7 of them received additional therapies (5 Inotuzumab/2 chemotherapy). Of these, 5pts were bridged to HSCT but only 2 remain in CR (7 and 17 months after HSCT).

One year overall and event-free survival were 15% and 0 in TP53mut patients, and 83% and 61% in patients without TP53 mut ($p < 0.001$), respectively (Figure 1).

Variables	TP53 mutated (n = 11)	TP53 no-mutated (n = 32)	p-value
Female,N(%)	8 (72.7)	14 (43.8)	0.01
Median age at infusion (range),IQR(years)	7.7 (4.1–24.8) IQR:4.6	9.3 (2.7–24.2) IQR: 7.4	0.22
Baseline disease status			
- Primary refractory disease,N(%)	0	2(6.3)	0.19
- 1st Refractory relapse,N(%)	6(54.5)	8(25)	
- ≥ 2nd Relapse,N(%)	5(45.5)	22(68.7)	
Prior HSCT, N (%)	3 (27.3)	17 (53.1)	0.14
Prior CNS, N (%)	2 (18.2)	9 (28.1)	0.4
Extramedullary disease	2(18.2)	17(53.2)	0.1

Previous lines, median(range)IQR	3 (2–4) IQR:1	2 (1–5) IQR:1	0.13
Prior Inotuzumab/Blinatumomab,N(%)	2/0	1/2	
%Blasts pre-lymphodepletion in BM (median, min-max, IQR)	73 (2–99) IQR:65	66 (0–98) IQR:65	0.62
Dose of CAR-T(x10E6CAR-T/kg)	3.2 (1–4) IQR: 1.4	2.5 (0.4–5) IQR: 1.7	0.45



Conclusions:

Pediatric and AYA patients with R/R B-ALL and TP53 mutation treated with CAR-T have very poor prognosis. In our series, all patients, but one with very short follow-up, were refractory or had CD19 negative relapses, most of them before 6 months after CAR-T. The role of early consolidation with HSCT in patients achieving complete remission after CAR-T should be explored.

O 18 - Identification of biomarkers predictive of relapse in pediatric and Young adults (Ya) patients with relapsed/ refractory B-Cell Acute Lymphoblastic Leukemia (R/R pB-ALL) after CD19-CAR T-cell therapy

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Background:

CAR T-cell therapy has improved survival in pediatrics and YA patients with relapsed/refractory precursor B-cell acute lymphoblastic leukaemia (r/r pB-ALL). However, a significant portion of patients relapse, and prognostic factors are needed. In adults, lower LDH and higher platelet counts prior to lymphodepletion chemotherapy (LD), as well as the use of fludarabine during LD, have been found to be associated with longer event-free survival (EFS). Data in paediatric patients are scarce.

Objectives:

The aim of this study is to describe whether there is an association between LDH and platelet counts and the risk of relapse in a paediatric population.

Methods:

An observational, descriptive, retrospective, single-centre study was conducted. Patients aged 1–25 years with R/R B-ALL treated with CD19-CAR T-cell therapy (tisagenlecleucel y ARI-0001), from April-2016 to January-2023 were included.

Results:

Seventy-seven patients were included (Table). All received cyclophosphamide and fludarabine as lymphodepletion chemotherapy. Probability of EFS and overall survival (OS) at 5 years were 39,2% (95% CI 26,6–51,5%) and 50,7% (95% CI 34,2–65,0%), respectively. Of the 77 patients, three patients died duet tocytokine release syndrome and six were refractory to CD19-CAR T-cell therapy. Of the 68 patients who achieved complete remission (CR), 28 relapsed with a median time of 8,6 months (2,1–28,4; IQR:14,7 2 died in CR due to transplant-related toxicity and 38 remain alive in CR. Univariate analysis showed that a pre-lymphodepletion platelet count ≥75,000/mm3 was associated with higher EFS (p = 0.0020) and platelet count ≥100,000/mm3 with higher OS (p = 0.009). LDH levels above the normal range was not associated with a decrease in EFS (p = 0.14) nor OS (p = 0.083).

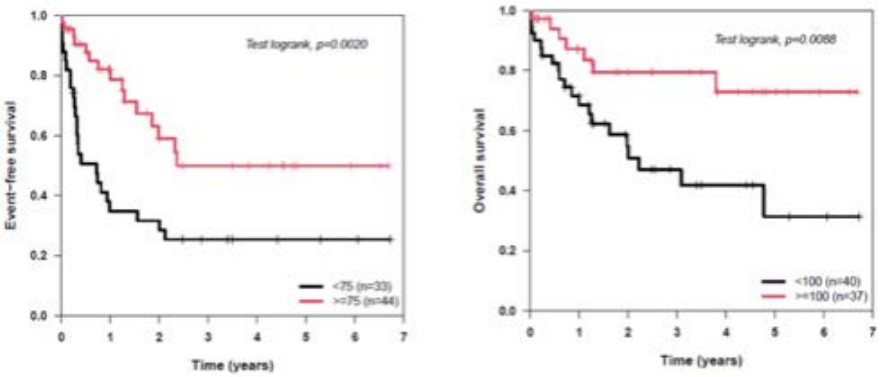

Image: EFS and OS according to platelet count.

Conclusion:

In our series of patients, the platelet count prior to LD was a survival biomarker; however, LDH does not seem to have a prognostic value. The identification of relapse prognostic factors would allow the selection of patients who could benefit from post-CAR-T consolidation strategies.

Table: Pre-infusion patient characteristics.

Variables	N = 77
Female, n(%)	38(49)
Median age at infusion, years (range)	8,4 (1,3–24,8) IQR:6,2
Baseline disease status: • Primary refractory, n(%) • 1st refractory relapse, n(%) • ≥2nd relapse, n(%)	2(2,6) 20(26) 55(71,4)
Prior central nervous system infiltration, n(%)	26(33,8)
Prior extramedullary infiltration, n(%)	42(52,5)
Previous lines, median (range)	2 (1–5) IQR:1
Prior HSCT, n(%)	38(49,4)
Prior Blinatumomab, n(%)	6(7,8)
Prior Inotuzumab, n(%)	5(6,5)
%Blasts pre-lymphodepletion: • <5%blasts, n(%) • ≥5% blasts, n(%)	35(45,5) 42(54,5)
Tisagenleucleucel/ARI-0001, n(%)	57(74) /20(26)
Dose of CAR-T(X10E6CAR-T/Kg), median (range)	2,8 (0,4–5) IQR:2

Poster presentations

Biology and translational research of primary and relapsed / refractory acute leukemias and non-Hodgkin lymphoma

P 01 - FLA plus liposomal Doxorubicin can comparably reduce MRD in refractory ALL like liposomal Daunorubicin, but is also associated with severe toxicity (AIEOP-BFM ALL 2017 – interim analysis BFM-Germany)

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Children with acute lymphoblastic leukemia (ALL) nowadays have an overall survival of above 90%, which was mainly improved over the past few decades by continuous refinement of risk stratification (e.g. treatment response including detection of minimal residual disease (MRD) as well as cytogenetic and molecular genetic features) along with individualized risk-adaptation of treatment. However, despite intensified polychemotherapy, patients with high-risk (HR) criteria and inadequate MRD response to HR courses (MRD ≥ $5x10^{-4}$ after the third HR block) still had a very poor prognosis despite allogeneic stem cell transplantation (alloHSCT) in ALL-BFM 2000 (5-y-pEFS 24% +/- 7%, n = 38). Therefore; in AIEOP-BFM ALL 2009 DNX-FLA (liposomal Daunorubicin (DaunoXome), Fludarabin and high-dose Cytarabin) was introduced as a salvage chemotherapy element with the aim to further reduce the MRD load in such patients prior to alloHSCT. Out of 64 eligible patients, 38 were treated with DNX-FLA, and subsequent MRD results were available for 36 patients. MRD reduction by at least one log-step could be achieved in 70% of patients, whereas in 55% MRD was even reduced to < $5x10^{4}$ (20/36). In contrast, rising or unchanged MRD levels (≥ $5x10^{-4}$) were observed in 8% and 22%, respectively. Additively, almost one third of the analyzed group received one or more additional chemotherapy elements subsequent to DNX-FLA.

Regarding the safety and toxicity profile of DNX-FLA, 13 serious adverse events (SAE) of special interest as specified in the study protocol were reported in 11 patients (30%) including six invasive pulmonary fungal infections. In addition, three non-fungal life-threatening infections along with four other SAE of special interest (severe soft-tissue infection, renal failure, seizure and elevation of liver enzymes (>20xUNL)) were reported, however no directly DNX-FLA associated fatalities occurred.

Given the effectiveness and favorable toxicity profile, the salvage element strategy was therefore intended to be continued in the successor trial AIEOP-BFM ALL 2017. However, due to temporary shortages and terminal unavailability, DNX was replaced by non-pegylated liposomal Doxorubicin (Myocet; MYO) during the ongoing trial.

Interim analysis of MRD response in 13 German BFM patients with MYO-FLA indication by protocol (18 patients treated in total) showed MRD reduction of at least one log step in 54% of the cases (7/13) and reduction to < $5x10^{-4}$ in 46% (6/13). Increasing or unchanged MRD levels were observed in 31% (4/13) and 15% (2/13), respectively. Again, about one third of the patients received other additional therapy elements in order to further reduce MRD prior to alloHSCT.

Concerning toxicity, a SAE of special interest (n = 7) was reported in six patients (46%) including three life-threatening events (cardiac insufficiency/arrhythmia, acute pancreatitis and venous occlusive disease) as well as four other non-life-threatening events (severe soft-tissue infection, bullous dermatitis and two invasive fungal infections). In patients with MYO-FLA indication by protocol no fatality was observed, though one patient without formal indication (MRD $1x10^{-4}$ after the third HR block) treated with MYO-FLA as bridge-to-transplant deceased due to a life-threatening infection and circulatory failure.

In summary, although the total number of evaluable patients is still low and further evaluation of the complete AIEOP-BFM ALL 2017 cohort is needed, MYO-FLA seems to been an adequate substitution for DNX-FLA in terms of MRD reduction in refractory ALL prior to alloHSCT in frontline therapy, however the toxicity profile needs further careful monitoring hereafter.

P 02 - Recurrent DNMT3B alterations are associated with unfavorable outcome in dicentric (9;20)-positive pediatric B-cell precursor acute lymphoblastic leukemia

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The dicentric chromosome dic(9;20)(p11~13;q11), occurs in 2% of the cases with pediatric acute lymphoblastic leukemia (ALL), almost exclusively of the B-cell lineage. Previous studies have reported discrepant results regarding outcome in the cases harboring dic(9;20). Thus, prognostic significance of the dic(9;20) alterations, as well as the mechanism in which these alterations drives leukemogenesis remains elusive. The aim of this study was to unravel the demographic, clinical, prognostic and molecular characteristics of the dic(9;20)-positive ALL in the cohort of 57 pediatric B-ALL patients.

Detection of dic(9;20) by conventional cytogenetic and molecular methods was the prerequisite for inclusion into the study. All 57 dic(9;20) cases, and 56 cases for which RNA samples were available, were subjected to genome-wide CGH+SNP array and targeted RNA-sequencing analysis. In addition, we performed whole transcriptome and whole genome bisulfite sequencing (WGBS) for a selected number of cases. Event-free survival (EFS) was estimated according to Kaplan-Meier, and the curves were compared by log-rank test.

Overall, the cohort consists of 22 male and 37 female children, with a median age at diagnosis of 3 years (range: 1–17 years). Patients were treated according to the AEIOP-BFM 2000, 2009 or 2017 treatment protocols and stratified in the standard risk (n = 15), medium risk (n = 37) or high risk (n = 5) treatment arms. In total, 44 patients achieved remission, 11 experienced relapses, while two patients in remission were lost during follow-up. Targeted RNA-sequencing and ArrayCGH analysis unraveled heterogeneous breakpoints on the chromosomes 9 and 20, as well as frequent deletions of the *IKZF1* gene. Together with the intrinsic susceptibility towards deletions of the *CDKN2A/B* and *PAX5* genes in the dic(9;20)-positive ALL, these patients are eligible for treatment escalation. Furthermore, we identified six dic(9;20)-positive cases with rearrangements involving *de novo* methyltransferase *DNMT3B* gene, and an abysmal outcome compared to dic(9;20)-positive cases without these rearrangements (5-year EFS 25%±20.4% VS 75.4%±8.2%; *P* = 0.015). The rearrangement between *DNMT3B* and *ZCCHC7* (n = 3) genes resulted in an in-frame chimeric fusion transcript, containing the 5′ end of the open reading frame of *DNMT3B* and the 3′ end of *ZCCHC7* genes. In contrast, due to opposing orientation of the *DNMT3B* and *PAX5* genes (n = 3), the resulting chimeric transcript contained the 5′ end of the open reading frame of *DNMT3B* or *PAX5* and a run-through transcript of the antisense strand of the partner gene. In five cases the breakpoint occurred upstream of the methyltransferase domain of the *DNM3TB* gene, suggesting that the altered DNA methylation may drive leukemogenesis and relapse in these cases. However, WGBS analysis did not unravel global changes in the methylation status of the genomic loci interacting with DNMT3B. In all four cases that relapsed, the breakpoints were clustered in the introns 6 and 7 of the *DNMT3B* gene, which together with the presence of the antisense transcript of *PAX5* gene suggests that the breakpoints in this region may cause disruption of the gene regulatory mechanisms. Therefore, we performed differential gene expression analysis between relapsed and non-relapsed *DNMT3B*-rearranged cases, which unraveled a dysregulation of genes involved in hematopoiesis, lymphocyte maturation and AKT/PI3K signaling pathway.

In this study we describe novel recurrent genomic rearrangements involving the *DNMT3B* and *PAX/ZCCHC7* genes in pediatric ALL with dic(9;20). Once confirmed in an independent cohort, dic(9;20)-positive cases with rearrangements involving *DNMT3B* and *PAX5/ZCCHC7* genes should be considered as high risk for relapse and treated accordingly.

P 03 - IGFBP7 enhances glycolytic metabolism via IGF1R-Akt axis in B-cell Precursor Acute Lymphoblastic Leukemia

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Fine-tuning of the glycolytic pathway is a critical step in the malignant transformation of B-cells. In B-cell precursor acute lymphoblastic leukemia (BCP-ALL), increased glycolytic metabolism has been linked to L-asparaginase and glucocorticoid resistance. In T-cell ALL, activation of the PI3K-Akt axis by receptor tyrosine kinases or oncogenic lesions (gain-of-function mutations) induces glucose transport 1 (GLUT1) accumulation at the cell surface and increases aerobic glycolysis. We have previously shown that IGFBP7 (insulin-like growth factor-binding protein 7) exerts mitogenic and prosurvival autocrine effects in ALL, by sustaining the activation of IGF1R>PI3K-Akt axis by insulin or IGFs (Artico et al. *Blood Adv*. 5:3633-3646, 2021). Here, to test whether IGF1R is indispensable for the transduction of the IGF1+IGFBP7 stimulus to downstream effectors such as Akt, we generated two *IGF1R* knockout (IGF1R^{ko}) cell lines. As expected, NTC cells (no target control) maintained detectable levels of IGF1R and Akt phosphorylation 4 h after IGF1+IGFBP7 co-treatment, while no such sustained signaling was seen in IGF1R^{ko} cells nor when IGF1 or IGFBP7 were used separately. To better understand the cellular consequences of prolonged IGF1R/Akt signaling in BCP-ALL, we performed microarray gene expression analysis followed by Gene Set Enrichment Analysis (GSEA). Hallmark gene sets characteristic of cell growth (PI3K/Akt/mTOR, mTORC1, MYC targets, E2F targets, and mitotic spindle) and metabolism (oxidative phosphorylation-OXPHOS, adipogenesis, fatty acid metabolism, and glycolysis) were upregulated upon IGF1+IGFBP7 treatment of BCP-ALL cell lines. To confirm the impact on energy metabolism, we determined the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in BCP-ALL cells upon IGF1+IGFBP7 stimulation to assess OXPHOS and glycolysis, respectively. Both the basal and maximal OCR were increased upon treatment, along with the OCR coupled to ATP synthesis. Besides, when mitochondrial OXPHOS was chemically inhibited, treated BCP-ALL cells showed a significantly increased glycolytic metabolism. To evaluate glucose uptake by orthogonal method, we cultured cells in medium supplemented with 2-deoxy-D-glucose (2-DG). Sensitivity to lethal 2-DG supplementation was clearly more pronounced when cells were pretreated with IGF1+IGFBP7 than by any of these factors individually. No such an additive effect was seen with the IGF1R^{ko} cells, indicating that IGF1R was indispensable for the increased glucose uptake triggered by IGF1+IGFBP7. Likewise, IGFBP7 neutralization with an anti-IGFBP7 monoclonal antibody or PI3K inhibition with Ly294002 protected IGF1+IGFBP7-stimulated BCP-ALL cells from the lethal effects of 2-DG. Chronic Akt activation has been shown to stabilize GLUT1 at the cell surface by inhibiting the endocytic machinery. Accordingly, 24 h after IGF1+IGFBP7 treatment, BCP-ALL cells showed ~60% more GLUT1 on their cell surface than untreated cells. As anticipated, this effect was completely abrogated when the cells were pretreated with anti-IGFBP7 antibody or Ly294002. Moreover, IGF1R^{ko} cells showed no changes in GLUT1 surface expression under any condition tested. GLUT1 overexpression (*SLC2A1*) has a negative prognostic impact in several types of cancer. In BCP-ALL, we found that high *SLC2A1* expression is also related to low overall survival curves. The metabolic effect described here may offer an additional mechanistic explanation for the strong negative impact seen in ALL cells *in vitro* and *in vivo* after the knockdown or antibody neutralization of IGFBP7 (Artico et al. *Blood Adv*. 5:3633-3646, 2021), while reinforcing the notion that it is a valid target for future therapeutic interventions. In conclusion, our observations reveal a hitherto unknown role for IGFBP7 in the glycolytic metabolism of BCP-ALL, opening doors for future investigations.

P 04 - Ph+ acute lymphoblastic leukemia in children and adolescents: Outcome of treatment in Slovakia in the period 2000–2022

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Background:

Approximately 3–5% of pediatric patients with acute lymphoblastic leukemia (ALL) have a philadelphia (Ph) chromosome, (9;22)(q34;q11.2), BCR-ABL1 fusion. Historically, these patients had a very poor prognosis and were candidates for allogeneic hematopoietic stem cell transplantation (HSCT) in the 1st complete remission (CR). Data of the American COG Group and the European EsPhALL Consortium have demonstrated that most pediatric Ph+ALL can be effectively treated with a combination of tyrosine kinase inhibitors (TKIs) and chemotherapy. Currently, consensus in the USA and Europe recommends treatment without HSCT in the 1st CR for patients with a favorable early response, and patients with an unfavorable response are candidates for HSCT in the 1st CR.

Methods:

Retrospective analysis of treatment results in children with Ph+ ALL. From 2000 to 2022, 748 pediatric patients with ALL were treated in Slovakia, of which were 20 (2.7%) patients with Ph+ ALL, 6 girls and 14 boys. Nine patients were aged <10 years, 11 patients ≥10 years, the median age was 11.1 years. The median follow-up was 9.1 years. Patients were treated heterogeneously according to currently used protocols.

Results:

The analysis was evaluated as of June 30, 2022. Out of 20 patients, 5 patients relapsed, 4 patients died. Assessed 5y and 10y OS±SE is 78% ± 9.6% and 5y and 10y EFS±SE is 55% ±11.8%. In the group of girls, 5y and 10y OS±SE is 100%, 5y and 10y EFS±SE 83, ± 15%. Patients in the ≥10y age group have a 5y and 10y OS±SE of 91% ± 8.7% and a 5y and 10y EFS±SE of 45% ± 16.6%.

Conclusion:

Cytotoxic chemotherapy administered to patients with Ph+ALL is much more intensive than standard treatment for non-Ph+ALL, which has an impact on higher treatment toxicity, mortality, and late effects of treatment. Since the addition of TKIs to chemotherapy, the management and prognosis of Ph+ALL patients have changed. The optimal standard treatment procedure has not yet been defined.

P 05 - The functional role of Musashi-2 in KMT2A-rearranged infant Acute Lymphoblastic Leukemia uncovers potentially targetable metabolic vulnerabilities of leukemic cells

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Introduction & Aim:

Acute Lymphoblastic Leukemia occurring in infants and carrying the rearrangement of KMT2A gene (KMT2Ar infant ALL) is a rare but very aggressive leukemia typically associated with poor outcome (overall survival: 20–40%) due to drug resistance and high incidence of relapse. The identification of druggable genes involved in KMT2Ar leukemogenesis is urgently needed for the development of novel therapeutic approaches. Herein we aimed at unravelling the functional role of the RNA-binding protein Musashi-2 (MSI2), in order to validate this gene as a novel biomarker and potential target for KMT2Ar infant ALL.

Results:

A cell line model with MSI2 knock-out was successfully generated by CRISPR/CAS9 genome editing in the KMT2A::AFF1+ ALL cell line (SEM MSI2 KO) to address the effect of MSI2 abrogation. In a long-term competitive assay in vitro, we observed that MSI2 KO cells have an impaired proliferation. Upon xenotransplantation into immunodeficient mice, the lack of MSI2 impairs the leukemia-initiating capacity in vivo of KMT2A::AFF1+ cells and mice transplanted with MSI2 KO show a lower disease burden and a prolonged survival. Moreover, we observed that MSI2 KO cells show an increased (100-fold) sensitivity to Glucocorticoids (GCs, such as Prednisolone or Dexamethasone) compared to their MSI2-expressing counterpart. Also, the treatment of KMT2Ar ALL cell lines in vitro or patient-derived xenograft (PDX) samples ex vivo with the MSI2 inhibitor Ro 08–2750 have a synergistic effect with GCs. To further investigate the biological mechanisms involved in GC-resistance/sensitization, we performed confocal analysis and Seahorse bioenergetics profiling. Our results revealed that mitochondria of MSI2 KO cells are hyperactivated already at steady-state compared to controls. Upon GCs exposure, glycolysis is inhibited in both MSI2 KO and CNTRL cells. However, in response to GC administration, mitochondria activity is increased in CNTRL cells to compensate ATP production and ensure cell survival; while it is severely impaired in MSI2 KO leading to energy failure and apoptosis. Also, by using the Glucose analogous 2-DG which blocks the glycolytic cascade, we observed that the lack or the inhibition of MSI2 make the cells more susceptible to metabolic perturbations such as 2-DG induced inhibition of glycolysis; thus suggesting a putative role of MIS2 in the metabolic rewiring of leukemia in stress conditions. Indeed, the treatment with MSI2 inhibitor Ro 08–2750 suppresses the mitochondrial respiration of KMT2Ar ALL cells acting as a "Mitocans" agent. To address whether targeting the mitochondria of leukemic cells might circumvent drug resistance of KMT2Ar ALL, we treated the cells with Oxphos inhibitors (like Metformin or Tigecycline) together with GCs, and we observed that this combination treatment has anti-leukemic effects both in vitro as well as in vivo. Finally, we also demonstrated that the MSI2 inhibitor Ro 08–2750 has a synergistic effect in combination with the Bcl-2 inhibitor Venetoclax, and the triple treatment with Ro 08–2750+Venetoclax+GCs completely eradicates leukemia in KMT2Ar ALL cell lines in vitro or in PDX samples ex vivo.

Conclusions:

Overall, we demonstrated that MSI2 sustains the growth in vitro and the leukemogenic potential in vivo of KMT2Ar ALL and it is responsible for GC-resistance. Moreover, our study unraveled the novel role of MSI2 in the metabolic plasticity of leukemia, provided new insights into the correlation of mitochondrial metabolism and drug resistance and paves the way for targeting the metabolic vulnerabilities of leukemic cells as a novel approach to increase the treatment response of infants with KMT2Ar ALL.

P 06 - Copy-number based characterisation of Acute Lymphoblastic Leukemia cell lines using digitalMLPA

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Background:

Cancer cell lines are valuable *in vitro* models that are widely used for studying cancer biology, validating novel cancer targets and defining drug efficacy. Knowing the genetic background of these cell lines used is hereby of utmost importance. As copy number aberrations (CNAs) are common in Acute Lymphoblastic Leukemia (ALL), in this study we performed copy-number based characterisation of a cohort of well-known ALL cell lines.

Objectives:

Showcase the ability of SALSA digitalMLPA Probemix D007 Acute Lymphoblastic Leukemia (MRC Holland) to detect deletions, gains or amplifications in specific genes and chromosomal regions, using ALL cell lines as a model.

Methods:

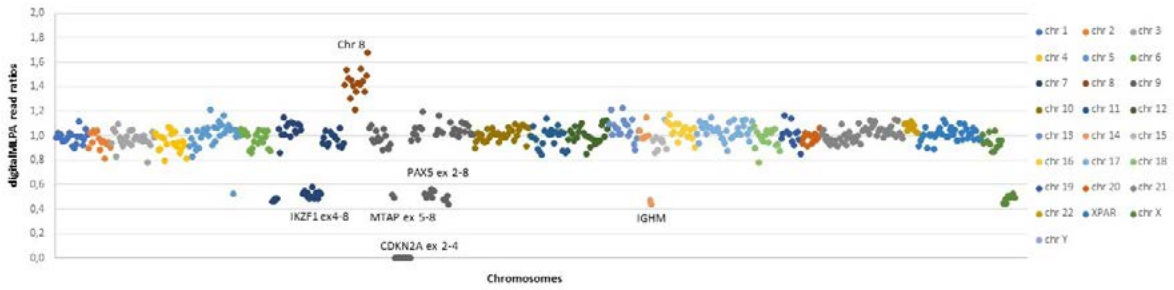
Leukemia cell lines from the LL-100 panel and other sources were ordered from DSMZ-Leibniz Institute. digitalMLPA testing was performed using SALSA digitalMLPA Probemix D007 Acute Lymphoblastic Leukemia, which includes >50 target genes and regions of known importance in B-cell Acute Lymphoblastic Leukemia (B-ALL) and T-cell Acute Lymphoblastic Leukemia (T-ALL). digitalMLPA-derived PCR amplicons were sequenced on a NextSeq 1000 Sequencing System (Illumina). FastQ files were analysed using Coffalyser digitalMLPA™ (MRC Holland).

Results:

digitalMLPA data of 30 cell lines was analysed and inferred copy number data was compared to 1) cytogenetics information available on these cell lines, 2) other information on these cell lines available from scientific literature and 3) gene-level copy number data available from the Cancer Cell Line Encyclopedia (CCLE; Broad Institute). digitalMLPA was able to reliably detect gross chromosomal or regional aberrations and exon-level deletions, gains or amplifications. Examples of CNAs observed in this leukemia cell line cohort include intragenic deletions or gains (i.e. heterozygous deletion of *NR3C1* exons 2–8 in B-Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL) cell line REH; or heterozygous deletion of *IKZF1* exons 4–8 in BCP-ALL cell line TOM-1, see *Figure 1*), co-deleted genes grouped in the IKZF1plus profile (i.e. intragenic *IKZF1*, intragenic *CDKN2A* and intragenic *PAX5* deletions in BCP-ALL cell line TOM-1, see *Figure 1*), presence of possible fusion genes (i.e. heterozygous deletion of *TAL1* exon 1 and *STIL* exons 2–12 in T-ALL cell lines CTV-1 and MOLT-16), and gains and deletions of chromosomal arms (i.e. heterozygous deletion of 7p arm and duplication of 7q arm, indicative of i(7q) in T-ALL cell lines RS4;11 and KASUMI-2).

Significance of the study: The copy number-based genetic characterization of leukemia cell lines can be reliably performed by digitalMLPA. This provides key information for future cell line studies unravelling the biology and translational research of primary and relapsed/refractory acute leukemias.

Figure 1: *Copy numbers in BCP-ALL cell line TOM-1.*



digitalMLPA read ratios in BCP-ALL cell line TOM-1 (female). Chromosomes are shown on the X-axis, digitalMLPA read ratios are shown on the Y-axis. A read ratio of 1,0 indicates a normal copy number, a read ratio of 0,5 indicates a heterozygous deletion, a read ratio of 1,5 indicated a heterozygous gain/homozygous triplication of the DNA sequence targeted by the probe.

P 07 - Targeted treatment options for pediatric B-cell precursor acute lymphoblastic leukemia patients with constitutional or somatic chromosome 21 alterations

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Background:

Chromosome 21 is affected in ~60% of pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL) patients and includes somatic and constitutional gains, intrachromosomal amplification of chromosome 21 (iAMP21), and the translocation t(12;21) resulting in the *ETV6-RUNX1* gene fusion. Despite ongoing improvements in the outcome of pediatric patients with BCP-ALL, relapse still occurs. High-risk relapse patients are insufficiently rescued with chemotherapy and hematopoietic stem cell transplantation, illustrating the need for more targeted treatment directed to biological features of these therapy-resistant leukemic cells.

Objectives:

We analyzed the type and frequency of yet-proven targetable lesions and expressed CD markers in the context of chromosome 21 alterations, which may serve as a roadmap to develop more efficacious treatment for these patients.

Methods:

Initial diagnosis samples of pediatric BCP-ALL patients were analyzed by RNA sequencing and flow cytometry. Chromosome 21 gains were called based on karyotype or DNA arrays. Somatic gene fusions and mutations in *CRLF2*, *EPOR*, *IL7R*, *JAK1*, *JAK2*, *KRAS*, *NRAS*, *PTPN11* and *FLT3* were studied in the context of constitutional gain of chromosome 21 (Down syndrome (DS)-ALL, n = 68), iAMP21 (n = 22), high hyperdiploidy (HeH, n = 108), *ETV6-RUNX1* (n = 47), somatic chromosome 21 gain (n = 15) and no chromosome 21 alteration (n = 44).

Results:

Among 304 primary pediatric BCP-ALL cases, 58 gene rearrangements were detected and 188 mutations, of which 69% clonal (≥25% VAF) and 31% subclonal (<25% VAF). The ratio of clonal:subclonal lesions did not significantly differ per pathway or per chromosome 21 alteration subtype.

The frequency of JAK/STAT pathway lesions ranged from 50% in DS-ALL to 3% in HeH cases. All but one of 34 DS-ALL patients with a JAK/STAT pathway lesion had a *CRLF2* rearrangement; the remaining one had an activating *CRLF2* mutation. *CRLF2* lesions were also frequent in iAMP21 (27%), samples with chromosome 21 gain (33%) and samples without chromosome 21 gain (18%). In total, 20% of chromosome 21-altered cases had a JAK/STAT pathway lesion compared with 23% of non-chromosome 21-altered cases.

RAS pathway lesions ranged from 57% in HeH to 13% in other somatic chromosome 21-altered cases. In total, 35% of chromosome 21-altered cases had a RAS pathway mutation compared with 20% of non-chromosome 21-altered cases.

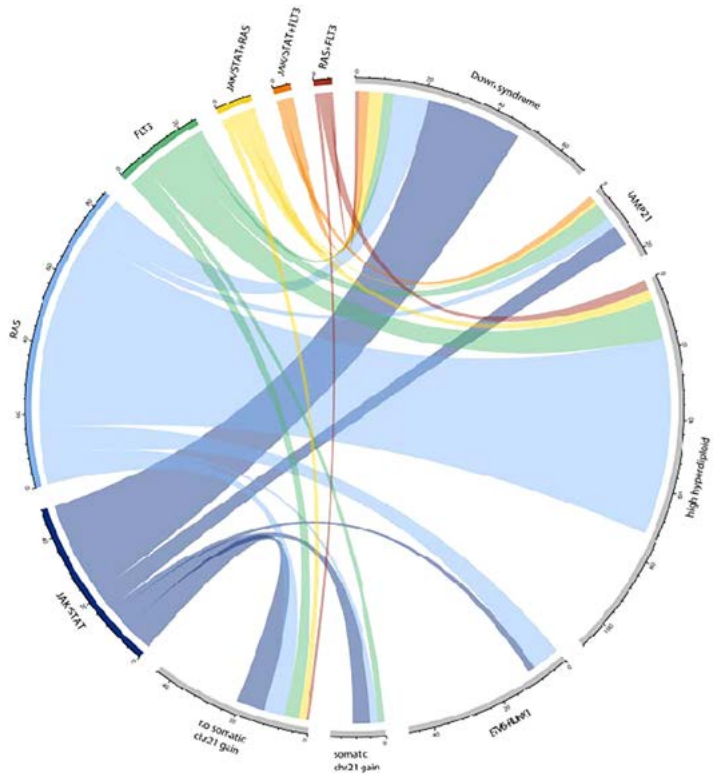
FLT3 lesions, including missense mutations, internal tandem duplications, and in frame insertions, were most frequent in iAMP21 (32%). In total, 12% of chromosome 21-altered cases had a FLT3 lesion compared with 11% of non-chromosome 21-altered cases.

Virtually all cases expressed CD19 and CD22 at the cell surface. Positivity for CD20 surface expression ranged from 73% in iAMP21 to 23% in *ETV6-RUNX1*.

Conclusion:

We found at least one targetable genetic lesion affecting the RAS, JAK/STAT or FLT3 pathway in 77% of iAMP21, 71% of DS-ALL, 68% of HeH, 21% of *ETV6-RUNX1*, 60% of other cases with a chromosome 21 gain, and 48% of BCP-ALL cases without a chromosome 21 alteration. CD19 and/or CD22 flow positivity was observed for nearly 100% of cases. These results suggest that the far majority of chromosome 21-altered BCP-ALL cases have one or more actionable events for precision medicines or immunotherapy.

Figure: Circosplot showing the frequency of RAS, JAK/STAT and/or FLT3 pathway activation per chromosome 21-aberrant BCP-ALL subtype.



P 08 - The impact of an additional copy of chromosome 21 in B-cell acute lymphoblastic leukemia

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Background:

A common finding in pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is that chromosome 21 is never lost and often gained, either in the context of high hyperdiploidy (>50 chromosomes) or in addition to translocations such as *BCR::ABL1*, *ETV6::RUNX1* and *IGH::DUX4*. Research into the role of chromosome 21 in the pathobiology of BCP-ALL requires appropriate models. Primary patient material is less suitable since leukemic cells do not proliferate or survive for extended periods of time *ex vivo*. In addition, patient cells often show secondary genetic aberrations that can hamper comparison of results obtained from different patients.

Objectives:

We aimed to develop and validate a trisomy 21 BCP-ALL cell line and investigate whether the presence of an extra chromosome 21 influences cell proliferation and drug sensitivity.

Methods:

We transferred chromosome 21 from A9(21–16) donor cells to the *DUX4*-rearranged BCP-ALL cell line NALM-6 using microcell-mediated chromosome transfer modified from Meguro-Horike *et al.* (Methods Mol Biol 2015). Cells were cultured on G418 selection medium until exponential growth and seeded at limited dilution to obtain single clones. Presence of chromosome 21 was confirmed by Multiplex ligation-dependent probe amplification (MLPA), whole exome sequencing and gene expression evaluated by RNA sequencing of four randomly selected clones. Drug sensitivity assays were performed for 4 days, and viability measured by MTT assay. Proliferation was evaluated using CFSE staining at 24, 48, 72 and 96 hours and measured using flow cytometry.

Results:

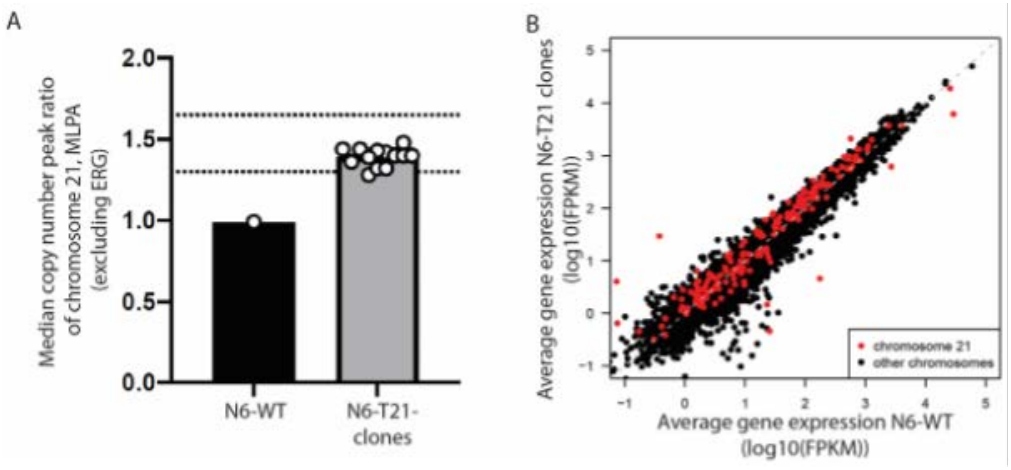
An additional chromosome 21 was successfully transferred to NALM-6 cells (N6-T21), confirmed by PCR for the selection marker, MLPA for chromosome 21 (Figure panel A), and whole exome sequencing. RNA sequencing confirmed that the extra chromosome in N6-T21 cells is actively transcribed, with a median 1.3-fold increased expression of 198 expressed genes (Figure panel B). 1919 genes were differentially expressed (FDR <0.05) in the N6-T21 clones (754 up and 1165 down), of which 66 (3.4%) were located on chromosome 21 (56 up and 10 down). Chromosome 21 genes including *IFNGR2*, *HMGN1*, *SON*, and *CHAF1B* showed increased gene expression. We confirmed that the increase of *IFNGR2* expression on RNA level led to increased surface protein expression. On the other hand, *RUNX1*, *DYRK1A*, and *ERG* were not upregulated compared with parental NALM-6, indicating tight transcriptional regulation.

N6-T21 cells showed a decreased proliferation rate compared with parental NALM-6 observed at three consecutive timepoints (48, 72 and 96 hours) and a slightly increased drug sensitivity to two induction chemotherapeutic drugs. Based on an average of three concordant N6-T21 clones, sensitivity increased 4-fold to prednisolone (IC50 from 0.24 µg/ml to 0.051 µg/ml) and 3-fold to asparaginase (IC50 from 1.2 Units/ml to 0.43 Units/ml). There was no change in drug sensitivity to daunorubicin. The extra copy of chromosome 21 did not confer sensitivity to targeted signaling inhibitors gilteritinib, ruxolitinib or trametinib.

Conclusion:

We transfected NALM-6 with an additional copy of chromosome 21 and found that this chromosome is actively transcribed and has an effect on genome-wide expression. Comparing to the parental NALM-6, we showed that an additional chromosome 21 results in slower proliferation, as well as increased prednisolone and asparaginase sensitivity. This model system can be used to further study the role of chromosome 21 in leukemia and developing more targeted treatments for chromosome 21-altered leukemia.

Figure: Validation of additional chromosome 21 in N6-T21 clones at (A) DNA and (B) RNA level



P 09 - Comprehensive genomic landscape of pediatric B-cell precursor Acute Lymphoblastic Leukemia analyzed by optical genome mapping and next-generation sequencing

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Background:

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common pediatric cancer, including the most frequent genetic subtypes: high hyperdiploid (HHD) and *ETV6::RUNX1* translocated BCP-ALL. ALL susceptibility may be caused by constitutional alterations in ~4% of the patients (Klco & Mullighan 2020). Secondary alterations are usually required to transform a preleukemic clone to overt ALL.

The mutational landscape of BCP-ALL has been described by conventional methods including cyto- and molecular genetics as well as short-read next generation sequencing (NGS). However, a comprehensive analysis of germline and somatic single nucleotide variants (SNVs), indels and structural variants (SVs) in HHD and *ETV6::RUNX1* BCP-ALL patients is lacking. Recently, novel methods were developed to specifically target SV detection including optical genome mapping (OGM) and long-read sequencing (LRS).

Our study aimed to decipher the subtype-defining genomic landscapes of HHD and *ETV6::RUNX1* BCP-ALL by combining OGM and NGS to gain novel insights on ALL predisposition and leukemogenesis.

Methods:

We collected tumor and matched germline samples of 60 pediatric patients diagnosed with *ETV6::RUNX1* or HHD BCP-ALL. From each sample, short-read whole exome sequencing (WES, Illumina) and OGM was performed. For OGM, high molecular weight was extracted, labeled and imaged using a Saphyr instrument (Bionano Genomics). We integrated datasets of each patient to identify somatically acquired SNVs, small indels and SVs >500bp. Further, the occurrence of rare germline SVs (<1% of 160 healthy samples [Bionano] and dbVar) potentially affecting known cancer predisposition genes (CPG) was determined.

Results:

In our cohort of 60 pediatric BCP-ALLs, we detected a total of 1.148 SNVs/indels by WES and 1.203 SVs by OGM, including all hallmark alterations t(12;21)(p13;q22) and hyperdiploidy.

By combining WES and OGM, we identified 54 recurrent minimal altered regions (MARs). 41 MARs included a therein located potential target gene with 12 genes altered by SV and/or SNV. Double hits of SV and SNV in the same patient affected *ETV6, BTG1, STAG2, MANBA, TBL1XR1* and *NSD2*. Frequent MARs occurring in >10% of the BCP-ALL cases included: 12p13.2 (*ETV6*, 16/60), 9p21.3 (*CDKN2A/B*, 14/60), 9p13.2 (*PAX5*, 13/60) and Xq25 (*STAG2*, 7/60). Interestingly, *STAG2* was altered by focal intragenic deletions in four cases, an intragenic deletion and concomitant missense mutation (p.Ile118Met) in one case and by a t(X;13)(q25;q13.1) leading to potential fusion of the cohesin members *PDS5B* and *STAG2*. We further detected MARs potentially affecting novel target genes including: 9p21.3 (*FOCAD/HACD4*, 5/60), 8p11.21 (*IKBKB*, 4/60), 4q24 (*MANBA*, 3/60) and 10p14 (*SFMBT2*, 3/60). 17 *ETV6::RUNX1* subtype-specific MARs with known target genes were identified including: 12q21.22 (*BTG1*, 10/30), 12p13.1 (*ATF7IP*, 10/30), 19p13.3 (*UHRF1*, 5/30), 19q13.11 (*UBA2*, 5/30) as well as MARs with potential novel target genes: 12p13.1 (*GPRC5A* 5/30), 12q24.21 (*MED13L*, 3/30), 18q11.2 (*MIB1*, 3/30) and 20q11.22 (*NCOA6*, 3/30). In HHD leukemia, one recurrent MAR on 6q25.2 (*MYCT1*, 3/30) was identified.

In 35% (21/60) of the patients, 28 rare germline SVs targeting 22 known CPGs were detected using OGM. 40% (9/22) of the affected genes were involved in hematopoietic or lymphoid organ development as *ETV6*, *FANCA*, *IKZF1 or ITCH*. In 11 children, rare germline alteration potentially altered a coding region of a CPG such as a focal duplication on 16p13.13 (*CIITA, SOCS2)* in an *ETV6::RUNX1* patient.

Conclusion:

Our study demonstrates the power of combining OGM and NGS to generate comprehensive germline/leukemia genomes in childhood BCP-ALL to reveal recurrently altered regions and novel potential target genes in ALL leukemogenesis and predisposition.

P 10 - Histone deacetylase inhibition restores therapy response in TP53-mutated acute lymphoblastic leukemia

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Background:

Although TP53 is the most frequently mutated gene in cancer, in Acute Lymphoblastic Leukemia (ALL), mutations or deletions affecting TP53 are rare, with an incidence of only 3% at diagnosis. However, at relapse TP53 aberrations are found in about 12% of the patients and predict a very poor outcome. As a consequence, relapsed TP53 deleted ALL is now classified as 'very high risk', together with relapsed t(1;19) and t(17;19) ALL.

Since p53-mediated apoptosis is an endpoint for many (cytotoxic) drugs, loss of p53 function induces therapy resistance to both classical chemotherapeutics and newly introduced targeted therapies. Therefore, there is an urgent clinical need for more effective strategies to treat TP53 aberrant relapsed ALL.

Methods:

Using CRISPR/Cas9, we introduced frameshift mutations in the TP53 gene to generate isogenic wildtype and knockout models in the $TP53^{WT}$ B-cell precursor ALL (BCP-ALL) cell lines Nalm6 and RCH-ACV. After confirming the presence of TP53 deletions, we tested these isogenic models for sensitivity toward a panel of treatment protocol drugs. To identify drug combinations that may reverse TP53 mediated therapy resistance, we assessed the viability of these isogenic models in a drug screen containing 198 (pre)clinical drugs. The most interesting targets were subsequently combined with the previously tested treatment protocol drugs to determine potential synergies. Effects on cell viability were determined by amine exposure and MTT assay and confirmed by measuring PARP cleavage. Additionally, positive drug interactions were validated *in vivo* using immunocompromised mice engrafted with RCH-ACV $p53^{KO}$ cells expressing a luciferase reporter gene.

Results:

We observed that loss of p53 protects BCP-ALL cell lines Nalm6 and RCH-ACV from drug-induced apoptosis, leading to increased resistance to all drugs currently used to treat relapsed ALL, except for asparaginase. Of note, P53 mutant cells were also less responsive to treatment with inotuzumab ozogamicin, a drug frequently used in salvage protocols. In a subsequent drug screen, we found that the histone deacetylase (HDAC) 1/2 inhibitor romidepsin shows strong single-agent activity at single nanomolar concentrations. Moreover, romidepsin strongly synergizes with contemporary ALL treatment drugs in both $TP53^{KO}$ and $TP53^{WT}$ cells. This effect was strongest in $TP53^{KO}$ cells, effectively reversing therapy resistance induced by TP53 loss. Gene expression profiling shows that several cellular processes are suppressed upon combination treatment, including genes involved in ribosomal biogenesis and proteasome composition. Efforts to connect these processes to enhanced cell death are ongoing. Finally, preliminary in vivo experiments show that romidepsin improves the response to cytarabine of mice transplanted with a TP53 aberrant leukemia without signs of added toxicity. A mouse trial is currently underway to test a potential synergy between romidepsin and cytarabine using TP53-deleted patient-derived xenografts (PDX).

Conclusions:

Loss of p53 protects BCP-ALL cells from currently used (chemo)therapy strategies. Romidepsin can improve response to many therapies, which was most prominently observed in TP53 deleted ALL. Further, romidepsin does so without added toxicity *in vivo* and may therefore be a valuable addition to current protocols. Since romidepsin already has FDA approval for use in other hematological malignancies, our findings may be rapidly translated into clinical practice.

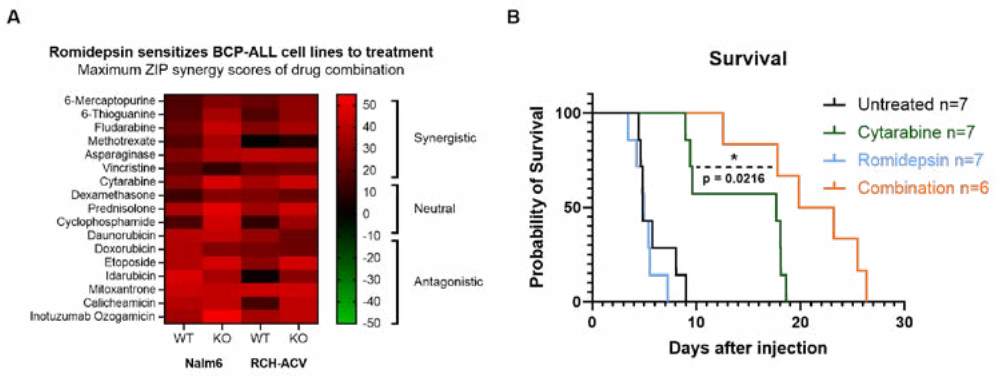

Figure 1 - (A) Heatmap of maximum ZIP synergy scores. Nalm6 and RCHACV p53^{KO} cells were exposed to five concentrations of romidepsin and five concentrations of the drug listed per row for 72 hours. Amine exposure was used as a measure of viability; synergy calculations using SynergyFinder web package. (B) Survival analysis of NRG-SM3 mice transplanted with RCH-ACV p53^{KO} cells. Event is defined as the interpolated day that tumor load reached total photon flux of 5x10^{6} photons per second. Mice were included in untreated (double vehicle), cytarabine (17.5 mg/kg bodyweight, Monday-Friday), romidepsin (1.5 mg/kg bodyweight, every Monday and Thursday) or the combination of both drug treatments. P-value from log-rank test comparing cytarabine and combination group. * $p < 0.05$.

P 11 - A patient-specific approach for monitoring MRD in ABL-class acute lymphoblastic leukemia by genomic breakpoint Q-PCR

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Background:

ABL-class fusions, involving tyrosine kinase genes *ABL1*, *ABL2*, *PDGFRB* and *CSF1R*, occur in ~3% of children and ~5% of adults with newly diagnosed acute lymphoblastic leukemia (ALL). Like *BCR-ABL1*-positive ALL, ABL-class patients are nowadays treated with imatinib which requires accurate residual disease monitoring. Measurable residual disease (MRD) is generally monitored based on immunoglobulin and T-cell receptor (IG/TR) rearrangements. This method is suboptimal due to the lack of identifiable IG/TR targets in about 5% of patients and the loss of the selected IG/TR gene rearrangement due to ongoing rearrangements and/or oligoclonality. Moreover, IG/TR MRD determination is not suitable to detect CML-like disease.

Objectives:

We aim to improve response monitoring of ABL-class patients, therefore we developed patient-specific genomic breakpoint Q-PCR assays and assessed whether this can overcome the limitations of IG/TR Q-PCR.

Methods:

Genomic breakpoints were determined using left-over diagnostic DNA by various next generation sequencing techniques, like targeted locus amplification (TLA), genomic capture high-throughput sequencing, and fusion detection by gene enrichment (FUDGE). Patient-specific Q-PCR assays were developed based on the identified genomic breakpoints. The quantitative range, sensitivity, and MRD values on follow-up time points were determined according to the EuroMRD guidelines.

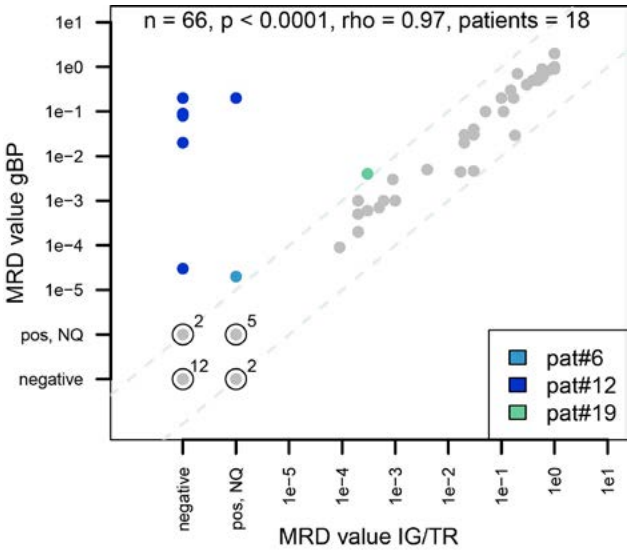
Results:

We included 22 ABL-class ALL patients, with *PDGFRB* (50%) being the most frequently fused kinase, followed by *ABL1* (36%), *CSF1R* (9%), and *ABL2* (5%). For all patients the genomic breakpoints were identified (with the largest breakpoint region spanning 150 kb) and patient-specific Q-PCR assays with high sensitivity (≤1E-4) and a sufficient quantitative range (≤5E-4) were developed. The MRD values of three patients lacking clonal IG/TR targets at diagnosis could effectively be determined using the genomic breakpoint. The patients with quantifiable MRD results based on IG/TR and the genomic breakpoint assays were highly correlated (Spearman correlation rank test $p < 0.0001$, $\rho = 0.97$, $n = 66$, patients = 18). However, three patients showed discrepant results, defined as >1 log10 difference between the two methods. One case (#6) had an excellent quantitative range for genomic breakpoint (QR 1E-5) resulting in quantifiable results, while the IG/TR MRD was positive but not quantifiable (QR 1E-4). Another case (#19) had higher genomic breakpoint levels compared to IG/TR levels due to a subclone of leukemic cells which could not be captured by IG/TR Q-PCR. Lastly, one case (#12) had high levels of genomic breakpoint MRD during treatment while IG/TR MRD was negative suggesting CML-like disease.

Summary/Conclusion:

For all ABL-class fusion types in our study, it was possible to identify the genomic breakpoint and to develop a patient-specific Q-PCR assay for MRD monitoring. MRD levels measured by genomic breakpoint and IG/TR Q-PCR were highly correlated. Moreover, we showed that the MRD response of patients lacking conventional IG/TR targets at diagnosis, those having oligoclonality, as well as those for which the selected IG/TR targets were lost during treatment, could be successfully monitored using the genomic breakpoint.

Figure 1: ***Correlation between IG/TR MRD level and genomic breakpoint MRD level.*** Comparison between clinical reported MRD based on IG/TR and patient-specific Q-PCR MRD based on genomic breakpoint (n = 66 samples from 18 patients). All samples with discordant MRD results >1 log10 difference (outside the dotted lines) are colored. Pos, NQ: positive but below the quantitative range of the assay.



P 12 - Relapsed/refractory Acute Lymphoblastic Leukaemia (R/R ALL) in a single-centre paediatric population

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Background:

ALL is the most common childhood cancer. Around 85% of children can be cured after contemporary treatment protocols. Relapse is the most frequent cause of treatment failure. Only 50% of children with relapsed ALL will survive long-term despite intensive chemo-radiotherapy, hematopoietic stem cell transplantation (HSCT) and novel salvage regimens. Most ALL relapses occur within 2 years (y) after treatment and most often in the bone marrow (BM), either isolated or combined with an extramedullary (EM) site, typically the central nervous system (CNS) or the testes. The most important prognostic factors at 1st relapse are time from diagnosis, relapse site, immunophenotype, disease genetics and minimal residual disease (MRD) post-reinduction therapy.

Aims:

Characterizing a single-centre paediatric R/R ALL population.

Methods:

Single-centre retrospective analysis of paediatric R/R ALL patients, clinical features, treatment, response, and outcomes. Data was collected from clinical records and analysed with IBM SPSS 25.0.

Results:

A total of 82 children with R/R ALL diagnosed between 2008–2021 were identified. Here we report the first 33 patients with complete data reviewed. Median age at diagnosis was 8y and 70% (23/33) were male. All underwent intensive front-line chemo-radiotherapy regimens. Most (88%; 29/33) achieved complete remission after induction (CR1). Refractory disease was observed in 12% (4/33) of children. In patients achieving CR1, positive MRD was detectable by molecular techniques in 21% (6/29).

Very early relapses (<18 months (M) from diagnosis) were observed in 28% (8/29), early relapses (18–30M) in 10% (3/29) and late relapses (>30M) in 62% (18/29). Most were isolated BM relapses (69%; 20/29). CNS was the most frequent EM site affected (14%; 4/29) followed by the testes (7%; 2/29).

Relapsed patients had a probability of 2y overall survival (OS) of 53%, with a median follow-up time (mFU) of 28M. 2y OS for refractory patients was 100% with a mFU of 85M.

B-cell relapses had a 2y event-free survival (EFS) of 61% and OS of 70%, while T-cell relapses had a 2y EFS and OS of 13% ($p = 0.001$). Very early relapses had a 2y EFS and OS of 0% (median EFS of 3M and OS of 5M) compared to 64% and 70%, respectively, in late relapses ($p < 0.001$). BM relapses had a 2y EFS of 52% compared to 40% for EM relapses (combined or isolated) ($p>0.001$). High-risk relapses had a 2y EFS 8% and 2y OS of 13%, compared to standard-risk 2y EFS of 75% and OS of 82% ($p < 0.001$). Most deaths were due to primary disease progression (67%; 10/15).

Over half of the 1st relapses (59%; 17/29) had indication for HSCT, 24% (4/17) were ultimately transplanted. After HSCT 2y EFS was 75% and OS 100%, with a mFU of 58M. 2nd relapses were observed in 18% (10/29) of patients. Salvage treatments included intensive chemo-radiotherapy with immunotherapies, such as CAR-T cell (2/10), incorporated in recent years.

Conclusion:

Survival rates among relapsed patients in our R/R cohort were in line with those previously published. B-cell ALL and late relapses were associated with better outcomes and HSCT provided long survival. All refractory patients survived in this cohort, and most were transplanted. Refractory disease criteria are currently under discussion and redefined in newer protocols. Treatment of R/R ALL remains deeply challenging. There is an unmet need for better and less toxic treatments. Novel immunotherapies are increasingly incorporated into salvage treatments to improve survival for children with R/R ALL.

P 13 - Next generation care next to the bedside: A centralized, comprehensive sequencing approach for children with acute lymphoblastic leukemia in Hungary

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Background:

Survival rates in some genetically defined subgroups of pediatric leukemia lag far behind the average. In this prospective study, we aimed to establish the deep genomic and transcriptomic profiles of Hungarian children (age of 0–18 years) with acute lymhoblastic leukemia/lymphoma (ALL) diagnosed between 2018 and 2021 to identify prognostically or therapeutically relevant alterations.

Methods:

Blast-rich diagnostic or relapse samples of 180 patients with ALL were analyzed using self-developed QIAseq Targeted DNA Custom Panel (Qiagen, Hilden, Germany) covering 102 disease-relevant genes. Targeted RNA sequencing was also performed using the TruSight RNA Pan-Cancer Panel (Illumina, San Diego, United States). Next-generation sequencing on Illumina MiSeq/NextSeq platforms and data analysis were centrally conducted at the Semmelweis University (Budapest).

Results:

Targeted mutation screening revealed an average of 2.7 mutations (range: 0–11) per sample. Strikingly, 18.6% of B-ALL patients harbored mutations in genes encoding actionable proteins including FLT3, JAK2, IL7R and CRLF2. In a multiple relapse case, we identified a novel germline mutation in *SH2B3* (c.1085A>C) associated with central nervous system involvement and probably predisposing to ALL. We also identified novel fusions involving *JAK2*, *KMT2A*, *PAX5*, *NOTCH1* and *RUNX1* in 5.3% of patients, with some of them affecting disease prognosis. The vast majority (75%) of *UBA2* mutations accompanied *ETV6::RUNX1* fusion. As a newly defined mutational subgroup, patients harboring *TP53* and/or *RUNX1* ‹high-burden› mutations with a variant allele frequency ≥25% showed a significantly shorter 3-year overall and event-free survival (OS: 92.3% vs. 50.0%, p = 0.0001; EFS: 87.0 % vs. 50.0%, p = 0.0016). Rare, patient-specific fusion transcripts (e.g. *KMT2A::MLLT3*, *DUSP22::IRF4*) were utilized for measurable residual disease monitoring by individualized molecular assays.

Conclusion:

Our clinical sequencing set-up deepens our understanding of the biology of treatment-sensitive versus aggressive disease course and identifies patients eligible for genomics-driven targeted therapy.

Funding:

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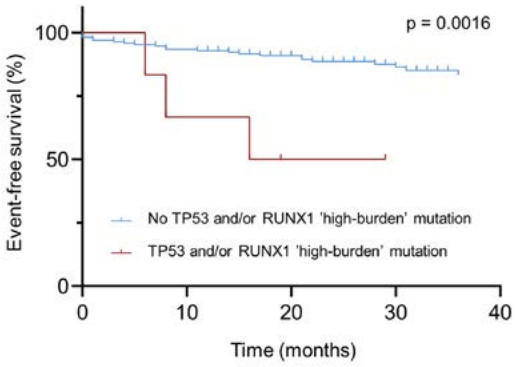
Figure: Inferior survival in childhood ALL with TP53 and/or RUNX1 high-burden mutations.



P 14 - T-ALL in vitro sensitivity to steroids is affected by Interleukin-7 (IL-7) and is effectively modulated by JAK/STAT inhibitor ruxolitinib

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Background:

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy arising from transformation and clonal expansion of T-cell precursors. While TALL accounts for only 15% of childhood and 25% of adult ALL, 30% of patients relapse with a poor outcome. Targeted therapy of resistant and high-risk pediatric T-ALL is therefore urgently needed. Several mechanisms can be identified as cause of chemoresistance in T-ALL. Indeed glucocorticoids (GCs) represent a substantial therapeutic option for T-ALL patients and a marked proportion of them show acquired GCs resistance whose molecular and cellular basis is still poorly understood. In childhood, treatment of T-ALL is based on a pre-phase of Prednisone (PDN) treatment in the first 7 days after diagnosis and patients can be distinguished between PDN Good Responders (PGR) and PDN poor Responders (PPR).

Objective:

We aimed to study the in vitro response of T-ALL to PDN with particular focus on the role of Interleukin-7 (IL-7) in the modulation JAK / STAT and PI3K / Akt / mTor pathways, both activated by IL-7, in T-ALL primary samples collected from either PGR or PPR patients.

Methods:

Eight T-ALL primary samples collected at diagnosis were studied. Samples were treated with IL-7, PDN and/or various inhibitors, and cultured in vitro for 48 hours. Furthermore, we studied the effect of two inhibitors, Ruxolitinib (RUXO) and NVP-BEZ 235 (BEZ), specific for the JAK/STAT and PI3K/Akt /mTor pathways, respectively, in the context of the response to PDN. For the evaluation of the signaling, the single cell proteomic and phosphoproteomic profile was evaluated by flow cytometry, while cell viability was assessed by toxicity assay.

Results:

In presence of IL-7 we observed an increased viability of cultured T-ALL cells, associated with activation of the proliferation markers such as Ki67, and of JAK/STAT and PI3K /AkT/mTOR signaling pathways, along with a general decrease of PDN sensitivity. In this condition the PDN + RUXO combination contributed significantly to the response PDN as compared to the conditions with PDN alone or with the single inhibitors. Such combination was even more effective than the PDN+BEZ combination regardless whether the samples were from PPR or PGR patients. Furthermore, the presence of IL-7, by directly activating the JAK/STAT pathway, allowed to increase the fraction of cycling cells which were particularly sensitive to the combined action of PDN and RUXO inhibitor.

Conclusions / Application to practice:

The *in vitro* administration of IL-7 prevents cell death, stimulates proliferation and inhibits sensitivity to PDN in T-ALL, in line with literature (Delgado-Martin et al. Leukemia, 2017). Moreover IL-7 is able to significantly support the action of Ruxolitinib (but not BEZ) both alone and in combination with PDN. PDN+RUXO was the most effective drug combination *in vitro* (Meyer et. al. JCI, 2020) even in PPR samples and regardless of their IL-7 responsiveness as compared to PDN alone or PDN+BEZ. Our data, further extended may contribute to the identification of new therapeutic approaches for T-ALL patients who currently do not respond adequately to conventional steroid therapy.

P 15 - Development of predictive scores for relapse and progression of pediatric acute lymphoblastic leukemia patients utilizing optical genome mapping technology

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Background:

Childhood acute lymphoblastic leukaemia (cALL) is the primary cancer found in children and constitutes 30% of paediatric cancers. Despite advancements in the combination of chemotherapy, hematopoietic cell transplantation, and new immunotherapies, a considerable percentage (10–20%) of patients do not survive due to treatment resistance or relapse. There is a pressing need for new valuable biological markers that can redefine the criteria for risk stratification and predict the clinical outcomes of current treatment protocols. This is particularly crucial for patients classified as "intermediate risk," which encompasses the majority of patients and lacks specific genetic characteristics.

The objective of this study was to develop a predictive prognostic score for stratifying and predicting relapse in acute lymphoblastic leukaemia (ALL) by analyzing global cytogenomic data obtained from whole genome mapping.

Methods and Results:

Samples of cells were collected from the bone marrow aspirates of 63 patients diagnosed and treated for acute lymphoblastic leukaemia (ALL) based on the SEHOP-PETHEMA 2013 protocol. Optical genome mapping (OGM) was utilized to perform cytogenetic studies on the cell samples following a specific protocol from Bionano Genomics. Only patients who were initially classified as intermediate risk were included in this study. The process involved extracting ultra-high molecular weight gDNA from the cALL samples, labeling it, and loading it onto the Saphyr platform using French fries. The data collected were analyzed using Bionano Access 1.6 software.

Using a binary matrix of genomic variations (presence/absence) across the entire genome, we employed Multi-Class Discriminant Analysis with Binary Predictors (binda 1.0.4 R package) to identify significant cytogenomic features that could differentiate patients who improved their risk group classification during treatment (score 1) or eventually relapsed (score 2) in acute lymphoblastic leukaemia (ALL). The binda.ranking function was utilized to select a set of chromosomal alterations, and score 1 was generated using 11 chromosomal alterations, with the highest scoring variables being a deletion in 9p21, an insertion in 6p22, an intratranslocation in chromosome 10 t(10q11–10q23), and a small increase in 7p13. Score 1 demonstrated 80% accuracy in our cohort, and CDKN2A was identified as the primary gene involved in at-risk group progression. Score 2 was developed using 21 chromosomal alterations, with the top five variables being a deletion in 21q22, gain of one copy of the entire chromosome 21, gain in 21q11, small insertion in 15q26, and a deletion in 22q11. Score 2 displayed 98% accuracy in our cohort, and relevant chromosomal changes revealed genes such as mTOR.

We are currently in the process of validating whether these scores can aid in clinical decision-making and improve the existing cytogenetic criteria for risk group classification in ALL.

P 16 - OMICS application to personalized medicine: Serum proteomic profile and bone marrow RNA-seq in pediatric precursor-B acute lymphoblastic leukemia

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Background:

Precursor-B Acute lymphoblastic leukemia (B-ALL) is the most frequent pediatric cancer. Genomic and cytometry techniques have improved treatment stratification. Nowadays, first-line treatment is based on chemotherapy and it is very toxic. About 15–20% of the patients suffers a relapse within the first 5 years. For this reason, it is necessary to develop new experimental approaches in order to increase the knowledge about the tumor cells features and the immunological surveillance mechanism against this hematological neoplasm.

Aim:

Development of clinical-molecular biomarkers panels using omics approaches for better classification and monitoring of the pediatric patients diagnosed with B-ALL.

Methods:

A Label Free nLC MS/MS proteomic approach was carried out to identify useful serum candidate biomarkers for diagnosis and B-ALL. For this purpose, serum proteomic profile from 18 pediatric B-ALL patients at diagnosis (day 0) and different stages of the disease (day +15, +33, and +78 of treatment) were compared to those of 16 healthy donors. Additionally, RNA-seq was performed in bone marrow samples from a smaller cohort of these patients at diagnosis, and compared to healthy donor B-cells as controls. Cytoscape and Ingenuity Pathway Analysis (IPA) were used for functional analysis. Statistical analysis was performed using different approaches, including non-parametric and parametric tests.

Results:

Analysis of serum proteomic profiles revealed 72 proteins significantly deregulated at diagnosis between patients and healthy donors, and they could be classified as potential diagnosis biomarkers. Most of these proteins reverted to healthy values at different stages of the disease: 23 proteins were classified as early reversion (day +15), 8 as intermediate reversion (day +33), and 36 as late reversion (day +78). These proteins could be also potential markers for B-ALL follow-up.

Transcriptomic bone marrow results showed concordance with serum proteomic data and a total of 29 proteins appeared significantly deregulated in both omics analyses. Interestingly, 17 of these proteins followed the same deregulation pattern, being either upregulation or downregulation.

In silico functional analysis of all serum deregulated proteins at diagnosis revealed clusters mainly related to lipid metabolism and inflammation. In addition, proteins deregulated in both proteomic and transcriptomic analyses also indicated lipid metabolism as the main molecular function affected.

Some of the proteins deregulated in both, proteomic and transcriptomic analysis, as well as other interesting immunological markers (CD300a, ROR1, SAA1, CD163 and CD25) were validated in a larger cohort of patients (n = 22) and healthy donors (n = 21) by ELISA. Our results confirmed that SAA-1, CD25 and CD300a were diagnosis and early reversion biomarkers. Currently, a set of 7 proteins (PIK3CG, SPARC, LRG1, LCAT, PLTP, GPX3 and APOM) are in process of validation.

Conclusions:

Serum proteomic profile studies revealed a total of 72 potential serum biomarkers for diagnosis and 67 candidate for B-ALL monitoring. Interestingly, 17 of these proteins were significantly deregulated in bone marrow transcriptomic analysis with the same deregulation pattern.

The deregulated proteins at diagnosis, identified in both bone marrow RNA-seq and serum proteomics, were mainly related to lipid metabolism.

Our preliminary results suggest a 3-protein panel (SAA-1, CD25 and CD300a) that could be used by physicians for a better diagnosis and early monitoring of pediatric B-ALL.

P 17 - The impact of WT1 mutations in pediatric acute myeloid leukemia

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Background:

Acute myeloid leukemia (AML) is a genetically heterogeneous disease; the outcome depends on the cytogenetic and molecular genetic markers.

Mutations in *WT1* gene are found in 10% of adult AML and some studies have shown that they negatively affect survival prognosis. The data on prognostic impact of *WT1* mutations in pediatric AML remain insufficient.

Objectives:

The aim of our study was to investigate the frequency and prognostic implication of *WT1* mutations in pediatric AML.

Methods:

The cohort included 281 AML patients diagnosed in 2018–2022 in national multicenter study AML-MRD-2018, among them 147 were males and 134 were females. Median age was 9 years (2 months - 17.9 years).

All patients underwent conventional karyotyping, FISH, fragment analysis and Sanger sequencing for *FLT3*-ITD, *NPM1*, *CEBPA*. Targeted NGS (HMNP, Qiagen) was performed to evaluate the mutational status of *WT1* and other genes significant for the pathogenesis of myeloid neoplasms.

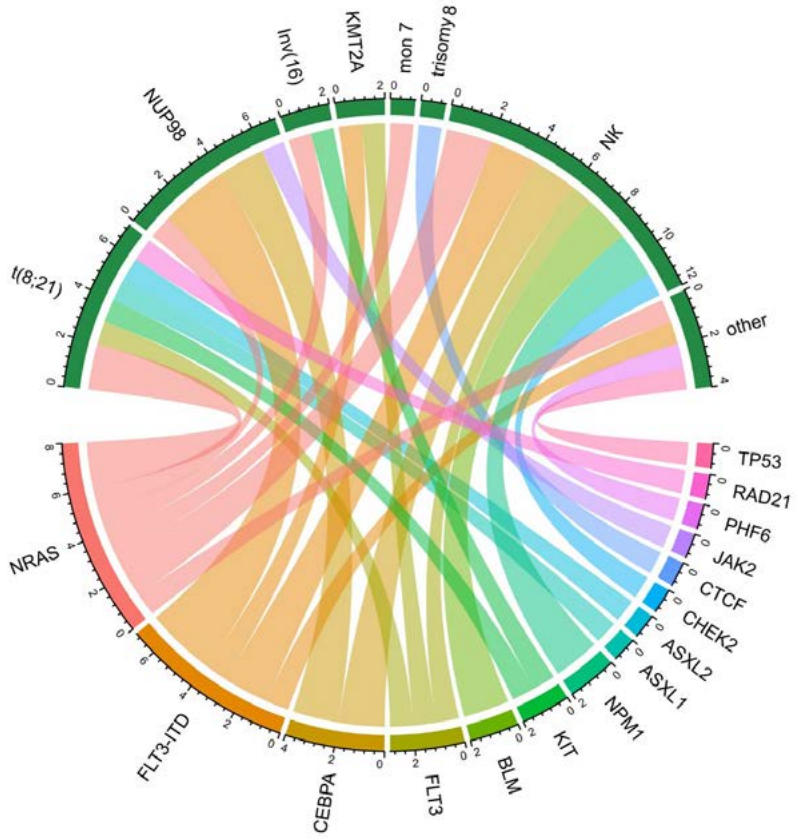
Results:

WT1 mutations were found in 27 patients (9,6%). These patients were older (11 vs 9 years in the whole cohort, p = 0.034). *WT1* mutations were significantly more frequent in DNA-binding domain affecting exon 7 (67%, n = 18), exon 9 (18%, n = 5), exon 8 (11%, n = 3), whereas only one patient had mutation in exon 3. Most mutations led to a shortened protein (frameshift n = 20, nonsense n = 1), while missense mutations were in minority (n = 6).

FAB M2 (n = 7) and M4 (n = 8) were the most common morphological subtype.

A substantial number of *WT1*^{mut} patients had normal karyotype (26%, n = 7), followed by *RUNX1::RUNX1T1* and *NUP98* rearrangements (18,5%, n = 5 each). *KMT2A* rearrangements and *CBFB::MYH1* were rear in patients with *WT1*^{mut} (7%, n = 2 each). No recurrent chromosomal abnormalities were found in 4 patients.

Investigation of gene mutations interactions has revealed that majority of patients with *WT1*^{mut} harbored at least one co-occurring mutation (figure 1).



The most frequent gene alterations accompanied by *WT1*^{mut} were in tyrosine-protein kinase receptor genes (40%): *FLT3*-ITD (n = 7), *FLT3*-TKD (n = 1), *KIT* (n = 3). In addition, *NRAS* mutations were found in 30% (n = 8), *CEBPA* mutations - in 15% (n = 4), *NPM1* mutations - in 11% (n = 2).

Two years event-free survival (EFS) was significantly worse for patients with *WT1*^{mut} compared to *WT1*^{wt}: 20% (n = 27, 95% CI 8,7–48%) vs 47% (n = 254, 95% CI 35–51%), respectively (p = 0,004). This difference was not statistically significant in intermediate risk group (EFS 35% vs 53%, p = 0.6) and statistically significant in high risk group (EFS 7% vs 41%, p < 0,001).

In multivariate analysis *WT1* was a factor negatively affecting EFS (HR = 1.9 p = 0.016) independently of *FLT3*-ITD mutations, *NUP98* translocations and hyperleukocytosis. When analyzed in subgroups, the results hold in high risk group, but not in intermediate risk group, where there was no significant difference between *WT1* and *FLT3*-ITD(table 1).

Conclusions:

In this study we showed the incidence of *WT1* mutations of 9,6% in pediatric AML. *WT1* mutations showed significant correlations with other cytogenetic and molecular alterations.

Multivariate analysis demonstrated that the *WT1* mutation was an independent poor prognostic factor for high risk AML.

Marker	HR	p
FLT3-ITD intermediate risk group	0.96	0.945
WT1mut intermediate risk group	1.28	0.562
t(8;21) intermediate risk group	0.68	0.159
FLT3-ITD high risk group	2.73	<0.001
WT1mut high risk group	2.68	0.004
NUP98r high risk group	0.95	0.878

P 18 - A rapid and comprehensive screening method for CRLF2 overexpression and its causing mechanism identification

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Background:

CRLF2 overexpression is frequent in pediatric acute lymphoblastic leukemia (ALL) and patients aggregate into the Philadelphia-like (Ph-like) subgroup. Different mechanisms contribute to *CRLF2* overexpression, such as *P2RY8::CRLF2* rearrangement, *CRLF2* translocation at @IGH locus, *CRLF2* point mutations (with p.F232C as the most frequent), and other JAK-STAT pathway activating mutations, like *JAK2* p.R683G. The identification of *CRLF2* overexpression mechanism is relevant since some of these patients may benefit from selective inhibitors.

Objectives:

Our objective was to develop a rapid RT-qPCR method to assess *CRLF2* overexpression and its causing mechanism in a single experiment.

Methods:

A RT-qPCR method simultaneously assessing *CRLF2* expression as well as its main causing mechanisms (*P2RY8::CRLF2*, *CRLF2* p.F232C and *JAK2* p.R683G) was set up.

CRLF2 expression was quantified by the 2^{-DDCt} method using *ABL1* as housekeeping gene and the median expression as the reference value. *CRLF2* overexpression cut-off was established at 20-fold interquartile range (IQR). Gene expression groups were compared using Mann–Whitney *U* test.

For *CRLF2* expression quantitation and *P2RY8::CRLF2* identification, SybrGreen on cDNA samples was used. In the same plate, high resolution melting (HRM) was used for *CRLF2* sensitive F232C and *JAK2* R683G screening on DNA samples. Samples showing divergent melting curves from negative controls were sequenced.

For technical validation, a cohort of 38 ALL patients previously characterised for *CRLF2* rearrangements by conventional cytogenetics was used.

Results:

A total of 7/38 (18,42%) samples showed *CRLF2* overexpression according to the established threshold. Median CRLF2 expression was significantly higher in these patients (p-value < 0.0001) compared with the rest (Figure 1).

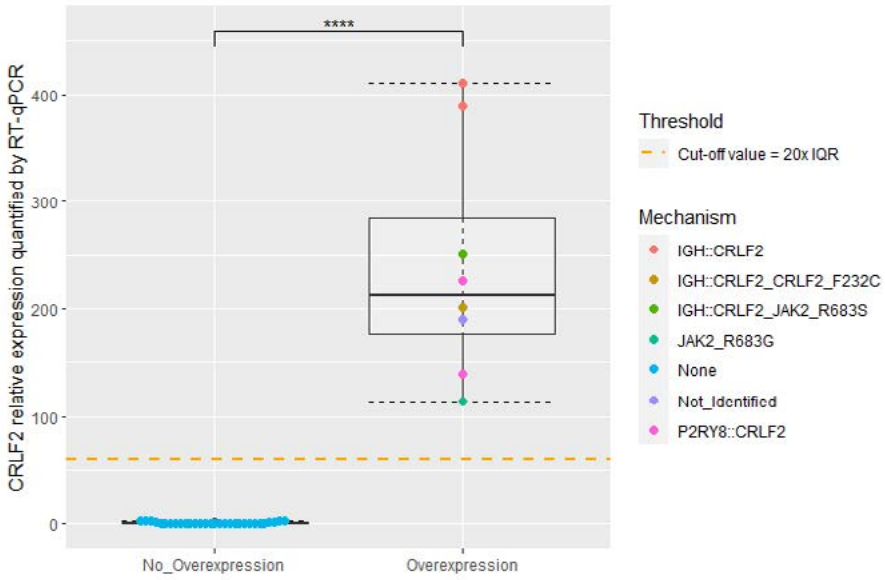

Figure 1: *CRLF2* expression levels clustered by groups, ** means significant differences (p-value < 0.0001).**

Among the overexpressing samples, the method detected the *P2RY8::CRLF2* rearrangement in 2/7 (28.6%) patients and point mutations in 2/7 (28.6%) patients. Additionally, the presence of *IGH::CRLF2* was identified by FISH in 4/7 (57.1%) patients. Of note, two patients carrying *IGH::CRLF2* also harboured point mutations. *CRLF2* overexpression mechanism could not be identified in one patient (14.3%) (Table 1).

Table 1: *CRLF2* expression levels and causative mechanisms in overexpressing patients.

Patient	*CRLF2* relative expresión (2^{-DDCt})	Mechanism	Moment
1	388.02	*IGH::CRLF2*	Diagnosis
2	225.97	*P2RY8::CRLF2*	Diagnosis
3	190.02	Not Identified	Diagnosis
4	410.15	*IGH::CRLF2*	Diagnosis
5	139.1	*P2RY8::CRLF2*	Diagnosis
6	114.56	*JAK2* R683G	Diagnosis
6	250.73	*IGH::CRLF2* + *JAK2* R683S	Relapse
7	200.85	*IGH::CRLF2* + *CRLF2* F232C	Diagnosis

Interestingly, the patient harbouring *JAK2* p.R683G mutation at diagnosis underwent an early relapse in which the R683G mutation was lost but a new *JAK2* variant (p.R683S) and the *IGH::CRLF2* fusion were acquired, resulting in two-fold *CRLF2* expression increase compared to diagnosis.

Conclusion:

Our method is fast, sensitive, and specific, to assess *CRLF2* expression as well as the main causative mechanisms, so it can be a useful tool for treatment selection, risk stratification and clonal evolution assessment.

P 19 - Revaccination of children with acute lymphoblastic leukemia following completion of chemotherapy

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Background:

Infection is an important cause of morbidity and mortality in children with acute lymphoblastic leukemia (ALL), even after the completion of chemotherapy. The use of intensive chemotherapy is known to have a significant impact upon humoral immunity, yet there are no uniform guidelines for post-chemotherapy vaccinations.

Objectives:

In this prospective multicenter study, we aimed to evaluate the immunity to measles, varicella, polio, pertussis, hepatitis A and hepatitis B among previously vaccinated children who completed ALL therapy and to assess the utility of revaccination in patients with insufficient antibody titers.

Methods:

Over a 6-year enrollment period (from March 2015 to March 2021), previously vaccinated children who had completed chemotherapy for ALL according to the AIEOP BFM ALL 2009 protocol (aged 1–18 years at leukemia diagnosis), were recruited from three large tertiary care hospitals in Israel. Serum antibody levels against measles, varicella, polio, pertussis, hepatitis A and hepatitis B were assessed 3–6 months after completion of chemotherapy. Children who did not have sufficient antibody titers to specific agents were revaccinated with a single dose accordingly. Antibody levels were assessed at least 4 weeks after revaccination.

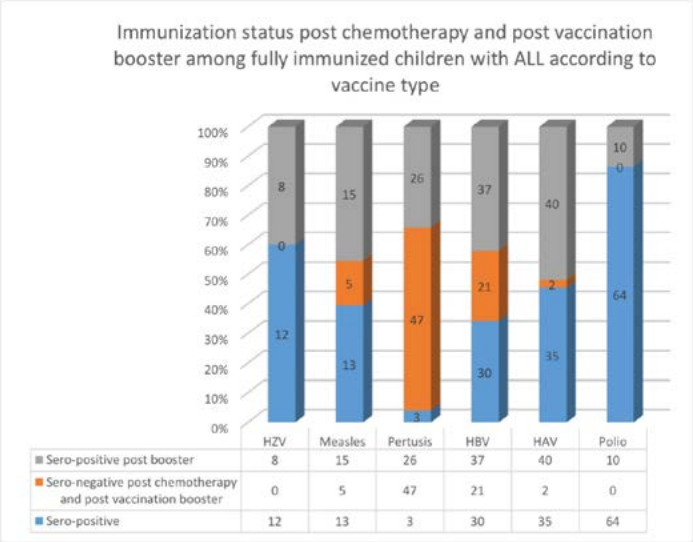
Results:

Ninety-six children who completed chemotherapy for ALL were recruited (mean age 6.2±4.6 years). Seventeen of these (18%) had been treated according to the high-risk arm of the AIEOP BFM ALL 2009 protocol.

At a median of 4.8 months after completion of chemotherapy, the majority of children in our study (96%) did not have protective antibody levels to at least one of the vaccine-preventable diseases that were examined. There were significant differences between the different types of vaccines with regard to the incidence of post-treatment pre-booster protective antibody levels. The highest percentage of protective antibody level was against polio (87%) and the lowest against pertussis (4%) ($P < 0.001$).

There were significant differences in antibody seronegativity rates between those who received therapy according to the high-risk arm and those who did not for the following vaccines: measles (100% vs 48%, $p = 0.012$), HBV (92 % vs 61%, $p = 0.031$), and HAV (92 % vs 47%, $p = 0.004$). Seronegativity rates were higher for the high-risk group for the other vaccines as well but did not reach statistical significance. There were no significant differences in seronegativy rates among the different vaccines according to patient age (<5 years vs. >5 years), with the exception of HAV (43% vs 68%, $p = 0.039$). We found a significant increase in antibody levels after administering a booster dose of all types of vaccines, with the highest response to the varicella and polio vaccine booster (100% response) and the lowest response to the pertussis booster (36% response).

Immunization status post chemotherapy and post vaccination booster among fully immunized children with ALL according to vaccine type.



	HZV	Measles	Pertusis	HBV	HAV	Polio
Sero-positive post booster	8	15	26	37	40	10
Sero-negative post chemotherapy and post vaccination booster	0	5	47	21	2	0
Sero-positive	12	13	3	30	35	64

Conclusions:

Our large, prospective, multicenter study evaluating immune status after childhood ALL therapy demonstrates that loss of humoral protection for vaccine-preventable diseases is common. Revaccination with a single vaccine dose after completion of chemotherapy achieved protective antibody responses in 34%-100% of the patients, depending on the type of vaccine. We recommend this revaccination schedule to all previously vaccinated children upon completion of ALL therapy.

P 20 - ERG deletions do not necessarily coincide with DUX4 rearrangements – A report of two cases

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Childhood B-cell acute lymphoblastic leukemia (B-ALL) is a genetically diverse disease, whose genetic subgroups are defined by gene fusions, ploidy changes, copy number alterations (CNAs) and sequence mutations. Several of these aberrations provide the basis for risk stratification and treatment decisions. The clinical interest currently focuses on subtypes presenting potential candidates for targeted therapy such as kinase-activating fusion genes and on those with more indistinct secondary abnormalities like the *IKZF1*^{plus} CNA profile, which is important for risk stratification. Due to its good outcome special attention is paid to *DUX4*-rearranged (*DUX4*-r) leukemia, which is diagnostically challenging, and therefore *ERG* deletion is often used as surrogate marker.

The prognostically adverse *IKZF1*^{plus} genotype is defined by *IKZF1* deletions co-occurring with any deletion in *CDKN2A*, *CDKN2B*, *PAX5* or in the PAR1 region in the absence of *ERG* deletions. *DUX4* rearrangements, mostly *IGH::DUX4*, lead to the overexpression of truncated *DUX4* isoforms and concur with deregulation and, in approximately half of the cases, a deletion of the *ERG* gene. Moreover, *DUX4*-r leukemia is typically associated with CD371 and frequently also CD2 antigen expression. *ERG* deletions are thus not only an essential exclusion criterion for defining the *IKZF1*^{plus} CNA profile but also for identifying *DUX4*-r B-ALL, which accounts for roughly 5% of all childhood cases.

We present two unique cases of girls with typical exons 3–10 *ERG* deletions but *DUX4*-negative B-ALL. In patient 1 (5.5-years-old), karyotyping revealed complex aberrations involving chromosomes 9p, 7p and Xp. Fluorescence in situ hybridization (FISH) analysis verified the presence of an isochromosome 9q together with complex rearrangements resulting in der(7)t(7;9) and der(X)t(X;9) chromosomes. As revealed by SNP array analysis, these aberrations concurred with a homozygous *CDKN2A/B*, a partially homozygous *PAX5* and heterozygous *IKZF1* and Xp deletions, respectively. At diagnosis, patient 2 (13.3-years-old) presented a normal karyotype and FISH showed neither any B-ALL typical gene fusion nor any changes in ploidy status but SNP array analysis revealed *PAX5* and *ERG* deletions.

Despite the presence of *ERG* deletions in both cases, whole transcriptome sequencing (RNA-seq) and gene expression profiling neither revealed the presence of an *IGH::DUX4* nor any other in-frame fusion gene nor the *DUX4*-r leukemia-specific gene expression signature. Moreover, the blast cells were negative for the cell surface markers CD371 and CD2, which are otherwise typically expressed in *DUX4*-r cases.

In patient 1 mutation calling in key genes involved in the RAS pathway, JAK/STAT signaling and lymphoid development using the RNA-seq data, identified only a PTPN11 p.T73I missense mutation. In patient 2 only relapse material was available and RNA-seq data analysis revealed a KRAS p.Gly12Asp and a ZEB2 p.His1038Arg mutation, and a second relapse-specific rare NR3C1 p.Phe624Cys mutation.

Patient 1 showed a good prednisone response and achieved complete remission on day 33 of treatment. Minimal residual disease on day 33 was $<10^{-4}$ and negative on day 78. The patient was stratified into the low/standard risk-group of the ALL-BFM 2000 clinical trial and remains in complete remission for twenty-one years. Patient 2 was stratified into the high-risk group of the ALL-BFM 2009 clinical trial and despite intensive treatment including stem cell transplantation and CAR-T cell therapy experienced two relapses.

In summary, we show that *ERG* deletions do not exclusively occur in *DUX4* leukemia and that their application as surrogate marker might in rare cases lead to misclassifications into the *DUX4* subtype.

P 21 - Ultimate clarification of the relationship between asparagine synthetase activity and sensitivity of leukemia to L-asparaginase

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Background:

L-asparaginase (ASNase) is one of the crucial drugs used in treatment of childhood acute lymphoblastic leukemia (ALL). Leukemia cells are sensitive to ASNase because they have lower asparagine synthetase (ASNS) level compared to healthy cells and other tumors. However, correlations of ASNS gene expression or enzyme production with sensitivity of leukemia to ASNase are inconsistent. Notably, ASNase used *in vitro* depletes all asparagine (Asn) and glutamine (Gln) whereas it is probably only Asn that is completely depleted *in vivo* after ASNase administration in ALL treatment with unchanged or lowered Gln concentration.

Objectives:

The main aim is to evaluate the role of ASNS enzymatic activity, rather than protein level, in predicting sensitivity of ALL cells to ASNase. To do that, ASNS enzymatic activity in ALL cells was measured together with sensitivity of these cells both to ASNase and Asn-depletion only.

Methods:

We employed stable isotope tracing to study the activity of ASNS under different nutrient conditions. Sensitivity of ALL cells to ASNase, Asn-depletion and chloroquine (CQ) was measured using MTS assay. Protein levels were detected using western blot (WB).

Results:

We measured the activity of ASNS together with ASNS protein levels in four B-ALL and five TALL cell lines. Six of them had ASNS detectable on WB (ASNS-*plus*). Surprisingly, none of the tested cell line synthesized Asn when it was present in the culture media, even in case when ASNS was clearly detectable on WB. On the other hand, when Asn was limited in the media, ASNS-*plus* cells were able to synthesize Asn whereas ASNS-*null* cells were not. We found out that all tested ASNS-*plus* cells used exclusively aspartate (Asp) synthesized from Gln via TCA cycle. Then, ASNS catalyzed the transfer of an amide group to Asp from the second Gln. Noteworthy, all of the tested ASNS-*plus* cell lines were able to import Asp in a dose-dependent manner. However, they did not use this imported Asp to synthesize Asn. These results indicate that ASNS prefers Asp produced via TCA cycle and that substrate channeling could play a role in ASNS activity.

Then, we measured the sensitivity of all studied cell lines to ASNase and Asn-depletion. In B-ALL, all ASNS-*plus* cell lines (NALM6, REH and UOC-B6) were less sensitive to Asn-depletion compared to ASNS-*null* cell line RS4;11. Furthermore, in T-ALL, ASNS-*plus* cell lines (JURKAT, MOLT4 and CCRFCEM) together with one ASNS-*null* cell line (ALL-SIL) were less sensitive to Asn-depletion compared to the second ASNS*null* cell line (DND-41). Since autophagy could provide Asn when it is limited in the media, we looked into autophagy activity. Interestingly, ALL-SIL cells were more sensitive to autophagy inhibitor CQ and also had higher autophagic flux compared to DND-41 cells. In accordance with that, we detected higher mTOR activity in DND-41 than in ALL-SIL cells. Regarding sensitivity to ASNase in both B-ALL and T-ALL, ASNS protein positivity only correlated with sensitivity to ASNase in mimicked *in vivo* conditions (Asn-depletion).

Conclusion:

Altogether, our study for the first time determined ASNS activity in leukemia cells under different nutrient availability. Importantly, ASNS activity correlates with ASNS protein levels in Asn-deplete conditions. In general, ASNS-*plus* cells are less sensitive to Asn-depletion than ASNS-*null* cells. However, the second mentioned could overcome ASNase treatment by high autophagy state and therefore bias the correlation between ASNS protein level and sensitivity to ASNase.

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P 22 - Allopurinol shifts 6-mercaptopurine metabolism in unselected patients with acute lymphoblastic leukemia in childhood

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Background:

6-mercaptopurine (6MP) is one of the key components of maintenance therapy (MT) in acute lymphoblastic leukemia (ALL). Inter-individual variation in 6MP metabolism causes significant differences in erythrocyte levels of 6-thioguanine (e-TGN), which is the proposed main mediator of the antileukemic effect, and 6-methylmercaptopurine (e-MeMP), associated with hepatotoxicity. Patients with excessive hepatotoxicity or difficulties achieving target white blood cell counts (WBC), often exhibit high e-MeMP and low e-TGN. Smaller studies have shown that allopurinol addition can shift the metabolite balance towards increased e-TGN and reduced e-MeMP in these patients.

Objectives:

Our main objective was to study the effects of allopurinol in an unselected cohort of children with ALL to investigate if a similar metabolite shift would be achieved also in patients without previous signs of skewed 6MP metabolism. We also wanted to evaluate if allopurinol is safe to use in this context.

Methods:

51 patients on NOPHO ALL-2008 protocols were included in a prospective crossover trial. 6MP metabolites were measured during 12 weeks of standard MT, followed by 12 weeks of MT with addition of allopurinol 50 mg/m^2 and finally 4 weeks of MT without allopurinol. The 6MP dose was reduced by 50% when initiating allopurinol. The primary endpoint was the fraction of patients with e-TGN > 200 nmol/mmol Hb after 12 weeks of allopurinol compared to before.

Results:

After 12 weeks of allopurinol, 91% of the patients had e-TGN > 200 vs 67% before ($p < 0.001$) with median e-TGN increasing from 247 (week 13) to 418 (week 25) ($p < 0.001$). The effect was rapid with e-TGN reaching a plateau within four weeks after initiating allopurinol. Median e-MeMP decreased from 8210 to 2742 nmol/mmol Hb four weeks after allopurinol initiation ($p < 0.001$). Alanine aminotransferase declined two weeks later.

The fraction of patients with WBC within target 1.5–3.0x10^9/L was slightly higher during the weeks on allopurinol (0.69) compared to the weeks before allopurinol, (0.53) ($p < 0.005$). Absolute neutrophil count (ANC) median was lower on allopurinol, 1.10 x 10^9/L, compared to 1.60 before ($p < 0.001$). Allopurinol was well tolerated causing no increased number of severe adverse events nor any life-threatening episodes.

The results are outlined in the table below:

					Allopurinol				
Study week	w1	w5	w9	w13	w17	w19	w21	w25	w29
e-TGN									
mean	254	282	251	280	485	447	461	440	185
(min-max)	(22–786)	(105–719)	(37–520)	(42–797)	(77–1065)	(91–1165)	(57–1224)	(100–978)	(64–427)
median	205	271	242	247	468	403	413	418	176
median whole phase	Median before allopurinol (w1-13) 241				Median on allopurinol (w17-25) 422				
>200 n/tot (%)	22/43 (51%)	35/47 (74%)	31/48 (65%)	**31/46 (67%)**	47/51 (92%)	46/48 (96%)	42/48 (88%)	**41/45 (91%)**	18/44 (41%)
e-MeMP									
mean	6993	9830	9753	9481	3395	2459	2230	2791	3459
(min-max)	(0–30962)	(322–26277)	(104–25382)	(139–31503)	(0–17511)	(116–14173)	(0–14545)	(0–16348)	(0–15430)
median	5127	9184	9267	8210	2742	1355	1547	1875	2095
median whole phase	Median before allopurinol (w1-13) 8149				Median on allopurinol (w17-25) 1677				
WBC									
mean	3.31	3.15	2.75	3.66	2.35	2.43	2.73	2.46	3.68
(min-max)	(1.49–6.00)	(1.30–8.00)	(1.40–5.60)	(0.90–25.00)	(1.00–9.30)	(0.95–4.70)	(1.30–8.40)	(1.10–5.20)	(1.80–14.40)
median	3.35	2.84	2.65	2.78	2.12	2.41	2.40	2.40	3.40
median whole phase	Median before allopurinol (w1-13) 2.90				Median on allopurinol (w17-25) 2.37				
1.5-3.0 n/tot (%)	18/48 (38%)	25/49 (51%)	32/48 (67%)	28/49 (57%)	34/45 (76%)	31/49 (63%)	30/47 (64%)	33/45 (73%)	19/45 (42%)
ANC									
mean	1.83	1.80	1.39	1.91	1.18	1.24	1.52	1.34	2.21
(min-max)	(0.30–4.40)	(0.20–6.60)	(0.02–4.00)	(0.53–8.90)	(0.10–6.80)	(0.24–3.20)	(0.30–6.80)	(0.38–3.44)	(0.78–13.20)
median	1.86	1.70	1.40	1.60	0.90	1.08	1.30	1.30	1.88
median whole phase	Median before allopurinol (w1-13) 1.60				Median on allopurinol (w17-25) 1.10				
ALT									
mean	2.17	2.08	2.84	3.07	2.21	2.14	1.87	1.64	1.83
(min-max)	(0.27–12.67)	(0.21–16.00)	(0.18–22.36)	(0.09–19.30)	(0.23–8.80)	(0.27–14.00)	(0.27–9.95)	(0.27–13.00)	(0.27–8.92)
median	1.35	1.30	1.67	1.64	1.40	0.81	0.69	0.77	1.40
median whole phase	Median before allopurinol (w1-13) 1.50				Median on allopurinol (w17-25) 0.87				
Creatinine									
mean	29	29	31	30	30	31	30	32	30
(min-max)	(15–70)	(15–70)	(17–71)	(15–63)	(17–64)	(16–62)	(16–62)	(17–63)	(16–71)
median	27	29	28	27	29	29	29	29	27
median whole phase	Median before allopurinol (w1-13) 28				Median on allopurinol (w17-25) 29				

Allopurinol is given study weeks 13-25, therefore weeks 17-25 are considered being influenced by allopurinol.
Units of measure: e-TGN (thioguanine in erythrocytes) and e-MeMP (methylmercaptopurin in erythrocytes) nmol/mmol Hb, WBC (white blood cells) and ANC (absolute neutrophil count) x10^9/L, ALT (alanine aminotransferase) µkat/L, creatinine µmol/L
Fraction of patients with e-TGN >200 nmol/mmol Hb after 12 weeks of allopurinol is primary endpoint. Weeks 13 and 25 are therefore highlighted.
1.5-3.0 is targeted WBC during maintenance therapy in the NOPHO ALL-2008 protocol.

Conclusion:

Allopurinol 50 mg/m^2 leads to increased e-TGN and reduced e-MeMP also in patients without previous signs of skewed 6MP metabolism and was well tolerated. WBC and ANC levels indicate that therapy intensity was maintained during the allopurinol phase. A larger prospective study is needed to examine if adding allopurinol could lead to better outcome and less toxicity.

Keywords:

allopurinol
acute lymphoblastic leukemia (ALL)
6-mercaptopurine (6MP)
6-thioguanine (TGN)
6-methylmercaptopurine (MeMP)
maintenance therapy (MT)
children
prospective
crossover

P 23 - Benefit of next-generation sequencing (NGS) in detection of fusion genes, immunome and ctDNA/MRN in patients with ALK-positive anaplastic large cell lymphoma

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Introduction:

Anaplastic large cell lymphoma ALK positive (ALCL, ALK+) belongs to the group of T lymphomas with pathologic activation of oncogene ALK. More than 10 fusion partners with gene ALK have been described, with the most common translocation t(2;5)(p23;q35), fusion NPM1::ALK. Analysis of T-cell receptor (TCR) rearrangement can aid to both diagnostic and research purposes. Analyses of TCR rearrangement in ALCL used qualitative methods with partially discordant results. Methods of NGS ease detection of fusion partners of ALK gene and enable characterisation of TCR not only in neoplastic cells, but also in infiltrating T lymphocytes. Analysis of circulating tumour DNA (ctDNA) in blood seems to be perspective for MRD assessment, its predictive value in ALCL is not yet characterized.

Aims:

By using NGS analyse fusion partners of ALK genes and profiles of rearrangements of TCR in a cohort of patients with ALCL, ALK+, further find out which markers are the best for MRD assessment and a pilot study for possibility of detection of MRD in ctDNA by TCR rearrangement analysis.

Methods:

Detection NPM1::ALK by PCR, quantitative PCR detection of expression of translocated part of ALK gene, detection of fusion transcripts by aimed NGS, NGS detection TCR beta (TCRB) a TCR gama (TCRG) rearrangements.

Results:

We analysed 96 cases of ALCL, ALK+ patients. In 71 patients we detected fusion gene NPM1::ALK, in 9 patients fusion of ATIC::ALK, in 3 TPM3::ALK. In two patients we detected fusion CLTC::ALK, resp. MYH9:ALK, resp. RNF213::ALK. Further we detected fusion genes TPM4::ALK and SATB1::ALK and yet undescribed fusions in ALCL, ALK+: SQSTM1::ALK a CAPRIN1::ALK. In only three patients we were unable to identify translocation partner of ALK gene because of unavailability of tissue material. In all analysed samples (92) we detected high level of expression of translocated part of ALK gene. Analysis of immunome was performed in 23 patients with DNA available. In 15 (65%) we found at least one (maximally four) clonal TCR rearrangement; at least one rearrangement was always productive. In 13 patients we found simultaneous rearrangements of both TCRB and TCRG. We did not found shared physiologic clones aimed against neoantigen. According to sequential of clonal rearrangement we designed primers for detection of MRD in ctDNA. Levels of MRD in 32 samples analysed in cells by quantitative detection of expression of ALK gene showed higher sensitivity compared to levels of ctDNA.

Conclusion:

NGS enables to detect translocation partners (including those not yet described) of ALK gene. Quantitative detection of expression of translocated part of ALK gene can serve both for diagnosis and to MRD assessment. Analysis of TCR by NGS enables setting aims for MRD assessment in both ctDNA and in cells and enables to describe bystanders clones of nonneoplastic T lymphocytes, which are specific for every patient and cannot serve as a marker for disease assessment.

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P 24 - Sequential drug treatment targeting cell cycle and cell fate regulatory programs blocks non-genetic cancer evolution in acute lymphoblastic leukemia

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Targeted therapies exploiting vulnerabilities of cancer cells hold promise for improving patient outcome and reducing side effects of chemotherapy. Yet, mechanistic understanding linking drug effects and cancer cell state diversity is crucial for identifying effective combination therapies to overcome treatment-induced resistance. Here, we report that the WEE1 inhibitor AZD1775 that compromises cell cycle checkpoints, selectively inhibits recovery of proliferation of KMT2A-rearranged (KMT2A-r) ALL cells, compared to other leukemia subgroups. Despite this, rapid drug resistance arises in monotherapy setting. Single-cell transcriptome and chromatin accessibility profiling of (*KMT2A*::*AFF1*) RS4;11 cells treated with AZD1775 revealed strong activation of p53-driven processes linked to induction of apoptosis and senescence, and disruption of a core KMT2A-RUNX1-MYC regulatory network through CDK1-mediated RUNX1 degradation. In RS4;11 cells and in patient-derived xenograft (PDX) model, we uncovered that an epigenetic transition to a cell state characterized by activation of transcription factors regulating pre-B cell fate, lipid metabolism and pre-BCR signaling supported a drug resistant cell state. By sequential treatment targeting the induced cell state switching with dasatinib, ibrutinib, fatostatin or AZD2014 after AZD1775, drug resistance could be blocked. Collectively, our findings provide new insights into the tight connectivity of gene regulatory programs associated with cell cycle and cell fate regulation, and a rationale for sequential administration of WEE1 inhibitors with low toxicity inhibitors of pre-BCR signaling or metabolism.

P 25 - Apolipoproteins – New biomarkers of overweight and obesity among childhood acute lymphoblastic leukemia survivors?

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Background:

Patients suffering from childhood acute lymphoblastic leukemia (ALL) are at risk for a spectrum of late adverse treatment-related effects. The examination of targeted biomarkers might be used to improve the diagnosis and prediction of the life-threatening sequalae of ALL. It seems that abnormalities in apolipoprotein (Apo) levels are associated with the development of metabolic syndrome components. Thus, the purpose of this cross-sectional study was to search for treatment-related alterations in lipid metabolism and the potential markers in the occurrence of obesity in subjects treated for ALL in the past and assess the relationship between weight, gender, anticancer treatment, and Apo concentrations. Methods: Fifty-eight ALL survivors were included in the study. The mean time of follow-up after treatment cessation was 5.41 ± 4.29 years. Serum levels of nine Apo were measured by a multiplex assay kit. Results: Among ALL survivors, we observed a significant correlation of Apo-C1, -C3, -H, and -J levels depending on BMI status. Moreover, markedly differences in an area under curve (AUC) of Apo-A1, -A2, -C1, and -D were observed, indicating to a great value of mentioned parameters as predictors for overweight and obesity. Conclusions: Our study showed that patients with the history of childhood ALL developed alterations within Apo profile. Furthermore, this is the first study revealing that some apolipoproteins may act as valuable biomarkers useful to prognosis of metabolic imbalance. We believe that this paper at least partially, will highlight the importance of long-term prognosis of metabolic complications associated with the anticancer chemotherapy toward hematological malignancies among children.

P 26 - Chromothripsis-like pattern of copy number abnormalities in childhood precursor T-cell acute lymphoblastic leukemia

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Background:

T-cell acute lymphoblastic leukemia (T-ALL) represents genetically heterogeneous disease distinct from B-ALL. Currently, risk-adapted therapy results in a relatively high 5-year overall survival rate of 80%. However, only the minority of patients with relapse T-ALL can be successfully cured with contemporary chemotherapy regimens. Therefore, significant effort is currently being aimed at preventing leukemia recurrence by deeper understanding of T-ALL biology. Chromothripsis is a form of genome instability leading to a massive de novo structural chromosome rearrangements within one-time catastrophic event. This phenomenon is associated with poor prognosis in diverse tumor entities.

Objectives:

The aim of this study was to identify the pattern of chromothripsis in 169 newly diagnosed childhood T-ALL using high-density single-nucleotide polymorphism microarray.

Patients and Methods:

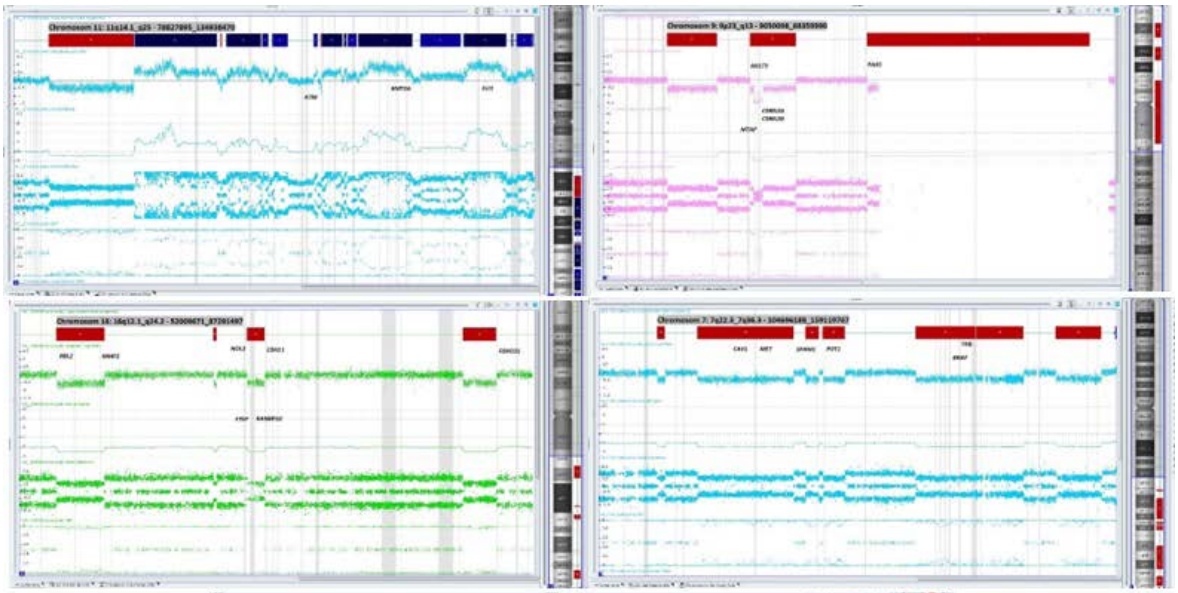
A total of 169 patients with newly diagnosed T-ALL, treated according to the ALL IC-BFM 2009 (n = 75) and AIEOP BFM 2017 (n = 94) protocol in Polish pediatric hematology centers from November 2011 to December 2021, were included in the study. The group consisted of 56 girls (33.1%) and 113 boys (66.9%). The age of patients ranged from 1.08 to 17.67 years (median age, 8.37 years). Immunophenotypic data enabled the classification of patients into ETP-ALL [n = 7 (4%)], pre-T ALL [n = 63 (37%)], cortical T-ALL [n = 53 (31%)], and mature T-ALL [n = 35 (21%)] based on the EGIL criteria. In 11 cases (7%), immunophenotypic data were not available. Analysis of CNAs was performed using CytoScan HD array (ThermoFisher). Chromothripsis was defined based on criteria provided by Korbel J and Campbell P based on an oscillating copy-number profile, requiring at least 10 copy-number alterations showing two or three copy-number states. Illumina TrueSight PanCancer panel was used for targeted RNA sequencing.

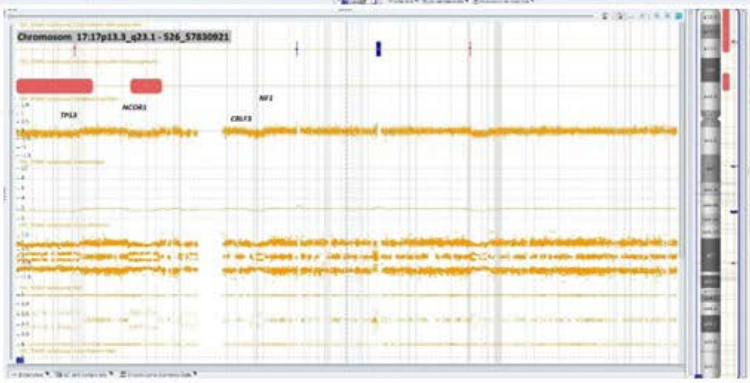
Results:

A total of 383 structural alterations (>5 Mb) copy number alterations (CNAs), including trisomies (n = 55), monosomies (n = 10), duplications (n = 67), deletions (n = 156), segmental loss of heterozygosity (95), were detected in 169 analyzed samples. The median number of structural CNAs per case was 2.2. Almost 55 of trisomies of whole chromosomes were observed, with chromosome 8 being the most frequently duplicated. The most common gains were observed in the following genes: *IGH@* (95%), *IGH* (50.8%), *L1CAM* (31.36%), *MYB* (13%), *PHF6* (11.2%) and deletions occurred in: *TRG@* (80.47%), *TRA* (76.92%), *TRD* (74.56%), *TRB* (63.31%), *TRD@* (57.4%), *CDKN2A*(70.41%), *CDKN2B* (54.43%), *STIL* (13%), *HOXA11* (11.24%), *RB1* (11.24%), *PTEN* (8.87%), *LEF1* (7.69%). To investigate the frequency of specific gene fusions and gene mutations in the study group, we performed targeted RNA sequencing in 50 patients with available RNA from ALL diagnosis. The following fusion genes were identified *PICALM::MLLT10* (n =5), *NUP214::ABL1* (n =4), *SET::NUP214* (n =2), *MYO18A::JAK2* (n = 1) and *ETV6::BLK* (n = 1). The most recurrent point mutations affected RAS pathway genes. We identified chromothriptic-like pattern of CNAs in five T-ALL (2.9%) all with a pre-/or cortical immunophenotypes (Figure 1). Chromothripsis affected chromosomes: 7q, 9p, 11q, 16 and 17p. Clinical and genetic characteristics of these patients are presented in Figure1. Chromothriptic pattern on chromosome 11 (region 55 Mb) resulted in deletion of *EED* and *PICALM* genes (M162). A least 12 genes related to leukemogenesis which are recurrently deleted in T-ALL, were lost due to chromothripsis in other cases.

Conclusion:

Chromothripsis in childhood precursor T-ALL is a rare genomic event associated high MRD during induction phase of the treatment. Further investigation needs to be done to estimate the clinical significance of chromothripsis in T-ALL.





Patients	M13	M162	M172	M97	M94
Age at ALL diagnosis (years)	11.5	1.5	6.67	3.75	5.30
Immunophenotype	T-ALL (pre) (T-II)	T ALL common cortical CD3- (T-III)	T-ALL (pre) (T-II)	T-ALL (pre) (T-II)	T ALL common cortical (T-III)
Sex	Male	Female	Male	Male	Male
WBC [n/ul]	30610	362300	7070	121550	6560
BM blasts at diagnosis (%)	85	96	94	73	26
CNS involvement	CNS status 1	CNS status 1	CNS status 2	CNS status 1	CNS status 1
Steroidoresistance n (%)	No	No	**Yes**	No	**Yes**
FCM-based MRD at day 15 (%)	0.9%	7.63%	90.19%	50.5%	45.24%
PCR-based MRD at day 33	Not available	Not available	positive >=5x10-4	positive=1x10-1	positive >=5x10-4
Risk group n (%)	IR	IR	HR	HR	HR
Complete Remission Achieved (days)	15 day	33 day	Not available	Late 92	33 day
Outcome	Alive	Alive	Relapse and allo-HSCT; death	Alive	Relapse, allo-HSCT
Karyotype GTG	46,XY	46,XX,t(6;7)(q23;q22),trp(11)(q13q12)	46,XY	46,XY	45,XY,dic(5;17)(q12;p173)[18]/45,XY,dic(5;17)(q12;p173),del(12)(p11.2p13),del(13)(q12q21)[1]/46,XY[11]
Chromosomal region affected by chromothripsis	7q22.3q36.3(104696188_159119709)	11q14.1q25(78827895_134938470)	9p23q13(9050098_68359990)	16q12.1q24.2(52009671_87291497)	17p13.3q23.1(526_57830921)
CNA	Deletion RPL22, NOTCH2, ETV6, CDKN1B	Deletion RAG2	monosomy X	Deletion SORBS2 ex6-7/15	Deletion SORBS2 ex6-7/15; monosomy 7;
Molecular karyotype	arr[GRCh37]1p36.33p36.31(849466_6445105)x1-2 hmz,1p36.22p31.1(10387929_76236452)x2-3,1p31.1p12(79915304_120001330)x1-2 hmz,1q21.2q44(148514235_249224684)x2-3,5q31.1q35.3(130697570_180719789)x1-2 hmz,7q22.3q36.3(104696188_159119709)cth,9p23p21.1(10407804_32282978)x1 hmz,9p21.1p13.2(32158191_38326473)x2-3,9q21.11q33.2(71512667_123009219)x1-2 hmz,9q33.2q34.3(124629919_141020389)x2-3	arr[GRCh37]11q14.1q25(78827895_134938470) cth	arr[GRCh37](X)x0,Yp11.31q11.21(2650141_14564367)x1,9p23q13(9050098_68359990) cth	arr[GRCh37]9p21.3p21.2(21206424_27114166)x1,16q12.1q24.2(52009671_87291497) cth	arr[GRCh37]5q21.2q35.3(103831721_180719789)x1-2,(7)x2~<2,(8)x2~>2,12p13.32p11.23(4627330_27619036)x1-2,13q13.1q21.2(32894228_62292434)x1-2,17p13.3p13.1(525_9395965) cth

P 27 - Long-term infection-drives changes in the bone marrow micro-environment of Pax5+/- mice predisposed to B cell precursor acute lymphoblastic leukemia

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Keywords:

Pax5+/- mice, Lymphocytic choriomeningitis virus (LCMV), bone marrow microenvironment, effector CD8⁺ T cells, microbiome.

Background:

Pax5 heterozygosity (*Pax5*$^{+/-}$) in mice mimics germline or somatic Pax5 dysregulation (resulting in reduced Pax5 levels) observed in childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL). While a link between general non-specific infectious exposure and BCP-ALL has been demonstrated in the *Pax5*$^{+/-}$ mouse model, the effects of a specifically tractable infection are poorly understood. In particular, little is known about potential interactions of pre-leukemic early progenitor B cell populations and immune cells present in the bone marrow microenvironment (BME) during a chronic viral infection.

Aims:

We aimed to characterize the effects of a chronic viral infection in the BME of the *Pax5*$^{+/-}$ mouse model employing the Lymphocytic choriomeningitis virus (LCMV). LCMV is a non-cytopathic virus that has been extensively utilized to investigate virus-induced immunopathology, effector responses and immune tolerance.

Methods:

Pax5$^{+/-}$ mice backcrossed to the C57BL/6J background (N10) were infected with LCMV. Using flow cytometry, ELISA and plaque assay, innate and late adaptive immune responses in infected *Pax5*$^{+/-}$ and WT (*Pax5*$^{+/+}$) were assessed. Pre-leukemic early progenitor B cell populations, antigen-specific CD8⁺ T cells and regulatory T cells (Treg) were evaluated in the BME and the microbiome via stool samples was assessed throughout the course of infection.

Results:

When we infected *Pax5-/+* and WT mice with LCMV-Docile (1 x 10^6 PFU), we found that early innate (interferon production 24 hours post infection and multiple cytokines (CXCL1, MCP-1 and CXCL10) 15 days post infection) and day 15 viral titers were not different in *Pax5-/+* compared to WT mice in the spleen, kidney, bone marrow liver and lymph nodes. However, at day 90 post-infection, LCMV-specific T cells were present within the BME and tetramer specific CD8⁺ T cells raised against the LCMV nucleocapsid protein (np 396) were higher in the BM of *Pax5-/+* infected mice. Furthermore, as ascertained by increased PD1 expression, CD8⁺ T and tet⁺ CD8⁺ T cells were more exhausted in the BME of *Pax5-/+* compared to WT infected mice. Infection caused a significant upregulation of Major Histocompatibility Complex Class II (MHC-II) expression in Pre-BI cells of WT but not *Pax5-/+* mice and an upregulation of IL-7r in Pre-BII cells in both WT and *Pax5-/+* mice. *Pax5-/+* mice which, were susceptible to LCMV infection resulting in shorter long-term survival compared to infected WT mice, had a specifically distinct gut microbiota compared to their infected and uninfected WT counterparts. *Pax5-/+* mice did not survive past day day 356 post-infection (observation time 500 days) and many of the mice that did not survive had detectable viral titres in their organs pointing to an inability to clear the virus.

Conclusion/Summary:

This study demonstrates that the BME environment, microbiome as well as survival of *Pax5-/+* mice is differently affected by LCMV infection supporting the observations that Pax5 heterozygosity shapes distinct long-term responses to chronic infection.

P 28 - Subcutaneous panniculitis-like T-cell lymphoma (SPTCL): A case report

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Subcutaneous panniculitis T-cell lymphoma (SPTCL) is a rare, cytotoxic T-cell lymphoma. It usually presents as erythematous, painless, multiple nodules of variable size, frequently located on the trunk and extremities. 50% present with systemic symptoms such as fever or general malaise, or with cytopenias.15–20% of cases present with haemophagocytic syndrome. There is no standard treatment regimen. In the past, treatment used to be multidrug chemotherapy. However, some articles suggest that immunosuppressive treatment could be effective.

We present the case of a 13-year-old girl, who came to the emergency department with a 3-week history of fever.

Physical examination revealed 12 palpable, indurated nodules of „recent onset", randomly distributed over the trunk and limbs. Basic laboratory tests (haemogram, ionogram, C reactive protein, clotting, and urinalysis) and chest X-ray were normal.

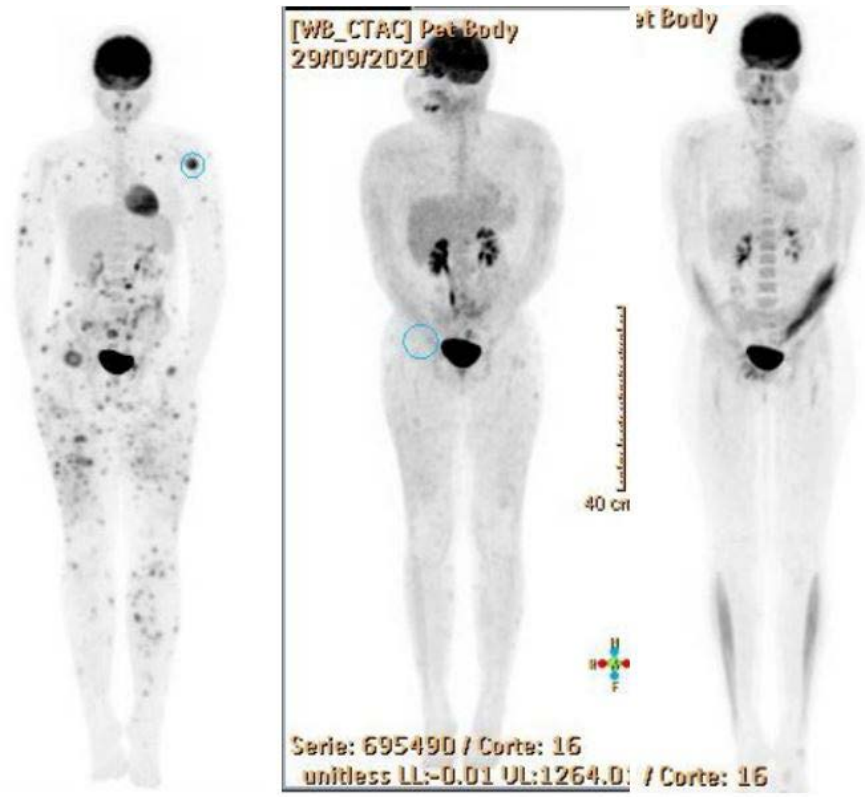
The initial differential diagnosis included septic nodules due to endocarditis, chronic gonococcaemia or erythema nodosum, all of which were ruled out.

An initial 4-mm punch biopsy was performed, which appeared not representative of the lesion and, therefore, an excisional biopsy of an inguinal subcutaneous nodule was performed. This biopsy demonstrated a moderate lymphocytic panniculitis with a lobular pattern with adipocytic rimming associated with a perivascular and periannexal dermal infiltrate and a lichenoid pattern. The lymphocytic infiltrate corresponded to T lymphocytes, predominantly CD8+, with expression of cytotoxic markers TIA-1 and Granzyme-B, without expression of CD56 or CD30. The Ki67 proliferation index was 40%. There were associated histiocytes, foamy macrophages, scattered plasma cells, and nuclear debris. The histopathologic findings favoured the possibility of a **subcutaneous panniculitis-like T-cell lymphoma**. Molecular studies demonstrated an alpha-beta phenotype. T-cell Receptor beta was performed and showed 2 reproducible clonal peaks, and the diagnosis was that of **subcutaneous panniculitis-like T-cell lymphoma**.

An extension study was performed: bone marrow (normal, without haemophagocytosis) and 18F-FDG PET-CT scan, which showed multiple subcutaneous hypermetabolic lesions, bilateral iliac and right inguinal hypermetabolic lymphadenopathies, mesenteric and retroperitoneal abdominal affected areas (see figure 1).

Figure 1:

a) PET-CT at diagnosis. Multiple lesions.
b) PET-CT scan one month after treatment. Signs of complete metabolic response (DS3- qPET in most active lesion 0.91). Persistence of subcutaneous lesions with minimal metabolic activity predominantly in the posterior thigh and gluteal region.
c) PET-CT at 7 months. No lesions were observed.



Oral methotrexate 20 mg/m2/week and prednisone 1 mg/kg/day were started. A complete metabolic response was seen one month later.

The prednisone dose was progressively reduced until it was withdrawn after 6 months of treatment and methotrexate was maintained for 2 years.

The patient is currently 6 months out of treatment, with no signs of recurrence.

Discussion:

PSCTL is a real challenge, often requiring multiple biopsies, with diagnostic delays of months or years. The cells have a mature alpha beta T-cell phenotype, usually CD8-positive, with expression of cytotoxic molecules. The neoplastic cells show rearrangement of TR genes and are negative for EBV sequences. No specific cytogenetic features or mutation patters have been reported.

The prognosis is favourable, with overall survival above 80%. However, if haemophagocytic syndrome is present, the prognosis worsens.

Literature shows that immunosuppressive treatment with oral steroids alone or in combination with low-dose methotrexate can be considered for first-line treatment. We describe our experience in this rare entity, allowing patient to maintain a good quality of life. The appropriate duration of treatment remains to be determined.

P 29 - Relapsed anaplastic large-cell lymphoma treated with Vinblastine monotherapy: A series of two cases

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Background:

Anaplastic large-cell lymphoma (ALCL) is a rare disease in children. Approximately 25–35% of patients progress during or after front-lines chemotherapy. There is no consensus regarding prognostic factors that determine the optimal treatment in relapses: treatment can be as different as autologous or allogeneic hematopoietic stem-cell transplantation (HSCT), ALK inhibitors or vinblastine monotherapy. The French Society of Pediatric Oncology showed that some of ALCL high-risk patients could be rescued with prolonged weekly vinblastine therapy. We present our experience with two relapsed ALCL cases achieving remission with vinblastine.

Objectives/Methods:

We present a clinical case review of two pediatric patients with relapsed ALCL treated with vinblastine monotherapy in our center.

Results:

Eligible two patients received first-line chemotherapy based on the protocol used in the International ALCL99 Trial. Treatment relapse with vinblastine (6 mg/m2) weekly for 12 months and every two weeks for 12 more months.

The first case was an 11 year-old boy diagnosed with an early relapse (11 months from the diagnosis) with multiple lymphadenopathies. Bone marrow aspirate was negative. Tumoral cells were ALK and CD3 positive. He did not have HLA-identical related donor. He received weekly vinblastine until an early evaluation was done with PET-TC. It was demonstrated morphological and metabolic remission, so weekly VBL was continued during the first 12 months and thereafter biweekly utility completing two years of treatment. After a follow-up for 9 years, he remains in remission.

The second patient, a 13 years old boy, was diagnosed of a late relapse (12 months from the diagnosis) with multiple infradiaphragmatic lymphadenopathies. Bone marrow infiltration was detected by molecular study (NPM-ALK positive). Tumoral cells were CD3 and ALK positive. He did not have HLA-identical related donor. He received weekly vinblastine until an early evaluation was done. A PET-TC demonstrated metabolic and morphological remission and the bone marrow aspirate was negative. We went on with weekly VBL during 5 months until the present time with the aim of completing 12 months and the biweekly during 12 more months. Nowadays he is still in remission.

Both patients received outpatient treatment and had no major adverse effects.

Conclusion:

Based on bibliography, 5 years event-free survival of relapsed ALCL treated with VBL monotherapy is around 30%.

Based on our experience vinblastine monotherapy could be efficient in some high-risk relapsed ALCL and may produce durable remissions. It is an outpatient treatment, well tolerated and with few adverse effects. Those patients without HLA-identical related donors could benefit from trying to be treated with weekly vinblastine, assessing early response after 6 weeks.

Our conclusions are the result of an observational series of two cases. It still remains unknown whether this treatment will be a cure or it only postpones relapses.

P 30 - Loss of 17p (TP53) and CASP8AP2-BACH2 at diagnosis are associated with poor outcome in pediatric B-other Acute Lymphoblastic Leukemia

Natacha Azussa Migita[1,2], Patricia Yoshioka Jotta[3], Natália Paiva do Nascimento[3], Danielle Ribeiro Lucon[4], Felipe Luz Torres Silva[2,5], Mariana Camargo Maschietto[5], Amilcar Cardoso de Azevedo[6], Silvia Regina Brandalise[6], José Andrés Yunes[1,3]

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Acute lymphoblastic leukemia of B-cell precursor origin (BCP-ALL) has been traditionally classified into six molecular entities defined by high-hyperdiploidy, hypodiploidy, t(12;21), t(9;22), t(1;19), and 11q23 (KMT2A) alterations. Cases (~30%) without such features were classified as B-other ALL. Today, some B-other cases can be classified into new genetic subgroups (iAMP21, CRLF2-high, ABL-class fusions, JAK2-, EPOR-, DUX4-, PAX5-, ZNF384-, MEF2D-, or NUTM1-rearranged). Some of these genetic lesions are of prognostic significance and/or targets for novel therapeutic interventions. Still, almost half of B-other do not have any of these recurrent genetic alterations. In this work, we analyzed copy-number alterations (CNAs) in key leukemia-related genes by NGS-based digital MLPA on a series of 136 cases of pediatric B-other ALL at diagnosis. Besides associations previously described, as the ERGdel in DUX4-r or IKZF1del in Ph-like ALL, we found some interesting ones: RUNX1del in MEF2D-r, ETV6del in iAMP21, VPREB1del in JAK-STAT-activating alterations (ABL/JAK2/EPOR-fusions and CRLF2 high), EBF1del and PAX5del in CRLF2 high. Loss of 17q11.2 (NF1, SUZ12) was frequent in ZNF384-r, loss of 7q34–36 (EPHA1, EZH2) in PAX5-r, and loss of 3q13.2 (CD200, BTLA) in iAMP21 and JAK-STAT-activating alterations. Importantly, the UKALL-CNA classifier held its prognostic value in B-other ALL, defining groups with leukemia free survival (LFS) rates of 90%, 78%, and 60%. ZNF384-r tended to group into UKALL-CNA-GR (Good Risk), PAX5-r and CRLF2-high into UKALL-CNA-IR (Intermediate Risk) and ABL/JAK2/EPOR-fusions into UKALL-CNA-PR (Poor Risk). Deletion of 17p (TP53) and CASP8AP2-BACH2, two pro-apoptotic genes, occurred in 7 cases (4 and 3 cases, respectively) which showed a dismal outcome (30% LFS).

P 31 - Early subclinical CNS manifestation in B-cell acute lymphoblastic leukemia modelled in vivo

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Involvement of the central nervous system (CNS) is a major clinical concern in acute lymphoblastic leukemia (ALL). By routine cytomorphology of the cerebrospinal fluid (CSF), CNS-ALL is only diagnosed in few patients. However, for all patients CNS-directed treatment is indispensable for relapse-free survival, indicating subclinical CNS manifestation in most cases. CNS-ALL is a leptomeningeal disease and several factors contributing to ALL manifestation within the CNS have been described, however the precise mechanism of leukemia cell entry remains unclear.

Identification of novel CNS-directed treatment strategies is of major interest to improve patient outcome. Patient-derived xenograft (PDX) mouse models, in which PDX-ALL cells are transplanted onto immunodeficient recipients, allow to study leukemia biology and preclinical evaluation of novel therapies. PDX-models recapitulate the human disease with ALL manifestation in different organ compartments including the CNS, though, precise dynamics of leukemia engraftment are unknown.

Hence, we addressed engraftment phenotypes of PDX-ALL samples (n = 3) intravenously transplanted onto NOD/SCID mice (n = 45 per group) and characterized leukemia manifestation in different organ compartments over time employing different methods of ALL detection at distinct sensitivity levels. At serial timepoints after transplant, ALL manifestation was assessed in peripheral blood (PB), bone marrow (BM), spleen, and leptomeninges in a subset of mice using flowcytometry (detection of huCD19-positive cells) or quantification of PCR-based minimal residual disease (MRD). For selected recipients, flowcytometry of sampled CSF or cranial magnetic resonance imaging (MRI) were performed.

Using flowcytometry, in all three cases leukemia infiltration was first detected in BM and last in PB. Interestingly, leukemia appearance was detected in the CNS at the same time as in the spleen. Importantly, equivalent leukemia load was found in the CNS analyzing either leptomeningeal manifestation or CSF sampled at the same time, indicating that leptomeningeal leukemia infiltration is precisely reflected by proportions of ALL cells drifting in the CSF.

We also performed cranial MRI at consecutive timepoints showing clearly enlarged meninges at later timepoints corresponding to the macroscopic presentation upon autopsy. Interestingly, first defined changes with circumscribed meningeal infiltrations were detectable earlier, however one or two weeks after flowcytometric detection of CNS involvement.

Moreover, we analyzed MRD levels in the different organ compartments over time by PCR-based detection of three patient-specific Immunoglobulin- (Ig-)rearrangements in one case, a T-cell receptor- (TCR-)rearrangement in the other and a patient-specific *KMT2A*-rearrangement in the third case. Parallel analysis of the three Ig-rearrangements in one leukemia showed almost identical levels, indicating reliable MRD quantification in our model system. In line with our data by flowcytometry, ALL was first detected in the BM, however already early on after one to three weeks post-transplantation and one or two weeks earlier than by flowcytometry, reflecting the higher sensitivity of this method. Most interestingly, in all three leukemias first involvement of the CNS with positive MRD markers was detected early on, in some mice even along with positivity in the BM and earlier and/or at higher levels as compared to first infiltration in spleen.

Taken together, within the NOD/SCID/huALL PDX transplantation model we identified along with BM manifestation very early invasion of ALL cells into the CNS before spleen engraftment indicating a high and early propensity of ALL cells to transmigrate via the blood-CSF barrier into the meninges. These findings contribute to the understanding of ALL biology required for faithful disease modeling including pre-clinical evaluation of directed therapeutic approaches.

P 32 - IGH::IGF2BP1 – A rare but recurrent IGH translocation associated with Down syndrome and ETV6::RUNX1-like B-ALL

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Chromosomal translocations of the immunoglobulin heavy chain locus (*IGH*) are generally a hallmark of mature B-cell malignancies, represent important drivers of pathogenesis and serve as diagnostic and prognostic markers. Due to the nature of *IGH* translocations, which result in activation of proto-oncogenes by their juxtaposition to *IGH* regulatory elements, the identification and characterization of the partner genes is challenging. In childhood B-cell acute lymphoblastic leukemia (B-ALL), *IGH* translocations have been identified in about 5% of the cases and in approximately 16% of Down syndrome (DS) associated B-ALL. However, considering the cryptic *IGH::DUX4* rearrangements, the incidence of *IGH* translocation might be significantly higher and account for up-to 10–15% of childhood B-ALL cases. Over the last decades a multitude of *IGH* translocation partner genes has been identified, the most frequently recurring ones being *DUX4, CRLF2, EPOR*, and *ID4*. The t(14;17)(q32;q21)/*IGH::IGF2BP1* rearrangement is very rare and has so far only been described in a few patients.

We present three B-ALL patients with an *IGH::IGF2BP1* translocation, two males (2.4 and 18.1 years old; patients 1 and 2, respectively) and one female (8.7 years; patient 3), two of whom (patients 2 and 3) were DS-ALL. Patient 1 was stratified into the medium risk-group of the ALL-BFM 2000 clinical trial and patient 3 into the standard risk-group of the ALL-BFM 2009 trial and both remain in complete remission for 17.9 and 6.1 years, respectively. The 18.1-years old patient 2 died shortly after diagnosis while still on induction treatment.

Fluorescence in situ hybridization (FISH) analysis using an *IGH* break apart probe indicated a translocation in all three patients. Karyotyping was normal, except for the constitutional trisomy 21 in the two patients with DS B-ALL. In all three patients, SNP-array analysis revealed a (17)(q21.32q25.3) duplication with breakpoints upstream of the *IGF2BP1* gene associated with an unbalanced t(14;17)(q32;q21)/*IGH::IGF2BP1* translocation or an additional derivative chromosome 14. In addition, all three cases showed gain of chromosome 21, either present in the germline (+21c) or only in the blast cells, and gain of chromosome X (in one case partial Xp and Xq duplications) as well as deletions of the histone genes (H1 cluster) at chromosome 6p22.2. Further recurrent deletions, each present in two of the three cases, involved *PAX5, ETV6* and *STAG2*.

In two cases, which were subjected to whole transcriptome sequencing (RNA-seq), a set of fusion detection tools failed to clearly identify the *IGH::IGF2BP1* translocation. However, the samples showed high expression of *IGF2BP1* and the rearrangement could be confirmed by manual curation. IGF2BP1 (Insulin Like Growth Factor 2 mRNA Binding Protein 1), belongs to a family of regulatory RNA-binding proteins involved in localization, stability and translational control of their target genes. In B-ALL, *IGF2BP1* has been shown to be overexpressed in *ETV6::RUNX1* leukemia. Notably, unsupervised hierarchical clustering revealed co-clustering of the *IGH::IGF2BP1* cases with *ETV6::RUNX1* leukemia samples suggesting an *ETV6::RUNX1*-like phenotype.

In conclusion, we report three patients with a rare t(14;17)/*IGH::IGF2BP1* translocation, associated with copy number alterations at chromosome 17q21.32 upstream of *IGF2BP1* detectable by SNP-array analysis. The *IGH::IGF2BP1* fusion appears to result in *IGF2BP1* overexpression and to represent a recurrent translocation in *ETV6::RUNX1*-like B-ALL.

To further evaluate the functional role of *IGH::IGF2BP1* and the clinical significance of this rare B-ALL subtype, improved detection and characterization of additional cases is needed.

P 33 - Synergistic anti-leukemia activity of the dual mTOR/PI3K inhibitor NVP-BEZ235 combined with the BCL-2 inhibitor venetoclax

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Survival rates of over 90% have been reached in patients suffering from B-cell precursor acute lymphoblastic leukemia (BCP-ALL) due to intensive treatment regimens that induce cell death of leukemic blasts. Although treatment options have continuously improved over the last decades, deregulation of cell death pathways can lead to treatment failure.

Previously we showed that deficient apoptosis signaling is an indicator for poor outcome in ALL and is associated with inferior relapse-free patient survival. Programmed cell death induction is regulated by pro- (e.g. BIM, BAX) and anti-apoptotic molecules (e.g. BCL-2, MCL-1). Targeting anti-apoptotic regulators such as selective inhibition of BCL-2 by venetoclax showed clinical activity in chronic lymphocytic leukemia (CLL) and promising results in preclinical and first clinical studies in ALL. On the other hand, cellular proliferation is controlled by different pathways with mTOR signaling being a central regulatory switch. Previously, we also found that hyperactivation of the mammalian target of rapamycin (mTOR) signaling pathway in ALL cells is a feature of aggressive leukemia disease and associated with inferior relapse-free survival.

Here, we evaluated anti-leukemia activities of BCL-2 (venetoclax) and mTOR/PI3K pathway (NVP-BEZ-235) inhibition in BCP-ALL analyzing the interplay of both inhibitors.

Half maximal effective concentrations (EC_{50}) were assessed in BCP-ALL cell lines and patient-derived xenograft (PDX) samples. mRNA expression was assessed by qPCR and protein expression was assessed by immunoblotting. Cell cycle analysis was performed using a Nicoletti staining protocol. Synergies were analyzed in dose-response matrices (Bliss synergy model).

Both, cell lines and PDX samples showed heterogeneous responses with varying EC_{50} values when treated with venetoclax. Interestingly, we identified high expression of anti-apoptotic MCL-1 in venetoclax insensitive ALL cell lines and PDX samples pointing to MCL-1 as a mediator of venetoclax insensitivity. The dual mTOR/PI3K inhibitor NVP-BEZ235 inhibited proliferation in BCP-ALL cell lines concomitant with an arrest in G_0/G_1 cell cycle phases. NVP-BEZ235 induced cell death measured via FSC/SSC criteria by flow cytometry only in a small fraction of tested samples. Exposure of ALL cells to NVP-BEZ235 also resulted in significantly decreased phosphorylation of the downstream targets S6 and 4E-BP1 in a dose-dependent manner. We could also observe a significant dose-dependent upregulation of pro-apoptotic PUMA transcript levels as well as dose-dependent and significantly downregulated MCL-1 protein expression. 4E-BP1 has been described as a translational regulator of MCL-1 and mTOR inhibition associated MCL-1 downregulation might prime venetoclax insensitive ALL to cell death induction by BCL-2 inhibition. Interestingly, co-treatment with venetoclax and NVP-BEZ-235 synergistically reduced cellular proliferation and induced cell death (Bliss scores > 40) in cell line and PDX-ALL samples, most importantly including apoptosis deficient, venetoclax-insensitive, poor outcome ALL.

Taken together, we show that simultaneous PI3K/mTOR and BCL-2 inhibition leads to synergistic anti-leukemia activity, priming apoptosis deficient, venetoclax insensitive BCP-ALL samples to synergistic cell death induction along with downregulation of anti-apoptotic MCL-1. Thus, combining therapeutic strategies targeting mTOR/PI3K signaling and apoptosis induction seems to be an effective strategy in ALL, warranting future clinical evaluation.

P 34 - Venetoclax insensitivity in B-cell precursor acute lymphoblastic leukemia is characterized by increased mitochondrial metabolism and can be overcome by co-targeting oxidative phosphorylation

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Deregulation of cell death is a hallmark of many cancers including leukemia and contributes to leukemogenesis and treatment failure in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Programmed cell death (apoptosis) is controlled by different pro- and anti-apoptotic proteins either inducing or counteracting cell death induction. The small molecule inhibitor venetoclax (VEN) binds selectively to anti-apoptotic BCL-2 resulting in apoptosis induction. VEN has shown clinical activity in other lymphoid malignancies. In BCP-ALL, we and others have shown preclinical activity and VEN is evaluated in first clinical trials. However, insensitivity and development of resistance have been described previously, potentially hampering effectiveness of VEN treatment.

In this study, we have modeled and addressed VEN resistance in BCP-ALL cell lines and patient-derived xenograft (PDX) samples and investigated underlying mechanisms in order to identify potential strategies to overcome VEN insensitivity.

Starting from the BCP-ALL cell line RS4;11, five VEN-insensitive lines (VEN^{ins}) were generated by parallel exposure to increasing concentrations of VEN over time (49 passages with continuous treatment) and five control lines (VEN^{sens}) were exposed to corresponding concentrations of solvent. Measuring half maximal effective concentrations (EC_{50}) showed increasing EC_{50} values from 4 nM to 26.2 µM over time in all VEN^{ins} lines, indicating acquired insensitivity in our model. However, VEN^{ins} lines displayed unchanged proliferation and same sensitivities to the prototypic apoptosis inducer staurosporine, induction therapy drugs vincristine/dexamethasone/ asparaginase or daunorubicin as compared to controls, indicating acquisition of VEN specific insensitivity.

To further characterize the mechanism of VEN insensitivity, we performed RNA-*Seq* analysis comparing VEN^{ins} to VEN^{sens} lines. Interestingly, gene set enrichment analysis on significantly regulated genes showed a significant upregulation of gene sets annotated to the citric/tricarboxylic acid cycle and the respiratory electron transport chain, pointing to increased mitochondrial metabolism in VEN^{ins} cells.

We carried out metabolic profiling (Agilent Seahorse XF Cell Mito Stress Test) of VEN^{ins} and VEN^{sens} lines (N = 5+5). Most interestingly, in contrast to significant reduction of oxygen consumption rates (OCR) in VEN^{sens} lines, we found sustained high mitochondrial metabolism indicated by persistent high OCR in VEN^{ins} lines.

To further validate our findings, we investigated a series of BCP-ALL PDX samples (N = 30) identifiying heterogeneous VEN sensitivities. Metabolic profiling was performed on sensitive as compared to highly insensitive leukemias (N = 3+3). In line with our previous findings, VEN^{ins} PDX samples showed higher OCR and ATP production rates, further highlighting that increased mitochondrial activity is a characteristic feature of VEN-insensitive ALL.

Along this line, significant higher mitochondrial DNA content was found in VEN^{ins} PDX leukemias. Although numbers of mitochondria did not differ, the overall mitochondrial area and structure was found to be significantly larger and elongated as analyzed by electron microscopy, further corroborating our finding of augmented mitochondrial metabolism in VEN^{ins} ALL. Additionally, fission factor DRP-1 was found to be less expressed in VEN-insensitive PDX samples.

Finally, we addressed whether insensitivity to VEN can be overcome by targeting oxidative phosphorylation (OxPhos). We found synergistic induction of apoptosis in BCP-ALL cell lines and PDX samples using oligomycin, an inhibitor of the complex V/ATPase subunit, in combination with VEN. This shows that VEN insensitivity can be overcome by co-targeting the OxPhos pathway.

Taken together, we show that VEN insensitivity in BCP-ALL is characterized by high mitochondrial metabolic activity. Co-targeting of BCL-2 and OxPhos results in synergistic anti-leukemia activity setting the basis for further preclinical evaluation and potential clinical application.

P 35 - Phase 1b trial of subcutaneous epcoritamab in pediatric patients with relapsed or refractory (R/R) Aggressive mature B-cell neoplasms (EPCORE peds-1)

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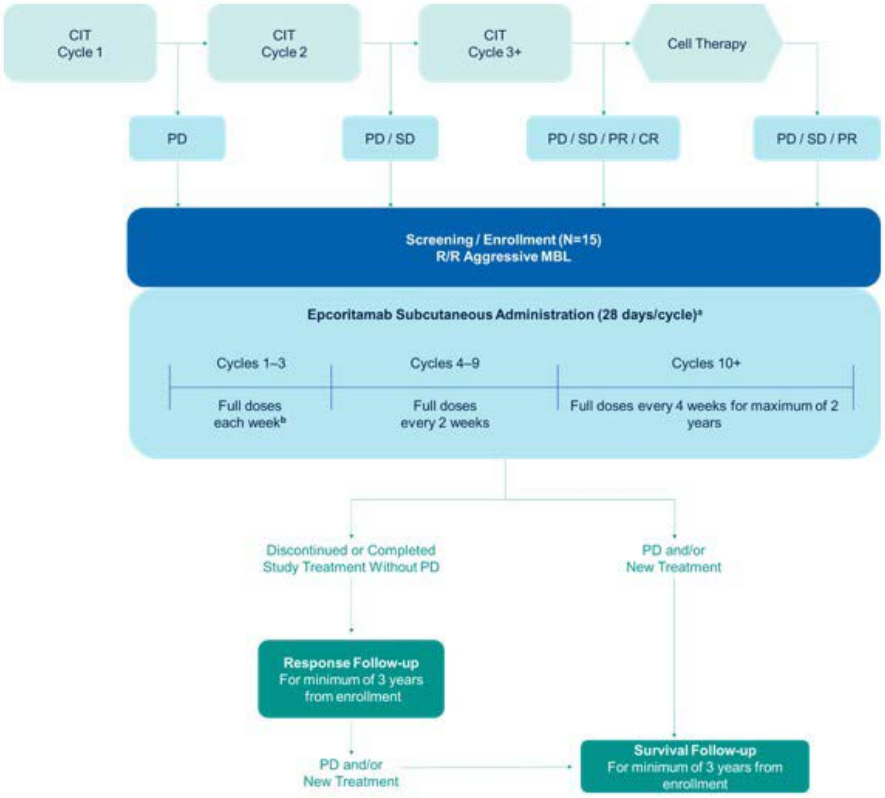
Background:

Burkitt lymphoma and diffuse large B-cell lymphoma (DLBCL) are the most common types of mature B-cell lymphoma (MBL) in children. A majority (90–95%) of children with these malignancies are cured with initial chemoimmunotherapy (CIT), leaving a small portion of patients with relapsed or refractory (R/R) disease who have a poor prognosis. R/R MBL is often treated with aggressive CIT regimens such as R-VICI or R-ICE, but a high, unmet need remains. Long-term survival is observed in ~20–30% of pediatric cases of R/R MBL, with better outcomes in patients who attain complete response (CR) with reinduction CIT and reach consolidation with stem cell transplantation (SCT) than those achieving partial response (PR) with or without SCT. Epcoritamab is a bispecific antibody that binds CD3 on T cells and CD20 on B cells, inducing potent and selective T-cell–mediated killing of malignant $CD20^+$ B cells. The phase 1/2 EPCORE NHL-1 study (NCT03625037) of epcoritamab monotherapy in adult patients with R/R B-cell non-Hodgkin lymphoma demonstrated safety and promising efficacy at the recommended phase 2 dose (RP2D), including deep and durable responses (overall response rate [ORR], 63%; CR rate, 39%; preliminary median duration of response [DOR], 12 months) in patients with highly refractory large B-cell lymphoma (n = 157) (Thieblemont et al, *J Clin Oncol* 2022). Because favorable safety and efficacy data were observed in adults, it is reasonable to investigate epcoritamab in children with R/R MBL who fail to achieve CR with reinduction CIT or are unable to receive further consolidation with SCT after CIT.

Methods:

EPCORE Peds-1 is a single-arm, open-label, phase 1b study (NCT05206357) evaluating the safety, pharmacokinetics, pharmacodynamics, preliminary efficacy, and immunogenicity of epcoritamab monotherapy in pediatric and young adult patients with R/R Burkitt lymphoma, Burkitt-like lymphoma/leukemia, DLBCL, or other aggressive $CD20^+$ MBL. Primary endpoints are safety/tolerability (including incidence and severity of cytokine release syndrome, immune cell–associated neurotoxicity syndrome, and clinical tumor lysis syndrome) and pharmacokinetic parameters. Secondary endpoints include CR per International Pediatric Non-Hodgkin Lymphoma response criteria, event-free survival, overall survival, initiation of SCT/chimeric antigen receptor T-cell therapy, ORR, DOR, duration of CR, and immunogenicity. At least 15 patients ≤25 years will be enrolled, with at least 12 patients ≥1–18 years who are evaluable for primary endpoint assessment. Eligible patients must not have reached remission with reinduction CIT or are ineligible for further consolidation with cell therapy and have acceptable performance status (Lansky, Karnofsky, or Eastern Cooperative Oncology Group, depending on age). Epcoritamab will be subcutaneously administered using step-up dosing (priming and intermediate doses on days 1 and 8 of cycle 1 [28 days/cycle], followed by 10 weekly full doses from day 15 of cycle 1 through cycle 3; Figure). Additional full doses will be administered every 2 weeks for cycles 4–9 and every 4 weeks for cycles 10 and beyond for a maximum of 2 years of treatment. Epcoritamab priming-, intermediate-, and full-dose regimens will be based on weight categories; patients weighing ≥40 kg will receive the adult RP2D. Disease status will be assessed by CT/MRI/PET prior to epcoritamab administration on day 1 and at a minimum at weeks 6, 12, 24, 36, and 48 and at 18 and 24 months. Patients will be followed for ≥3 years after enrollment. Enrollment is ongoing in North America, Asia, Europe, and Australia.

Figure: Study design.



[a]Disease status will be assessed by CT/MRI/PET prior to epcoritamab administration on day 1 and at a minimum at weeks 6, 12, 24, 36, and 48 and at months 18 and 24.
[b]Epcoritamab will be subcutaneously administered using step-up dosing (priming and intermediate doses on days 1 and 8 of cycle 1, followed by 10 weekly full doses from day 15 of cycle 1 through cycle 3). Dose regimens will be based on weight categories; patients weighing ≥40 kg will receive the adult RP2D.
CIT is second line or greater standard of care. Participants may receive cell therapy before receiving epcoritamab but not concomitantly.
CIT, chemoimmunotherapy; CR, complete response; MBL, mature B-cell lymphoma; PD, progressive disease; PR, partial response; R/R, relapsed/refractory; SD, stable disease.

P 36 - Modelling CREBBP or EP300 loss to identify new therapeutic vulnerabilities in BCP-ALL

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Background:

Epigenetic alterations play a substantial role in the pathogenesis of acute lymphoblastic leukemia (ALL), and mutations in the epigenetic modifiers have long-term prognostic implications for ALL patients. As such, the loss-of-function mutations in the CREB-binding protein CREBBP (or CBP) are detected in around 18 % of relapsed ALL and up to 30 % of lymphoma cases.[1,2] In general, CBP is involved in the regulation of key cellular processes by stabilizing protein-protein interactions and mediating catalytic acetyltransferase activities. The functional impairment of CBP has been shown to influence treatment response by implicating drug resistances.[1] Interestingly, EP300, the paralogous HAT-acetyltransferase displays similar functions to CBP, but is less frequently mutated in hematological malignancies. Studies demonstrated that the ablation of EP300 in CBP-deficient cells lead to synthetic lethality.[3] Thus, making EP300 an interesting target to overcome drug resistance caused by CBP mutant cancer types. However, due to the homology of CBP and EP300, it is difficult to study their function independently and to this day, there are no selective inhibitors available.[4]

Aim:

We aim to decipher the specific roles of CBP and EP300 in the pathogenesis of B-cell precursor ALL (BCP-ALL) by developing genetically edited models, in order to identify novel therapeutic vulnerabilities upon their ablation.

Methods:

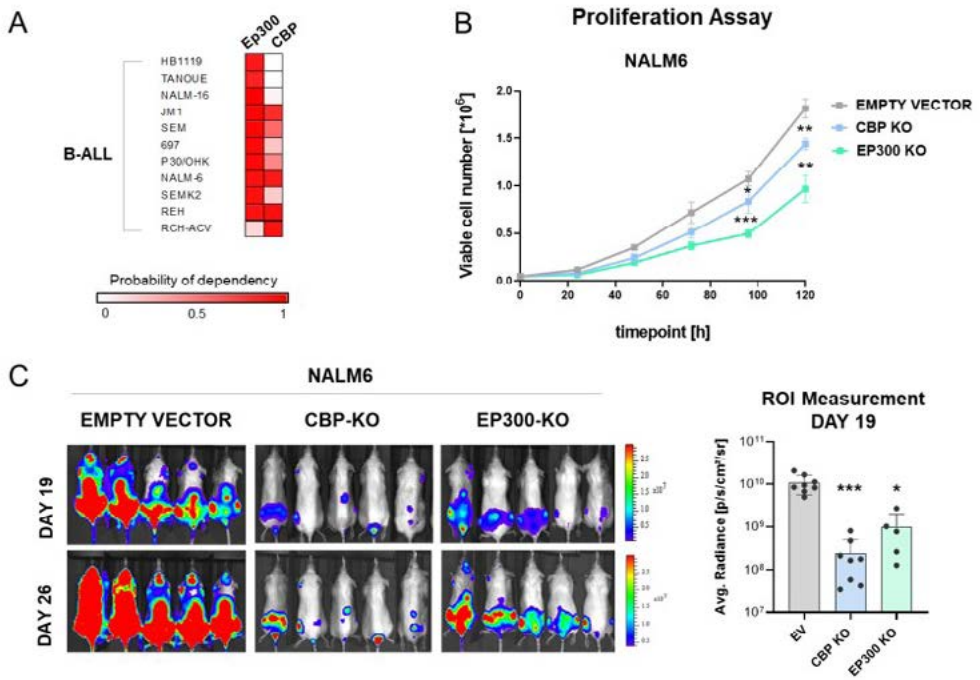
CRISPR-Cas9 mediated CBP and EP300 KO models were generated in two BCP-ALL cell lines (NALM6, REH). The KO cells were analysed in *ex vivo* functional assays (proliferation assay, colony formation assay and western blotting) and their transplantation efficiency was tested in immunodeficient NSG mice models. Additionally, a high throughput drug screening (HTDS) approach was employed, involving conventional chemotherapeutics and targeted inhibitors (n = 191).

Results:

Using data mining, we found that B-ALL cell lines exhibit an overall increased dependency on EP300 in comparison to CBP (Figure A, Depmap database 22Q1). CBP and EP300-KO cells have shown reduction in the *ex vivo* proliferation and colony-forming abilities (Figure B). In agreement, lower engraftment of CBP-KO and EP300-KO cells was noticed upon their transplantation into NSG mice, in comparison to the control group (CBP KO p = 0.0008; EP300 KO p = 0.0122) (Figure C). Interestingly upon HTDS, CBP-deficient cells displayed resistance toward HSP90-, BCL2- (Ventoclax), CDK-, PI3K/mTOR-inhibitors. In contrast, EP300-deficient cells did not display resistance toward any tested drugs, contrarily they demonstrated higher sensitivity to HDAC- and HSP90-inhibitors than the respective controls. In addition, EP300-KO cells displayed higher sensitive towards CBP/EP300 inhibitors (GNE-781. SGC-CBP30, A485) as well as PROTACS (dCBP-1, JQAD1) in comparison to the CBP-KO cells.

Conclusion:

Our results indicate that targeting EP300 in CREBBP mutated BCP-ALL cases can be a promising therapeutic strategy. Furthermore, a higher sensitivity of the EP300 deficient cells to HDAC- or HSP90-inhibitors warrants further preclinical validation studies.



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P 37 - Deep transfer learning approach for automated cell death classification reveals novel ferroptosis-inducing functions of volasertib in BCP-ALL

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Background:

The characterization of cell death is a crucial part of drug discovery and development in the field of cancer treatment. Correct determination type of cell death is important in linking the primary target of the drug with the effector pathway. Apoptosis, necroptosis, ferroptosis and autophagy are the most commonly regulated types of cell death, which are distinguished in the cell morphology, which can be potentially used for automated classification of cell death.

Deep transfer learning (DTL) is a part of machine learning, using artificial neural networks and is broadly applied in life science. A program built on DTL using off-label images to identify novel modulators of cell death has the potential to significantly accelerate the experimental approach for anti-cancer testing in a cost-effective and rapid manner.

Aims:

We aimed to train a neural network to characterize brightfield images of transformed cells undergoing different modes of cell death and to apply this methodology to uncover novel cell-death inducing functions of pharmacologically used anti-cancer therapeutics.

Methods:

Brightfield microscopy images of L929 tumor cells undergoing different modes of cell death (apoptosis, necroptosis, autophagy and ferroptosis) were taken every hour using the Incucyte live cell imager and classified using DTL with the Resnet50 as backbone model and finetune by retraining its last three layers. The resulting network was combined with high-throughput pharmacological screening approaches to identify novel cell death-inducing functions of anti-cancer therapeutics which were further validated *in vitro* in human 697, Nalm6, Halo1 and REH BCP-ALL cell lines and *in vivo* using the C1498 murine myeloid leukemia cell line.

Results:

Using this approach we identified several ferroptosis and autophagy inducing clinically relevant therapies which we proceeded to validate *in vitro* and *in vivo.* We discovered that the polo-like Kinase Inhibitor volasertib induced ferroptosis in the pharmacological screen assessed using DTL. As recent reports have suggested hematological malignancies to be sensitive to ferroptosis induction, we proceeded to validate volasertib further. Subsets of precursor B acute lymphoblastic leukemia (BCP-ALL) cell lines (697, Nalm6, Halo1 and REH) and 2 ETV6/RUNX1-positive primary patient samples, where ferroptosis genes were downregulated relative to insensitive cells B-ALL were sensitive to ferroptosis induction by volasertib. The phenotype was recapitulated with the ferroptosis-inducer RSL3 and reversed by ferrostatin-1 (Fer-1). Using intravenously injected leukemia cells, we determined that volasertib induced ferroptosis *in vivo* and importantly upregulated the immunogenic tumor ferroptosis mediator Acyl-CoA synthetase long-chain family member 4 (ACSL4) 16 days post tumor inoculation.

Conclusion / Summary:

Using DTL approaches we uncovered that volasertib has the ability to induce ferroptosis in subsets of human BCP-ALL cell lines and patient samples. Our methodology has the potential to significantly accelerate anti-cancer testing in a cost-effective and rapid manner and to identify previously unidentified functions of anti-cancer therapeutics.

P 38 - Ex vivo drug response profiling of pediatric ALAL

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A rare but significant and heterogeneous subset of acute leukemia (AL) constitutes AL of ambiguous lineage (ALAL). Despite recent recommendations that favor ALL type of therapy in most ALAL patients, there is still an uncertainty whether subtypes of ALAL exist that would benefit from AML drugs. The iBFM AMBI2018 Registry continues on recruiting patients' data, aiming at reappraisal of the treatment chosen. In addition, we established a drug response profiling (DRP) pipeline that can evaluate the ex vivo response to ALL- and AML-specific drugs side by side. We put together a panel of drugs used in the therapy of childhood acute leukemias. Particularly, the panel encompasses ALL-directed compounds (e.g., prednisone, vincristine and doxorubicin), AML- directed compounds (e.g., idarubicin) and compounds used in both treatment types or off protocol as a rescue (e.g., etoposide, cytarabine, venetoclax or nilotinib). Guided by a publication of Frismantas et al (1), we optimized a co-culture of diagnostic blasts and feeder mesenchymal stromal cells. This co-culture is incubated with varying concentrations of each compound followed by cell viability evaluation by high-content microscopy coupled with automated image analysis. Preliminary results on 35 patients (17 ALL, 8 AML, 10 ALAL at the time of the abstract submission) show variable response profiles. Bearing in mind the results are too premature to be evaluated statistically, there seems to be an AML>T ALL>B ALL gradient (resistant to sensitive) to ALL-directed drugs, AML = T ALL>B ALL gradient in in the group of AML-directed or combined AL drugs, AML>B ALL>T ALL gradient in a heterogeneous group of drugs often used in rescue treatment (e.g., fludarabin, busulphan or various tyrosine kinase inhibitors). Among the few ALAL patients so far analyzed, two patterns of DRP profiles were observed: a more resistant profile (included all 5 T/My patients and a B/T patient) and a less resistant profile (included all 4 B/My patients). The most profound differences between the two profiles corresponded to level of sensitivity to etoposide, clofarabine, fludarabine, daunomycin, doxorubicin and L-asparaginase. At this stage of investigation, the results are not yet sufficient to indicate promising drugs for the T/My subset of ALAL. We will continue investigating the DRP in ALALs and compare the results with other biological and clinical data, aiming at finding optimal treatment in ALAL subsets.

(1) Blood 2017 Mar 16;129(11):e26-e37

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P 39 - Deciphering the underlying molecular complexity of the IKZF1plus signature

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Background:

Acute lymphoblastic leukemia (ALL) is the most frequent pediatric cancer. Even though novel therapeutic strategies and stratification led to a ~90% long-term cure, there is still a proportion of patients experiencing relapse or therapy-related toxicities. Recently, the *IKZF1plus* profile, defined as the presence of a deletion in *IKZF1* together with one or more additional deletions in *PAX5* and/or *CDKN2A/B* and/or PAR1 and the absence of a deletion in *ERG*, was characterized as a very poor prognostic marker specifically in MRD-positive patients based on retrospective analyses of the AIEOP-BFM ALL 2000/2009 trials (PMID 29498923). Dependent on MRD at day 33, *IKZF1plus* is used for high-risk stratification in the ongoing AIEOP-BFM ALL 2017 trial.

Objectives:

This retrospective study aimed to decipher the underlying molecular complexity of the *IKZF1plus* profile by reanalyzing patients from the AIEOP-BFM ALL 2000 and 2009 trials with optical genome mapping (OGM), a molecular cytogenetic approach which allows the parallel detection of all kinds of structural variants including copy number variations, translocation as well as aneuploidies.

Methods:

In total, 142 patients with *IKZF1* deletion (73 with IKZF1*del*, 69 with *IKZF1plus* profile) as determined by MLPA in previous analyses were re-analyzed by means of OGM. Based on our findings seven patients were not eligible and eight patients were reclassified from *IKZF1del* to *IKZF1plus* or vice versa (94.1% concordance with MLPA). Results were validated by comparison to previously collected data and in case of new fusions by RT-PCR/RNA-Seq. Next, genetic markers were correlated with patient outcome (5-year event-free survival (EFS), overall survival and cumulative incidence of relapse).

Results:

In 45.9% of the analyzed patients either known prognostic markers (18/135; 13.3% with *ETV6::RUNX1*, high hyperdiploidy or iAMP21) or other gene fusions (44/135; 32.6%) were identified. These fusions were evenly distributed among the subgroups (21 *IKZF1del* and 23 *IKZF1del*) and categorized into: *ABL*-class fusions (12; 8.9%), *PAX5* fusions (9; 6.7%), *JAK2* fusions (10; 7.4%), *ZNF384* fusions (4; 3.0%) and other fusions (9; 6.7%).

Investigating the patient outcome, we reproduced the initial results (PMID 29498923) and show that *IKZF1del* and especially *IKZF1plus* positives did much worse compared to a cohort 845 other patients from the AIEOP-BFM ALL 2000 and 2009 trials without an *IKZF1* deletion (EFS 73.1±5.3 vs 60.6±6.3 vs 87.1±1.2). The dismal outcome was even more pronounced when we excluded patients with an established good prognostic marker from both groups. (EFS *IKZF1del* vs *IKZF1plus*; 68.3±6.2 vs 62.0±6.4). *IKZF1*-deleted patients with a favorable prognostic marker (high hyperdiploidy and *ETV6::RUNX1*) had an EFS of 100% and were exclusively found in the *IKZF1del* but not the *IKZF1plus* subgroup.

Furthermore, *IKZF1del/plus* patients had an inferior 5-year EFS in the presence of a gene fusion (55.9±7.6), especially with *ABL*-class (41.7±14.2), *JAK2* (60.0±15.5) and *PAX5* (50.0±17.7) fusions when compared to the absence of fusions (70.5±5.4). Validation of our results in an independent cohort is ongoing.

Conclusion:

By using OGM, we show that the genomic landscape in ALL is complex and that ~46% of the *IKZF1del/plus* patients carry an established marker or other gene fusions which may be the underlying leukemogenic driver and contribute to an (un-) favorable outcome. Thus, a comprehensive genetic characterization could be valuable to further improve risk-adapted treatment stratification.

P 40 - Intermittent lorlatinib dosing in relapsed/refractory ALCL provides rapid and durable responses. Frequent MRD measurement suggests drug-driven dependency deprivation mechanism

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Background:

About 100 new children pediatric patients present with anaplastic large-cell lymphoma (ALCL) in the US and Europe each year. Approximately 90% harbor ALK gene rearrangements, with NPM1-ALK being the most prevalent. Despite the use of intensive chemotherapy approaches, about 1/3 of patients will experience a relapse of the disease. In relapsed patients, multiple therapeutic strategies can be considered. The frequency of ALK gene rearrangements in ALCL and the success of ALK inhibitors in other cancer types led to early use of TKIs in patients with ALK gene rearrangement positive ALCL (ALK+ ALCL).

Objectives:

Inhibition of ALK gene rearrangements represents a promising therapeutic option for ALK+ ALCL. Intermittent dosing of TKI as means to prevent drug resistance has been previously explored in the past, however different mechanisms of resistance to different TKIs in various malignancies need to be considered.

Design/Methods:

For ALK+ ALCL, upregulated ALK signaling may lead to a resistance to continuous TKI administration. After TKI withdrawal, increased ALK signaling may results in cell death, which should allow for prolonged disease control, compared to continuous dosing. In relapsed/refractory ALCL where the best treatment is not yet defined, we commenced intermittent lorlatinib treatment with frequent MRD monitoring to assess the efficacy of this approach. We present 2 children who relapsed one during and the second one shortly after intensive chemo-immunotherapy as per protocol ANHL12P1 with anti CD30 antibody and both were successfully treated using lorlatinib, intermittent dosing, without stem cell transplant.

Results:

One child had only non-quantifiable positive minimal residual disease (MRD) level at the time of clinical relapse, but frequent MRD monitoring was available for the other patient and revealed that the most significant drop of MRD was repeatedly caused by interruptions of lorlatinib treatment. Intermittent dosing seems to be a very effective approach in ALK fusion-positive ALCL.

Conclusion:

Using this strategy, we were able to achieve 2nd EFS several times longer, then the 1st EFS achieved by intensive chemo-immunotherapy using BFM backbone with anti CD30 antibody. Different mechanisms of resistance to TKI, together with different biomarkers like CD48 and PDGFR expression in relapsed ALCL should be taken into consideration to guide personalized treatment of such patients. /published in JCO Precision oncology, June 2022, https://pubmed.ncbi.nlm.nih.gov/35700412/

P 41 - Combining Pyrimidine de novo synthesis blockade with replication checkpoint inhibitors represents a targetable vulnerability in TP53 aberrant BCP-ALL

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Background:

Unlike most solid tumors, the incidence of TP53 aberrations in ALL is only 3% at diagnosis, however in relapsed ALL this number increases to 12%. Moreover, at relapse, loss of TP53 function is associated with therapy failure, with an overall survival of only 20%. Therefore, alternative therapies for TP53 deleted ALL are much needed. Owing to their high proliferative state, leukemic blasts show a high dependency on pyrimidines for DNA synthesis. In fully differentiated cells, pyrimidines are mostly obtained through the salvage pathway, while activated lymphocytes as well as leukemic blasts are dependent on de novo synthesis. A lack of pyrimidines leads to nucleotide stress and replication fork stalling, causing activation of p53 and the DNA damage response pathway. This is mediated by checkpoint kinases such as ATM and ATR. Therefore, combining pyrimidine synthesis blockade with checkpoint inhibitors may provide an attractive therapy for these highly proliferative tumors.

Aim:

To determine the sensitivity of TP53 deleted ALL to pyrimidine synthesis blockade either as a single agent, or in combination with DNA replication checkpoint inhibitors.

Results:

Nalm6 and RCH-ACV were genetically engineered using CRISPR/Cas9, to generate variants that were either wild type or TP53 deficient. To explore responses of TP53 ALL to pyrimidine synthesis blockade, we used an inhibitor of Dihydroorotate Dehydrogenase (DHODH), a mitochondrial enzyme involved in de novo pyrimidine biosynthesis. This clinical grade inhibitor, known as Orludodstat (BAY-2402234), was evaluated in either wild type or TP53 aberrant BCP-ALL and T-ALL cells, including cell line models and patient derived xenograft samples (PDX).

Orludodstat treatment is capable of inducing apoptosis without causing significant DNA damage, as determined by microscopic examination of γH2AX and 51BP1 foci. In contrast, antimetabolite-based chemotherapy (cytarabine) treated samples display much higher levels of γH2AX and 51BP1 foci. Despite the absence of detectable DNA damage, Orludodstat appears to induce replication stress, leading to activation of the ATR pathway. Consistent with this notion, a high-throughput drug screen comparing effects of Orludodstat in genetically engineered models that were either wild type or TP53 deficient, identified the inhibitors of the checkpoint kinases WEE1, CHK1, and ATR to strongly synergize with Orludodstat treatment, specifically in a TP53 deficient context.

In most models a strong apoptotic response was observed when Orludodstat was combined with the ATR inhibitor Berzosertib, most notably in TP53 aberrant BCP-ALL. This p53 dependence was confirmed using ex vivo cultures of patient derived xenografts (PDX), representing diagnosis-relapse pairs where each diagnostic sample was wild type for p53, and the relapse carried a TP53 aberration. While little or no synergy was observed in the diagnostic samples, strong synergy was observed in the matched TP53 aberrant relapsed samples (Figure 1).

Conclusion:

Together, our findings suggest combined targeting of de novo pyrimidine synthesis and ATR signaling may present a specific and non-genotoxic vulnerability for TP53 aberrant ALL. Current efforts are aimed at further defining the molecular mechanisms responsible for this observed synergy.

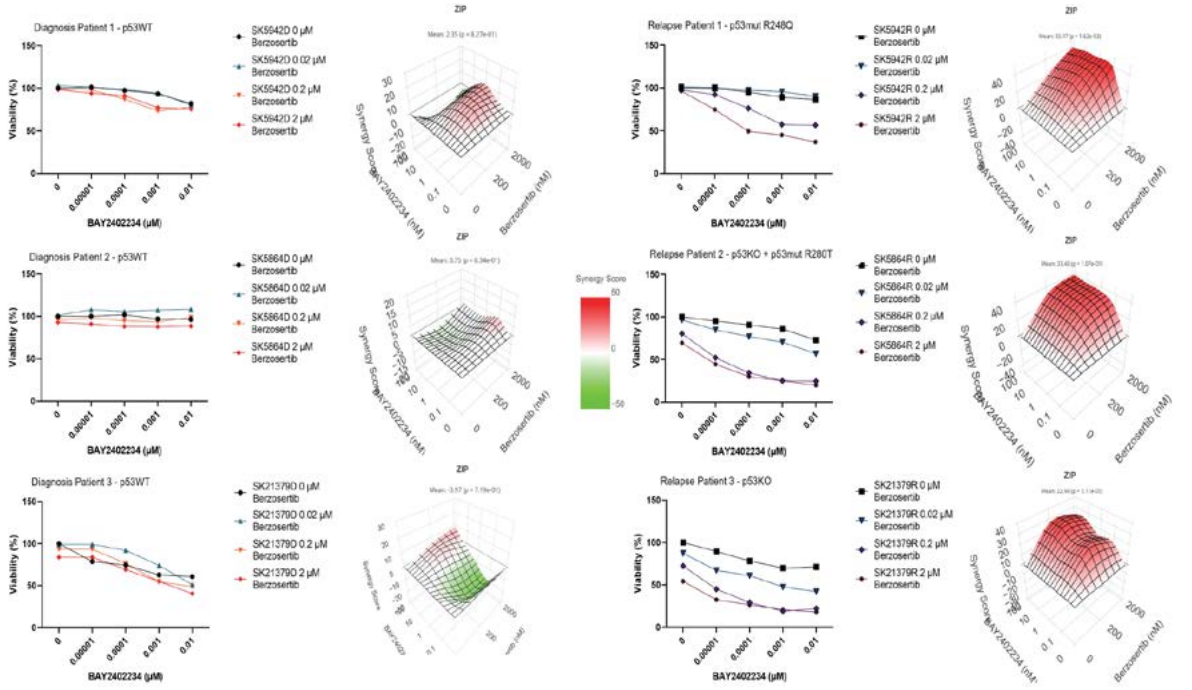

Figure 1. DHODHi (BAY2402234) strongly synergizes with ATRi (Berzosertib) in patient derived xenograft (PDX) samples, affecting a resistant phenotype (p53 mutated) beneficially at relapse. ZIP Synergy scores were calculated for the relapsed patient samples; Patient 1: ZIP = 33.47, p=1.62e-3. Patient 2: ZIP = 33.48, p=1.07e-9. Patient 3 ZIP = 22.94, p=3.11e-5 (n=2).

P 42 - Are minimal residual disease measurements after consolidation therapy useful in children with acute lymphoblastic leukemia?

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Purpose:

Bone marrow examinations are often performed after consolidation in patients with negative or low MRD levels end of first consolidation (EOC). The question remains whether these measurements are useful for detecting (molecular) relapse and predicting outcome. We investigated the prognostic significance and clinical relevance of MRD measurements after EOC in medium risk patients with low MRD levels (< 0.05%) at EOC.

Methods:

MRD measurements were analyzed in 271 medium risk (MR) children with ALL treated in DCOG ALL-10. MRD results were available at EOC (Time point (TP)2; n = 269), at end of consolidation II/start of intensification(TP3; n = 252), week 19 maintenance (TP4; n = 251), and week 37 maintenance (TP5; n = 263). Results were validated in a cohort of patients treated in DCOG ALL-9 (n = 121). MRD was measured by PCR analysis of immunoglobulin and/or T-cell receptor genes. Relapse free survival (RFS) was used as endpoint.

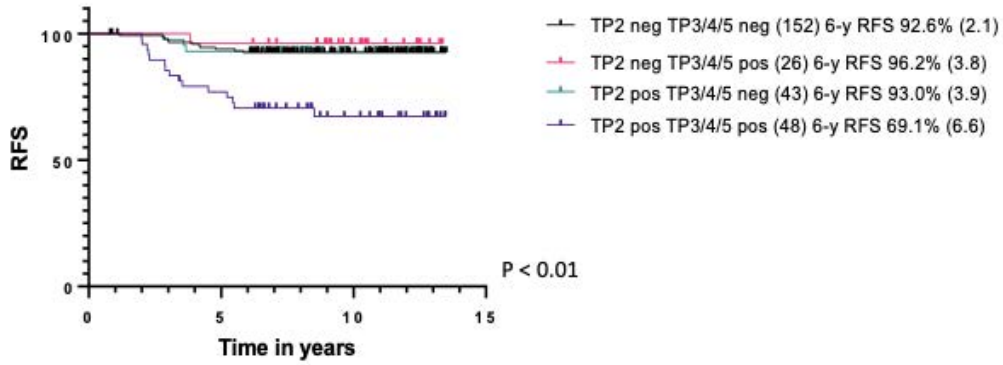
Results:

Patients being MRD negative EOC (n = 178) had excellent outcome, irrespective of the MRD results at later time points; 6-years RFS of 92.6% (SE 2.1) for those with MRD negativity at all later time points compared to 96.2% (3.8) for those with one or more later time points being positive (p = 0.46). Patients with positive EOC MRD (n = 91) of whom the subsequent time points were MRD negative (n = 43) had a comparable good outcome, with a 6-y RFS of 93.0% (SE 3.9). Patients being MRD positive at EOC and MRD positivity at one or more subsequent time points (n = 48) had a higher risk of relapse, 6y RFS 69.1% (SE 6.6), p =0.002. These findings were confirmed in the validation cohort of ALL-9 patients.

Conclusion:

We conclude that in patients with medium risk ALL, MRD measurements after the first consolidation can be abandoned in case of MRD negativity at EOC. For patients who are EOC MRD positive the subsequent MRD measurement might be informative for further risk stratification.

Figure 1. Prognostic significance of EOC MRD in combination with later time points (ALL-10).



P 43 - Molecular profiling based personalized, non-transplant approach for patients with limited treatment options - Three clinical cases of relapsed/refractory pediatric mature B-NHL

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Introduction:

In pediatric oncology, current standard intensive chemotherapy with anti-CD20 antibody regimens achieves long-term, and disease-free survival in about 95% of patients with mature B cell NHL, but the prognosis for relapsed refractory patients remains guarded. In the last 5 years (2017–2022) 48 patients with Non-Hodgkin lymphoma were treated at our department. Three mature B-NHL and two ALCL patients relapsed. All patients treated with molecular profiling-based therapy achieved a second complete remission.

The case series study presents three patients with relapsed, poor-prognosis mature B-NHL. The samples of their tumor tissue were analyzed using whole-exome sequencing, gene expression profiling, phosphoproteomic assays, and phosphoflow cytometry. They were treated according to the molecular analysis of the tumor tissue and achieved second complete remission with no need for high-dose chemotherapy with stem cell transplantation. This individualized therapy was mostly outpatient based and associated with minimal treatment-related toxicities.

Case 1

A 7-year-old boy with stage III Burkitt lymphoma was treated as per standard BFM NHL Registry 2012 protocol. 6 weeks after the last cycle the disease progression was diagnosed. Histology revealed relapsed BL with areas of sclerosing mesenteritis and a germ-line variant of the PI3K-delta. The functional significance of the PI3K-delta variant was confirmed by phosphoflow cytometry from the patient's peripheral blood. Two further lines of intensive chemotherapy led only to disease progression. An off-label combination of PI3K inhibitor idelalisib with ibrutinib, valproic acid, and nivolumab based on molecular profiling was commenced, with metronomic cyclophosphamide and 21-Gy local radiation to bulky side. 2 weeks after idelalisib started the phosphoflow from peripheral blood already normalized. 3 months later partial remission on FDG PET/CT was achieved and nivolumab and autologous dendritic cell vaccine were added. The patient had achieved complete remission after 11 months from initiation of the second-line treatment. The progression-free survival is 88 months now after this personalized therapy, compared to 6 months after the initial standard BFM protocol.

Case 2

A 12-year-old boy was treated for stage III Burkitt lymphoma with standard BFM B-NHL Registry 2012 chemotherapy and relapse three months after treatment. Gene expression profiling of tumor tissue samples detected strong expression of PD1, confirmed by immunohistochemistry. First, participation in the randomized ibrutinib retrieval trial was planned, however, based on molecular profiling, we have prioritized immune therapy. Treatment with nivolumab led to radiological partial remission after the third R-ICE cycle and then he continued with nivolumab single-agent treatment. After 12 weeks of nivolumab, he achieved first complete remission. His first PFS on standard intensive protocol was 7 months, second PFS using immunotherapy is 55 months.

Case 3

A 17-year-old boy with stage III PMLBCL was treated according to DA-R-EPOCH protocol and achieved complete remission after 8 cycles. 6 months later he developed intracranial metastatic relapse. He was successfully re-treated with 2 cycles of R-ICE and 2 cycles of blinatumomab, based on high CD19 positivity in the relapse tissue sample. He achieved second complete remission and is on maintenance therapy with nivolumab and taxanes.

Conclusion:

Our findings support a personalized, non-transplant approach for patients with limited treatment options like relapsed NHL.

Dedications:

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P 44 - MLL (KMT2A)-rearranged acute lymphoblastic leukemias are addicted to S-adenosylmethionine (SAM): implications for therapy

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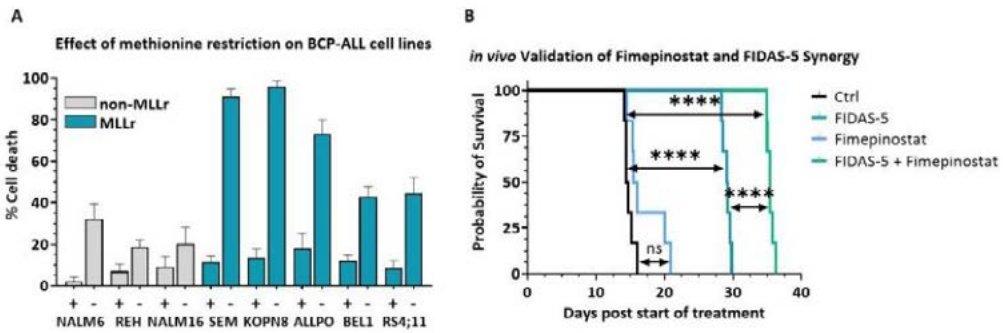
MLL (*KMT2A*)-rearranged acute lymphoblastic leukemia (MLLr ALL) is an aggressive subset associated with poor survival and relapse that represents 5% of childhood ALL cases, but accounts for 75% of infant leukemias. Though current intensive chemotherapy regimens have improved survival in childhood ALL overall, they fail to cure many of these high-risk patient subgroups and inflict significant long-term side effects, demonstrating the urgent need for novel therapies.

While exploring the effect of different amino acid depletion strategies, we observed that MLLr cells are particularly sensitive to methionine restriction (MR) in vitro. This observation was corroborated in vivo where we found that leukemia development was significantly delayed ($p = 0.015$) by a dietary intervention. Mice engrafted with MLLr SEM cells were fed a 95% MR diet which effectively reduced methionine levels in plasma by 50%. Tumor burden was monitored by flow cytometry and was reduced by 35% after 7 weeks. Interestingly, methionine levels in the control group also decreased over time, suggesting that more methionine was consumed by the leukemic cells as they proliferated.

To explore how MR impinges on MLLr cells' metabolic state we performed metabolic profiling of SEM cells and observed significant changes upon MR in 416 of the 650 metabolites measured. Parallel mRNA sequencing identified 1351 differentially expressed genes. Integrated omics analysis revealed key pathways interlinked with S-adenosylmethionine (SAM), a metabolite synthesized from methionine that is the sole methyl donor for all methylation reactions. We hypothesize that the histone methyltransferase function of the MLL fusion protein complex requires increased amounts of SAM to maintain its hypermethylated state, creating a selective sensitivity of MLLr ALL for perturbations in methionine availability. Supporting this, addition of SAM completely rescued MLLr cells from MR-induced apoptosis, an effect not observed in non-MLLr cells. Moreover, we found that MR suppresses global histone methylation, and identified key MLL fusion target genes associated with proliferation and maintenance of the oncogenic phenotype downregulated in response to MR.

The availability of an FDA approved methionine-free formula for infants allows for rapid translation to the clinic and compounds targeting MAT2A, the enzyme producing SAM from methionine, are currently in Phase 1 trials. We performed a drug screen using 214 compounds to identify potential synergizers with one such MAT2A inhibitor, FIDAS-5. Strikingly six of the top hits were histone deacetylase (HDAC) inhibitors, further tying together the epigenetic effect of MR and SAM depletion in MLLr leukemias. We performed follow-up validation experiments in patient derived xenograft (PDX) models and observed synergy between fimepinostat and FIDAS-5 in six of the eight MLLr samples tested. Compelling synergy was also found *in vivo* using an aggressive MLLr PDX model. The effect of fimepinostat treatment alone did not significantly slow leukemic growth, while FIDAS-5 as monotherapy was effective ($p < 0.0001$) and the combination treatment even more so ($p < 0.0001$).

In summary, MLLr leukemic cells are selectively vulnerable to perturbations of the methionine cycle due to their increased need for SAM to maintain hypermethylation and aberrant gene activation. Limiting methionine and/or SAM availability is an attractive adjuvant therapy that can potentiate the effects of current or novel therapies in MLLr leukemia patients.



MLLr ALL is uniquely sensitive to perturbations of the methionine cycle.

P 45 - Flow cytometry of cerebrospinal fluid for detection of central nervous system involvement in childhood non-Hodgkin lymphoma

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Background:

Owing to treatment advances, survival of children and adolescents with non-Hodgkin lymphoma (NHL) now approaches 90%. However, involvement of the central nervous system (CNS) represents a major therapeutic challenge, and patients with B-NHL developing a CNS relapse have a poor prognosis. Current methods cannot reliably identify patients with CNS involvement or patients at high risk of CNS relapse. Multicolor flow cytometry is a novel and highly sensitive method for identification of malignant cells in the cerebrospinal fluid (CSF).

Objectives:

In the current study, the aim was to investigate the frequency of CNS involvement by CSF flow cytometry compared to the frequency detected by conventional cytospin.

Methods:

This prospective study was initiated in 2018 by the NOPHO (19 centers in Denmark, Sweden, Norway and Finland), NHL-BFM (53 centers in Germany) and BSPHO (8 centers in Belgium) study groups. The aim is to include 360 children and adolescents (0–18 years) diagnosed with NHL. CSF samples were collected at the diagnostic lumbar puncture and at subsequent lumbar punctures until day 15 (only NOPHO and BSPHO centers). CSF was collected in cell-stabilizing tubes and shipped to the Department of Clinical Immunology, Rigshospitalet for flow cytometric analysis.

Results:

By December 2022, a total of 238 CSF samples from a total of 146 NHL patients have been included in the study. The median volume of CSF per sample is 2.3 mL (range: 0.3–5.8 mL) and the median time from sampling to analysis is 2 days (range: 0–9 days). At diagnosis, malignant cells were detected in 16 patients (11.0%) by CSF flow cytometry and in 13 patients (8.9%) by cytospin (see table 1). In total, 22 (15.1%) of patients were positive by either flow and/or cytospin. When comparing the level of malignant cells in CSF by flow cytometry in the 16 flow positive patients, the median number of cells per mL CSF were significantly higher in patients also positive by cytospin compared to patients only positive by flow (421 vs 7, P = <0.001). For patients with B-NHL (n = 130), seven (5.4%) were positive both by flow and cytospin and six (4.6%) were positive only by flow and six (4.6%) were positive only by cytospin. Of the six patients positive only by cytospin, malignant cells were detected for one patient by CSF flow, but below the threshold for classification as a positive sample (≥10 malignant cells in total). For four patients, more than three days from sampling to CSF flow analysis had passed, reducing sensitivity of the analysis. For patients with T-NHL (n = 16), three (18.8%) were flow positive whereas no patients were positive by cytospin.

Conclusion:

The preliminary results from this study shows that CSF flow cytometry identifies malignant cells in the CNS in 11% of patients with NHL. Interestingly, more than 50% of these patients only demonstrates low level CNS disease and were classified as CNS negative by cytospin. These data indicates that CSF flow cytometry could be of diagnostic value in childhood NHL, however more patients and follow-up data are needed to draw any conclusion regarding the clinical impact of CSF flow positivity in childhood NHL.

		FLOW		
		CNS+ n (%)	CNS- n (%)	Total n (%)
CYTOSPIN	CNS+ n (%)	7 (4.8%)	6 (4.1%)	**13 (8.9%)**
	CNS- n (%)	9 (6.2%)	108 (74%)	117 (80.1%)
	Data missing n (%)	0 (0%)	16 (11%)	16 (11%)
	Total n (%)	**16 (11%)**	130 (89%)	146 (100%)

Table 1: CNS frequencies at initial diagnosis by CSF flow and cytospin.

P 46 - The effect of immunoglobulin prophylaxis on infectious morbidity in pediatric patients with acute lymphoblastic leukemia: Results of a randomized controlled trial

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2 *University Hospitals Leuven, Department of Pediatric Hemato-Oncology, Leuven, Belgium*
3 *Leiden University, Applied Mathematics, Leiden, Netherlands*
4 *Hopp Children's Cancer Center Heidelberg KiTZ, Pediatric Hematology and Oncology, Heidelberg, Germany*

Background:

Infections lead to substantial morbidity during treatment of acute lymphoblastic leukemia (ALL). During ALL treatment, the adaptive immune system gets severely affected, leading to, amongst other things, declining serum immunoglobulin (IgG) levels. Theoretically, one may partially overcome the infection risk by supplementing the low IgG levels with intravenous immunoglobulins (IVIG).

Objective:

The aim of this trial was to investigate whether IVIG prophylaxis in pediatric patients with medium risk (MR) ALL can prevent admissions for fever.

Methods:

This randomized controlled trial was a subtrial of the national Dutch multicenter ALL study (DCOG ALL-11). Patients aged 1–19 years with MR ALL were randomized into either IVIG prophylaxis (receiving 0.7 g/kg IVIG every three weeks) or control group (receiving standard of care). The primary endpoint was number of admissions for fever. Secondary endpoints were antibiotic treatments during admission for fever, adaptation of chemotherapy after admission for fever, ICU admissions, relapse, event-free survival (EFS), and overall survival (OS). To account for possible correlations between episodes within the same patient, a generalized estimating equation (GEE) was fitted for admissions for fever, therapeutic antibiotics, chemotherapy adaptations and ICU admissions. Age was included in the GEE models as a factor.

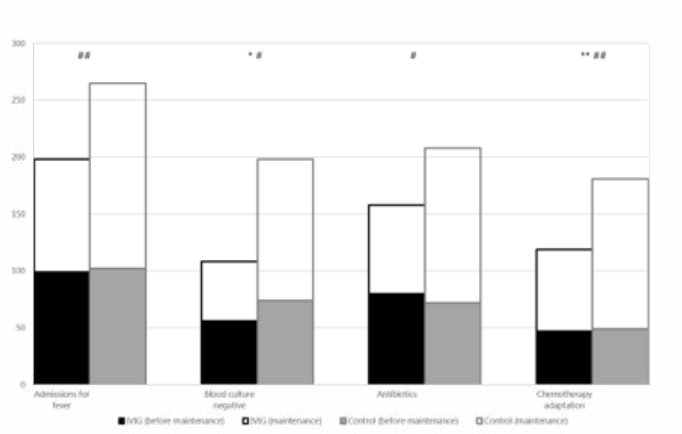
Results:

Between October 2012 until March 2019, 165 patients were enrolled; 82 (50%) patients were randomly assigned to IVIG prophylaxis and 83 (50%) to the control arm. There were no significant differences in baseline characteristics. In the IVIG prophylaxis group there were 198 admissions for fever versus 265 in the control group ($p = 0.055$, based on GEE models adjusted for age, Figure). During maintenance treatment of ALL, IVIG prophylaxis was associated with a significantly reduced number of admissions for fever (N = 99 versus 163, for IVIG prophylaxis versus control group, respectively, $p = 0.005$, Figure), resulting in significantly less antibiotic treatments (N = 78 versus 136, for IVIG prophylaxis versus control, respectively, $p = 0.011$, Figure). IVIG prophylaxis was associated with a significant reduction in admissions for fever with negative blood cultures compared to the control group (N = 108 versus 198, for IVIG prophylaxis versus control, respectively, $p = 0.009$, Figure), especially during maintenance treatment of ALL (N = 52 versus N = 124, for IVIG prophylaxis versus control, respectively, $p = 0.014$, Figure). Next, IVIG prophylaxis led to a significant decrease of chemotherapy adaptations after admission for fever (N = 119 versus 181, for IVIG prophylaxis versus control, respectively, $p = 0.024$, Figure), especially during maintenance treatment of ALL as well (N = 72 versus 132, for IVIG prophylaxis versus control, respectively, $p = 0.004$, Figure). There was a low number of relapse in this study and IVIG prophylaxis did not significantly impact 5-year incidence of relapse, EFS or OS.

Conclusion:

In pediatric patients with MR ALL, IVIG prophylaxis is associated with a significantly reduced number of admissions for fever (with a negative blood culture), especially during maintenance treatment of ALL, resulting in less antibiotic treatments and a decreased number of chemotherapy adaptations. Future studies should aim at identifying a subgroup of patients that benefit most from IVIG prophylaxis.

Figure: Admissions for fever; with negative blood cultures, courses of antibiotics, or chemotherapy adaptation for IVIG and control group.



P 47 - Various components of the bone marrow niche equally support B-Cell Precursor Acute Lymphoblastic Leukemia

Cesca Van De Ven[1,2], Mandy W.E. Smeets[1,2,3], Caitlin Reichert[1,2], Aurélie Boeree[1,2], Judith M. Boer[1,2], Monique L. Den Boer[1,2,3]

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Background:

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) originates from the bone marrow, which also represents the major site of relapse. In leukemia, the bone marrow microenvironment facilitates leukemogenesis and provides a survival benefit to leukemic cells that also affects the efficacy of chemotherapeutic drugs. The focus of studies into the relation of BCP-ALL and the bone marrow microenvironment has mostly been on the role of mesenchymal stromal cells (MSCs). However, little is known about the stability of gene expression, secreted cyto-/chemokines or CD-marker expression of MSCs in *ex vivo* studies and whether the source of MSCs has an impact on their function. Moreover, besides hematopoietic cells and MSCs, the bone marrow also contains other (supportive) cell types, including osteocytes, chondrocytes, fibroblasts, and adipocytes. Still, to what extend these bone marrow cell types provide support to BCP-ALL cells remains largely unknown.

Objectives:

We have assessed whether support of MSCs to BCP-ALL cells differs between MSCs collected at the time of diagnosis (full blown leukemia), in remission, or from healthy, non-leukemic controls. Additionally, we have evaluated the stability of expression of cell surface markers, gene expression, and secreted cyto-/chemokines of bone marrow derived MSCs in prolonged *ex vivo* culture. Furthermore, we have also studied whether bone marrow stromal components other than MSCs are supportive to BCP-ALL.

Results:

To validate primary bone marrow-derived MSCs as a stable component in functional studies of the leukemic niche, we measured CD marker expression, secreted cyto/-chemokines and RNA expression over time for primary MSCs. Our findings show that bone marrow-derived MSCs from different pediatric BCP-ALL patients or controls display a stable and uniform expression of the cell surface markers CD9, CD13, CD29, CD73, CD146 and CD166, up to cell passage 17 (equivalent to ~8 weeks of culture). The secreted level of 88% of the 171 cytokines quantified by Luminex technology did not significantly alter up to passage 10 (~4 weeks of culture). Total-RNA sequencing of MSCs revealed that gene expression levels of MSCs do not markedly alter up to passage 10, except for only 20 out of 16,105 genes. Moreover, the disease status at the time of MSC collection (leukemic/remission/control) did not have a differential effect on the survival benefit acquired by patients' BCP-ALL cells. All tested supportive cell types provided a survival benefit to BCP-ALL cells, with a median survival benefit ranging between 18–24% (chondrocytes and early adipocytes) and 30–36% (MSCs, osteocytes, and fibroblasts). Survival benefit was direct cell-cell contact dependent and decreased upon physical separation in a transwell setting. The secreted level of 8 pro-inflammatory cyto-/chemokines increased in co-cultures of BCP-ALL cells with most of the tested bone marrow components.

Conclusion:

Together, our results indicate that the characteristics of bone marrow derived MSCs remain highly stable for at least one month of culture, which fosters their use in functional studies. Most importantly, our data suggest that BCP-ALL cells manipulate different components of the bone marrow supportive tissues via direct cell-cell contact, which favors the survival of leukemic cells and creates a pro-inflammatory microenvironment. This strengthens our hypothesis that BCP-ALL cells hijack the bone marrow microenvironment and offers the perspective that interference with this stromal interaction and/or released cyto-/chemokines may be of additive value in the treatment of BCP-ALL.

P 48 - Mutational mechanisms in pediatric acute lymphoblastic leukemia with multiple relapses

Cédric Van Der Ham[1], Stefan Lelieveld[1], Zeljko Antic[1], Marley Yeong[1], Lianne Suurenbroek[1], Lennart Kester[1,2], Edwin Sonneveld[1,2], Peter Hoogerbrugge[1,2], Frank van Leeuwen[1], Roland Kuiper[1,3]

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Pediatric acute lymphoblastic leukemia (ALL) is marked by a low mutational load at initial diagnosis, which increases in subsequent relapses, partly because of hypermutation in a subset of cases (Waanders *et al.*, 2020, Blood Canc. Disc., 1:96). This increased mutational load can be driven by intrinsic mutational processes or could be therapy-related. To determine which processes are active in (relapsed) ALL and how they behave during disease progression before and after therapy, we have investigated these mutational processes in pediatric ALL patients who experienced multiple relapses.

We identified 63 historical cases of ALL with multiple relapses, and selected patients by availability of material and blast percentage above 70% in multiple leukemic samples. We collected and whole genome sequenced 97 tumor samples from 29 ALL patients (28 precursor B-ALL and 1 T-ALL) with 2 (n = 18) or more (n = 11) relapses, as well as 44 remission samples. Somatic single base substitutions (SBSs) were called by Mutect2. SBSs were clustered per sample based on their dynamics in time and all clusters in all patients were included for de novo signature extraction using the R package MutationalPatterns.

ALL patients with multiple relapses had a median mutational load of 1096 somatic SBSs at diagnosis, significantly higher than an unselected diagnosis cohort with median 455 somatic SBSs. Mutational loads increased upon relapse in 28/29 patients, often increasing at each relapse. We performed mutational signature analysis and extracted nine SBS signatures, of which six were known COSMIC signatures (cosine similarity >0.95) and two were similar to known COSMIC signatures (cosine similarity >0.8). We identified clock-like mutational signatures SBS1 and SBS5 in most samples. At time of diagnosis four other distinct mutational processes were found to be active alone or in combination in nine cases (31%), which is much higher than an unselected diagnosis cohort (13%). These processes included APOBEC-associated mutagenesis (SBS2 & SBS13), a mutational pattern resembling UV-like damage (SBS7a), a process that may be caused by reactive oxygen species (SBS18-like) and an unknown process. By clustering SBSs per patient in time we followed the activity of mutational processes and identified rising and falling (sub)clones. SBS7a was present at diagnosis in two patients but also arose at 3rd relapse in one patient. SBS18-like mutagenesis was restricted to initial diagnosis in four patients, but continuous in another patient. APOBEC mutagenesis was found to be an ongoing process and often appeared for the first time at first or second relapse. One patient, who relapsed five times, acquired at each presentation between 500–1500 new somatic mutations which were likely due to an unknown ongoing mutational mechanism.

At time of relapse, additional (mostly treatment-related) mutational processes became active, eventually affecting 22/29 (76%) patients, much higher than observed in cases with single relapses (~40%; Li *et al.*, 2020, Blood, 135:41). We observed the thiopurine-associated SBS87 in 16 (55%) patients, and an unexplained SBS86-like mutational signature in 4 patients. Furthermore, we noticed SBS17-like mutagenesis in one patient. The etiology of SBS17 is as yet not established but has been proposed to be caused by reactive oxygen species.

To conclude, ALL patients with multiple relapses showed an increased prevalence and large variety of interplaying mutational processes at diagnosis (31%) and final relapse (76%), with intrinsic as well as therapy induced origins.

P 49 - Transcriptomic characterization of Switching B-cell Precursor Acute Lymphoblastic Leukemia in childhood

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Background:

The recent advances in multi-omic techniques and multiparametric flow cytometry (MFC) allowed distinguishing different subtypes of pediatric acute lymphoblastic leukemia (ALL) based on chromosomal rearrangements, ploidy level, mutations, and gene expression profile, as well as immunophenotype at diagnosis and response to therapy. The accurate identification of different ALL subgroups is mandatory for a precision medicine approach since they are associated with specific therapeutic responses and outcomes.

We previously identified a pediatric B-cell precursor (BCP) ALL subtype showing a transient immunophenotypic switch towards the myelomonocytic lineage (mm-SW). The mm-SW was detected by MFC on Day 8 or Day 15 of Induction therapy and characterized by the presence of two distinct populations of blasts, one keeping the immunophenotype of the diagnosis and the second one showing downregulation of CD19, high expression of CD34, upregulation of CD45, and increase of SSC.

Objectives:

To characterize the transcriptome of mm-SW-positive (mm-SW^{pos}) BCP-ALL and potentially define the biological mechanism underlying mm-SW phenomenon.

Methods:

We performed transcriptomic analysis on a total of 264 bone marrow samples belonging to pediatric BCP-ALLs at diagnosis and enrolled in the AIEOP LAL 2000 and AIEOP-BFM 2009 ALL trials. Then we excluded 122 specimens showing one among KMT2A rearrangements, ETV6::RUNX1, TCF3::PBX1, and BCR::ABL1 translocation signature by the Diagnostic Classifier model and known to harbor the translocation. Seventy-seven of the remaining 142 BCP-ALLs (50.7%) had an available bone marrow sample on Day15 of Induction therapy to evaluate the presence/absence of the mm-SW by MFC. Of those, 19 samples (24.6%) were mm-SW^{pos} and 58 (75.3%) mm-SW-negative (mm-SW^{neg}).

Results:

In our cohort, most of the mm-SW^{pos} patients clustered on ERG-related subtype, one was HHD-like, one ZNF384r, one KMT2A-like, and one ETV6::RUNX1-like, based on an unsupervised analysis approach using the top 1000 variables genes. *CLEC12A,* a c-type lectin receptor usually expressed on myelomonocytic lineage cells, appeared among the most differentially expressed genes in mm-SW^{pos} patients. Notably, these patients showed a high expression of genes commonly found in CD34+ hematopoietic stem cells (HSC-positive enrichment of CD34^{+}HSC signature). Moreover, the interferon pathway, usually activated in myeloid cells as a response to infection or cell necrosis, was one of the most prevalent in the mm-SW^{pos} group, together with enrichment in genes typically regulated by myeloid transcription factors such as *CTNNB1*, *GATA2,* and *MYB*. Furthermore, preliminary ongoing data on simultaneous analysis of cell surface markers and whole transcriptome at single cells revealed a different maturative block of these patients in comparison to BCP-ALL mm-SW-negative.

Conclusion:

Pediatric mm-SW^{pos} BCP-ALLs showed a homogeneous transcriptome in our cohort, different from that of mm-SW^{neg} BCP-ALLs. Transient mm-SW may potentially rely on a biological plasticity linked to the aberrant myeloid and immaturity features detected in this subgroup of samples. Nonetheless, further studies are necessary to better correlate our findings with transient mm-SW during the first phase of Induction therapy.

P 50 - CD371-positive B-cell precursor acute lymphoblastic leukemia is associated with transient myelomonocytic switch and slow early response to treatment in childhood: an AIEOP-BFM Flow Network study

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Background:

Since 2014, the *Associazione Italiana di Ematologia ed Oncologia Pediatrica* (AIEOP) and Berlin-Frankfurt-Münster (BFM) Flow Cytometry Network has adopted the myelomonocytic marker CD371 in the panel for pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL) to ameliorate immunophenotyping definition and minimal residual disease (MRD) detection. That led us to define a new subgroup of BCP-ALL featuring the aberrant expression of marker CD371 at diagnosis.

Objectives:

To investigate the clinical and biological features of CD371-positive ($CD371^{pos}$) pediatric BCP-ALL.

Methods:

From June 2014 to February 2017, a total of 1948 pediatric patients with a new diagnosis of BCP-ALL (age 1–18 years) and enrolled in the AIEOP-BFM ALL 2009 trial were recruited to either a screening cohort (n = 843, AIEOP centers) or validation cohort [n = 1105, BFM centers: Austria (269), Czech Republic (163), and Germany (673)]. BCP-ALL diagnosis was performed on peripheral blood (PB) or bone marrow samples (BM) in all patients and relied on morphological, immunophenotypic, and genetic [standard karyotype; t(12;21)(p13.2;q22.1)/ETV6::RUNX1; t(1;19)(q23;p13.3)/TCF3::PBX1; t(4;11)(q21;q23)/KMT2A::AFF1] analysis. Additionally, the presence of any DUX4 fusions by RNA-Sequencing was assessed in the Austrian cohort at diagnosis. Response to therapy evaluation consisted of morphology, multiparametric flow cytometry (MFC) MRD, and polymerase chain reaction (PCR) MRD. Outcome assessment included patients from both screening and validation cohorts.

Results:

In the screening cohort, 76 of 843 (9%) BCP-ALLs were $CD371^{pos}$ at diagnosis and were associated with older age (≥10 years, $p < 0.001$), lower ETV6::RUNX1 frequency ($p < 0.001$), immunophenotypic immaturity (B-I as per EGIL classification, $p < 0.001$), strong expression of CD34, CD45, and CD58 (all $p < 0.05$). $CD371^{pos}$ BCP-ALLs were not associated with high-risk genetic features [hypodiploidy, t(4;11)(q21;q23)/KMT2A::AFF1]. During Induction, $CD371^{pos}$ BCP-ALLs showed a transient myelomonocytic switch in 26 of 43 (60.5%), 51 of 73 (69.9%), and 1 of 40 (2.5%) cases on Days 8, 15, and 33, respectively. No myelomonocytic switch was found on Day 78. $CD371^{pos}$ BCP-ALLs showed an inferior response to Induction chemotherapy (Slow Early Response, $p < 0.001$). The validation cohort confirmed these results. In the Austrian cohort, $CD371^{pos}$ BCP-ALLs were strongly associated with DUX4 fusions (13 of 17 $CD371^{pos}$ versus 1 of 248 $CD371^{neg}$ tested cases, sensitivity = 76.5%, specificity = 99.6%). Regarding outcome, $CD371^{pos}$ BCP-ALL showed a 5-year event-free survival and cumulative incidence of relapse of 86.9% and 6.8%, respectively.

Conclusion:

We comprehensively described the biological and clinical features of pediatric $CD371^{pos}$ BCP-ALL in the largest cohort until now. We confirmed the association of $CD371^{pos}$ BCP-ALL with DUX4 fusions in the Austrian cohort. CD371 positivity was associated with transient myelomonocytic switch, whose identification is critical for proper MFC-MRD detection. $CD371^{pos}$ BCP-ALL showed a poor response to Induction treatment. Regardless, continuing chemotherapy as per the AIEOP-BFM ALL 2009 protocol with no change to a myeloid-related treatment led to satisfactory outcomes. We strongly recommend adopting the CD371 marker in MFC panels for pediatric BCP-ALL.

P 51 - Genomic characterization of childhood B-Other Acute Leukemia patients by RNA sequencing in a single center

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Background:

B-cell precursor acute lymphoblastic leukemia (B-ALL) is the most frequent childhood malignancy. Genetic alterations have been used to stratify patients into recurrent leukemic subtypes impacting on diagnosis and prognosis. However, conventional methodologies fail to identify driver alterations in approximately 25–30% of B-ALL patients, and these cases are classified as B-other. A broad genomic landscape of childhood leukemia has emerged from new genomic analyses such as transcriptome sequencing (RNA-Seq). These techniques have helped to reveal new recurrent leukemogenic alterations (fusions, mutations, gene expression profiles (GEP)) with diagnostic, prognostic, and/or therapeutic value and constitute new leukemia entities.

Aim:

To study by RNA-Seq B-ALL patients lacking the main stratifying or recurrent gene abnormalities (B-other) to identify new biomarkers to reclassify these patients into the newly defined subtypes.

Methods:

The study included 44 B-other patients diagnosed at Hospital Sant Joan de Déu Barcelona from 2009 to 2022. The transcriptome of these patients was analyzed using RNA-seq in search of fusion genes and different GEPs. Fusion detection was performed using a combination of five pipelines included in the Fusion Integrative Pipeline (Fusion InPipe), and the t-SNE algorithm was used to visualize the differential GEP.

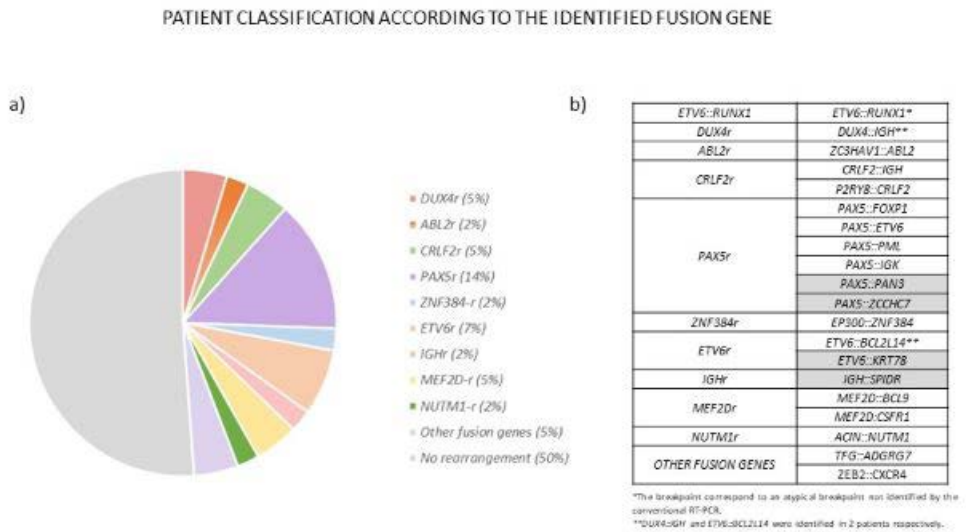
Results:

We included 44 pediatric B-other patients (19 female (43.2%), median age 6.7 (1.6–17.5). By RNA-seq, we were able to identify a chromosomal rearrangement in 22 cases (50%) (Figure 1). We identified one patient harboring an *ETV6::RUNX1* rearrangement presenting an atypical breakpoint and therefore not identified by conventional methodologies. Nineteen of the 22 patients presenting a rearrangement belonged to the recently established subtypes (*ABL2, CRLF2, DUX4*, *ETV6, MEF2D*, *NUTM1*, *PAX5,* or *ZNF384* rearrangements). In addition, 4 out of these 19 patients had fusion genes not previously reported: *PAX5::IGK, PAX5::PAN3, ETV6::KRT78,* and *IGH::SPIDR*. Finally, two of the 22 patients presented fusion involving other genes (*ZEB2::CXCR4* and *TFG::ADGRG7)* and therefore do not define the molecular category.

Overall, RNA-seq allowed to reclassify half of B-other patients, based on the detection of fusion genes, providing valuable information for disease classification and prognostic stratification.

Moreover, the ongoing differential expression analyses will help to refine the classification of patients in the different molecular categories based on their GEP.

Figure 1: Patient reclassification according to the fusion gene identified by RNA-seq. a) Distribution of the patients in the different established subtypes. b) Different chromosomal rearrangements identified by RNA-seq. Rearrangements colored in grey indicate not-previously reported fusion genes.



ETV6::RUNX1	ETV6::RUNX1*
DUX4r	DUX4::IGH**
ABL2r	ZC3HAV1::ABL2
CRLF2r	CRLF2::IGH
	P2RY8::CRLF2
PAX5r	PAX5::FOXP1
	PAX5::ETV6
	PAX5::PML
	PAX5::IGK
	PAX5::PAN3
	PAX5::ZCCHC7
ZNF384r	EP300::ZNF384
ETV6r	ETV6::BCL2L14**
	ETV6::KRT78
IGHr	IGH::SPIDR
MEF2Dr	MEF2D::BCL9
	MEF2D::CSFR1
NUTM1r	ACIN::NUTM1
OTHER FUSION GENES	TFG::ADGRG7
	ZEB2::CXCR4

*The breakpoint correspond to an atypical breakpoint not identified by the conventional RT-PCR.
**DUX4::IGH and ETV6::BCL2L14 were identified in 2 patients respectively.

Conclusions:

The use of RNA-seq has allowed to refine the classification of B-other patients, allowing a more refined diagnosis or the reclassification into novel molecular subtypes of B-ALL based on the identification of fusion genes. Our results support the application of RNA-seq to achieve a more accurate risk stratification and a better follow-up of B-other leukemia patients.

P 52 - Integration of transcriptomic and MLPA copy number analysis reveals discordant risk stratification in patients with high-risk lesions

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Objectives:

Multiple studies indicate that copy number alteration (CNA) profiles determined by multiplex ligation-dependent probe amplification (MLPA) can identify paediatric patients at higher relapse risk. Questions persist regarding whether high-risk CNA profiles identify all patients with high-risk lesions or remain predictive of relapse risk in older patients. In this study, we performed paired MLPA and transcriptomic analysis of a non-consecutive Australian cohort of paediatric and adolescent/young adult patients. We compared CNA profiles of patients with distinct B-ALL subtypes or high-risk lesions.

Methods:

B-ALL samples (n = 258) were transcriptionally sequenced, and subtypes were assigned according to identified fusion genes, single nucleotide variants and gene expression profiles. Deletions affecting nine genes were detected by MLPA (SALSA MLPA P335 and P202 kits). Samples represented 140 paediatric (CHI, 0–15 yrs) and 118 adolescent / young adult (AYA, 16–39 yrs) patients.

Results:

Subtype prevalence varied by age, with *ETV6::RUNX1* and hyperdiploidy representing 40% of paediatric cases. AYA patients demonstrated a high frequency of Ph+ALL (17.8%), JAK-STAT alterations (*JAK2*r, *CRLF2*r or *EPOR*r, 11%), *KMT2A*r (10.2%) and *DUX4*r (12.7%). Gene deletions were observed in 70% of samples (181/258), affecting a greater proportion of AYA cases (65% CHI vs 76.3% AYA). Deletions were rare among patients with *KMT2A*r (4/17, 24%) but occurred in all but one patient with JAK-STAT alterations (20/21, 95%). *IKZF1* deletions were present in all but the *ETV6::RUNX1* subtype, occurring most frequently in patients with *BCR::ABL1* (76%, 28/33), ABL-class rearrangements (50%, 3/6) or JAK-STAT alterations (76%, 16/21). Deletions of *CDKN2A/B* were common across the cohort but strongly associated with *PAX5* p.P80R (80%, 8/10) or PAX5alt (88%, 15/17). Co-deletion of *PAX5* and *CDKN2A/B* was also more frequent in PAX5-associated subtypes (78%, 21/27). *ERG* deletions predominately occurred in samples classified as *DUX4*r (39%, 11/28). The high-risk CNA-profile *IKZF1*plus was observed in 46 patients (17.8%), affecting 12.1% of CHI (17/140) compared with 24.6% of AYA cases (29/118, FDR < 0.001). *IKZF1*plus occurred with the greatest frequency in patients with *CRLF2*r (9/16, 56.3%) and *BCR::ABL1* (18/33, 54.5%). Transcriptomic analysis identified 18 patients (6/140 CHI, 12/118 AYA) with cryptic high-risk lesions, including *IDH1/2* mutations (2/258), the recently described *CDX2/UBTF* subgroup (2/258) or rearrangements of *PDGFRB, ABL2, EPOR, JAK2, MEF2D, NTRK3* and *HLF* (14/258). Of these, 6 patients demonstrated a UKALL-CNA intermediate-risk profile, 6 UKALL-CNA poor-risk and only 3 patients were classified *IKZF1*plus. Due to the small size of some subgroups and the variation in treatment protocols, the prognostic significance of CNA profiles remains to be determined.

Conclusion:

In our cohort, we identified distinct patterns of deletions associated with specific B-ALL subgroups. We also revealed that patients with high-risk lesions, cryptic to cytogenetic analysis and more frequent in AYA patients, do not consistently demonstrate high-risk CNA profiles. These results highlight the value of transcriptomic sequencing for a comprehensive assessment of patient disease and the need for additional research to understand the prognostic effect of focal gene deletions in newly established B-ALL subgroups.

P 53 - Pediatric AML with NUP98 gene rearrangements – Genetic characteristics and predictive value

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Introduction:

NUP98 is a component of nuclear pore complex. *NUP98* gene rearrangements (*NUP98*r) are found in about 4% of cases of AML in children and are associated with poor prognosis, when treated with chemotherapy alone.

Patients:

From 2006 to January 2023, we followed up 406 pediatric patients with high-risk *de novo* AML. *NUP98*r group consisted of 54 patients (m:f = 29:25; 13.3% of high-risk AML; age 6 months - 17.8 years, median 8.8 years). Median WBC at presentation was 64.5 x 109/l (0–561); 25 patients had the initial hyperleukocytosis (WBC > 50 x 109/l; 46%). Initial CNS involvement was present in 4.5% patients (2 of 44); extramedullary lesions – in 4.9% patients (2 of 41). FAB M4 and M5 variants prevailed (n = 12 and 11, respectively). All patients received myeloid-directed induction therapy: 25 patients (46%) – AML-MRD-2018, 16 (30%) – AML-BFM-2004, 8 (15%) – AML-MM-2006, 5 (9%) – other protocols.

Methods:

*NUP98*r were detected by FISH with break-apart probe (Leica, Netherlands) followed by *NUP98::NSD1* and *NUP98::KDM5A* translocational probes (CytoCell, UK). Double negative cases were subjected to NGS with either multiplex anchored PCR (ArcherDX, USA) or RNAseq (NEB, USA). Fusion sequences were determined with Arriba [Uhrig et al., 2021]. *FLT3* mutations were studied by fragment analysis and Sanger sequencing (exons 14–15, 20) in 48 patients.

Results:

NUP98 translocation partner was identified in 49 of 54 patients. The most common rearrangements were t(5;11)(q35;15.5)/*NUP98::NSD1* (n = 35, 64.8%) and t(11;12)(p15;p13)/*NUP98::KDM5A* (n = 8, 14.8%). Single cases of *NUP98::TNRC6C*, *NUP98::XRCC6*, *NUP98::BPTF*, *NUP98::KAT7*, *NUP98::TNRC18* and *NUP98::PSIP1* were also found (figure A). Additional abnormalities included trisomy 8 (n = 5) and trisomy 6 (n = 5). Complex karyotype was found in 12 patients, normal karyotype – in 14 patients. *FLT3* activating mutations were present in 46% patients (22 of 48: 18 ITD, 2 TKD, 2 both).

Of 54 patients, 40 reached CR1 (74%): 25 after induction with SD AraC, anthracycline and etoposide, 12 after salvage therapy with HD AraC, fludarabine and anthracycline and 3 (5.5%) after HSCT. Six patients died early, 3 from progression, 2 are alive in active disease. After reaching CR1, 15 patients relapsed (38%; 12 early and 3 late relapses, median 10 months, 5–16 months), one died in CR1 of infection and 23 are alive (22 disease-free, one in active disease). HSCT was performed in 29 patients (MUD - 4, MFD - 5, haploidentical - 20) in CR1 in 18 patients, CR2 in 3 and active disease in 8 (of those, 7 reached CR). Second transplant was performed for post-HSCT relapses. Thus, 23 of 29 transplanted patients are currently alive and leukemia-free. Totally, 31 of 54 patients are alive (57%; 18 after HSCT in CR1, 4 in CR1 without transplant, 6 in second or subsequent CR, 3 in active disease); 18 died (33%; 6 early deaths, 9 from progression, 3 in CR) and 5 were lost for follow-up. The median follow-up time was 1.5 years (1 month - 12.5 years). Overall survival (OS) for whole *NUP98*-positive cohort was 0.53[0.39–0.74], event-free survival (EFS) was 0.23[0.15–0.89] (figure B). EFS for *NUP98*r+*FLT3*-ITD was 0.1[0.09–0.84] vs 0.75[0.15–1.1] for *NUP98*r+*FLT3*^{wt} (p = 0.13). OS was 0.5[0.28–0.9] vs 0.64[0.47–0.88], respectively.

Conclusions:

HSCT in CR1 reduces relapse rate in children with *NUP98*-positive AML. Post-HSCT relapses are not uniformly fatal and patients may be salvaged with second and even third transplant. The presence of *FLT3*-ITD worsens the prognosis in *NUP98*-positive patients without the use of targeted therapy.

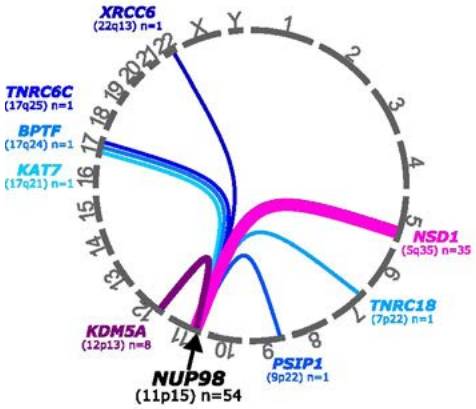

Figure A

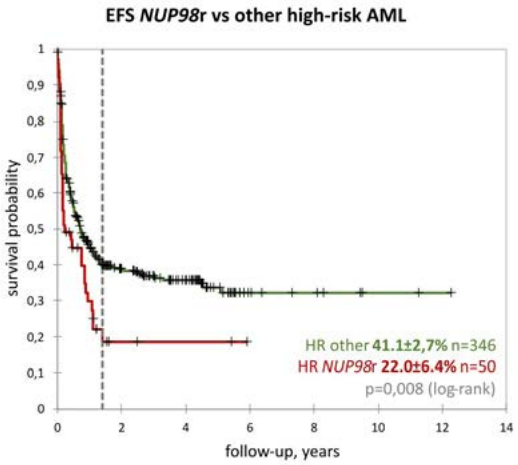

Figure B

P 94 - High chromosome instability across aneuploid subtypes of childhood B-cell ALL and potential association with disease progression in PDX models

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B-cell acute lymphoblastic leukemia (B-ALL) is the most common cancer in children and 35% of them show aneuploidy. Specific aneuploid subtypes have an important prognostic value and are classified according to chromosome numbers in high-Hyperdiploid (HeH), low-Hypodiploid (HoL) and near-haploid (nHap). Aneuploidy frequently associates with chromosome instability (CIN), a cell-to-cell variation in chromosome content, which is a widespread phenomenon in human cancers and usually correlates with poor prognosis. However, little is known about CIN in aneuploid subtypes of childhood B-ALL. This project aims to elucidate the presence and levels of CIN in the distinct aneuploid B-ALL subtypes and its specific contribution to disease outcome.

Computational analyses in a large cohort of B-ALL samples showed higher levels of chromosomal copy-number heterogeneity (chrCNH) in aneuploid subtypes compared with Euploid B-ALL samples. Single-cell DNAseq of primary samples confirmed these results and showed a correlation between aneuploidy scores and chrCNH. We next generated primary-derived xenograft (PDX) models from B-ALL samples with euploid, HeH, HoL and nHap karyotypes to accurately study the CIN phenotype and its contribution to disease progression (n = 57). Our results showed significant higher levels and a strong positive correlation between chromosome missegregation and CNH in the different aneuploid subtypes compared with euploid samples ($P < 0.0001$), demonstrating the presence of different levels of CIN in aneuploid subtypes of B-ALL. Remarkably, the levels of chromosome missegregation and CNH were highly correlated with overall survival rates in PDX. Moreover, proteomic analyses on paired PDX samples elucidated the CIN signature in aneuploid B-ALL, with specific proteins positively and negatively correlated with chrCNH.

Collectively, our results suggest that the levels of CIN in aneuploid subtypes of B-ALL are associated with disease progression. Future experiments aim to assess the potential use of CIN as a novel therapeutic target.

Predisposition in lymphoid and myeloid hematopoietic malignancies

P 54 - Pax5 heterozygosity affects B-cell differentiation and generates a deregulated precursor population in the bone marrow

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Introduction:

PAX5 is a master regulator for B-cell development, differentiation and identity. While the reduction of PAX5 transcriptional activity in the germline of humans and mice is known to predispose to B-cell precursor acute lymphoblastic leukemia (BCP-ALL), *Pax5* heterozygosity is mostly believed to be phenotypically unremarkable. Thus, the mechanistic process how leukemia evolves upon genetic predisposition affecting the *PAX5* locus is only insufficiently understood. Here, we deeply characterized the developing B-cell population in the bone marrow (BM) of *Pax5* heterozygous (*Pax5*$^{+/-}$) mice.

Methods:

Aiming to understand the etiology of pediatric BCP-ALL in the setting of reduced Pax5 germline levels, we used 9-color FACS staining, single-cell (scRNA) and bulk RNA-Seq to pinpoint deregulated populations in the BM of *Pax5*$^{+/-}$ mice.

Results:

A comprehensive FACS staining depicting all B-cell developmental stages within the murine BM showed a robust enrichment of the pre-BII population (B220^{+}CD19^{+}IgM^{-}CD25^{+}) in healthy *Pax5*$^{+/-}$ mice compared to their wildtype (WT) littermates (Student's t-test; p = 0.0026). These results could be validated across all ages (3, 6, 9 and 14 months) and the individual cells displayed a higher mean fluorescence intensity of CD25 and IL7-Receptor (Student's t-test p = 0.0001). Bulk RNA-Seq of sorted pre-BII populations (3 WT vs. 3 *Pax5*$^{+/-}$ mice) revealed downregulation in B-cell receptor (BCR) signaling components including Cd79a/b, Lyn, MTor and various ITIMs as well as upregulation of Jak3 in *Pax5*$^{+/-}$ preB-II cells. Additional analyses of the enriched pre-BII population via scRNA-Seq (4 WT vs. 4 *Pax5*$^{+/-}$ mice) furthermore highlighted a preferential distribution of *Pax5*$^{+/-}$ cells into clusters with lambda light chain (IGL) rearranged B-cells.

Conclusion:

In summary, in mice, reduced Pax5 germline levels generate a deregulated pre-BII population in the BM, which displays delays in their BCR assembly and skewing towards the less commonly used IGL rearrangements.

P 55 - PALB2 gene mutation as a predisposition to tumorigenesis in childhood - Case report

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Background:

Positive oncological family history, as well as the development of second malignancy during childhood, require germline genetic testing for inherited cancer predisposition. Constitutional mutations in the *PALB2* gene encoding tumor suppressor are associated with an increased risk of ovarian, breast and pancreatic cancer, whereas biallelic ones cause Fanconi anemia.

Objectives:

We present the case of a patient with a genetic malignancy predisposition, positive oncological family history and the second malignancy during childhood.

Methods:

We analyzed the patient's medical history of diagnostics and treatment of Hodgkin lymphoma (HL) and Ewing sarcoma (ES).

Results:

The 8-year-old patient presented with multiple focal lesions in the spleen, a mediastinal tumor, weight loss, night sweats and a history of fever (39 degrees Celsius). One week before admission pharyngitis was treated with cefaclor. Her father suffered from HL at the age of 27 years. She was diagnosed with nodular sclerosis classical HL, IIIB grade, therapeutic group TL-3 according to EuroNET-PHL-C1 protocol. The patient was administered two chemotherapy cycles of OEPA with adequate response and good tolerance. At the administration of the 1st OEPA cycle, she presented an allergy to etoposide manifesting with rash and breathlessness. It was exchanged successfully for etopophos. She was administered 4 cycles of COPDAC-28 with good tolerance and achieved complete remission. She remained under the care of the outpatient children's oncology clinic.

At the age of 16, she was complaining of leg pain for 2 months and then low back pain for a week. The patient was administered analgesic drugs after a consultation with a surgeon. After exacerbation of symptoms, she was diagnosed with ES of the 6th left rib with metastases in vertebrae and the head of the left femur, without bone marrow involvement. According to Euro Ewing 2012 protocol Arm B, she underwent preoperative chemotherapy and radiotherapy targeted on metastases in vertebrae with good tolerance. The mobilization of hematopoietic stem cells was successful only in the second trial after a double dose of filgrastim. After the rib tumor resection, she continues postoperative chemotherapy cycles.

We performed whole exome sequencing that revealed a heterozygous mutation of unknown significance in the *PALB2* gene (NM_024675.4:c.110G>A;p.Arg37His) in the patient and her father. The detected mutation was previously seen in families with early breast and ovarian cancer history. The functional analysis of the mutation proved the impairment of the homologous recombination activity of PALB2 without the reduction of the PALB2-BRCA1 interaction. However, in the other family with similar *PALB2* mutation, i.e. heterozygous deletion in *PALB2* (NM_024675.3:c.(1684 + ?_1685-?)_(2586 + ?_2587-?)del) affecting exons 5 and 6, the child of the mother suffering from renal cell cancer presented acute lymphoblastic leukemia and ES. It allows us to suspect the following *PALB2* gene mutation as a pathogenic variant inherited via an autosomal dominant manner.

Conclusion:

Whole exome sequencing for cancer predisposition in patients with multiple malignancies is an effective tool to identify possible inherited background of the clinical course of disease which may affect clinical management.

The case of our patients with multiple neoplasms and positive family history provides another observation supporting the possible link between heterozygous constitutional defect of *PALB2* gene and the risk of lymphoid malignancies.

P 56 - Prenatal origin of PAX5 fusion genes in identical twins affected by infant BCP-ALL

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Background:

PAX5 fusion genes are part of PAX5 alterations, which are recurrent in pediatric and adult BCP-ALL.Interestingly, we recently reported forthe first time that PAX5 fusions are recurrent in patients with BCP-ALL in the first year of age and with germline MLL configuration (6 cases out of 30). Further, PAX5 rearrangements have a specific signature in pediatric BCP-ALL and are associated to poor outcome. Moreover, in the recent revision of WHO, PAX5 alterations have been included as provisional entity. Nowadays, no studies are available on the origin of this disease.

Objective:

To characterize the origin of PAX5 fusion genes in childhood ALL.

Methods:

RNAseq was performed by Universal RNA Plus with Nuquant kit (Tecan), on Nextseq550 (75x2). IG/TR screening was done by NGS (250x2, Miseq). Digital-MLPA (MRC-Holland) testing 55 ALL hot spot genes was performed to detect CNAs, PAX5 included. Whole Exome Sequencing (WES) by Nextera Flex for enrichment and IDT WES probes (150x2, Nextseq550).

Results:

We have analyzed 309 consecutive diagnoses of both T- and BCP-ALL enrolled in Italy in the current AIEOP-BFM ALL 2017 protocol from 01/12/2021 to 31/12/2022. Among the of BCP-ALL, we identified 17 cases as PAX5-rearranged (PAX5r) (5.5%). Strikingly, 2 of 17 cases were identical twins and both resulted positive to the translocation t(9;12), giving origin to the *PAX5::FBRLS1* fusion gene. They carried the fusion of the same exons. IG/TR screening identified 5 rearrangements in twin A and 6 in twin B, respectively and none of them was shared. The two identical twins were born from healthy consanguineous parents; no familiar history of cancer was reported. Patient A was diagnosed with B-ALL at 7 months of age; at time of diagnosis no hyperleukocytosis (WBC 70200/mmc) was detected; he was enrolled in AIEOP-BFM ALL 2017 protocol. At day + 8 he was considered as prednisone good responder (PGR); he was stratified as high risk according to MRD values. Two month later, patient B was diagnosed with B-ALL at 9 month of age; he presented with hyperleukocytosis (WBC 535000/mmc); he was also considered as a PGR and stratified as MR according to MRD values. Further, Patient A had heterozygous deletion of PAX5 exons 9 and 10, as well as homozygous deletions of CDKN2A/CDKN2B (only ex2 as homozygous deletion, ex1 as heterozygous) and VPREB1 was also deleted. Twin B also has the deletion of PAX5 exons 9–10, and both homozygous CDKN2B exons 1–2, as well as CDKN2A. RNAseq profile was investigated by ALL Catch R pipeline and associated their profile to PAX5alt class. We further investigated their genomic profile to assess whether cancer predisposing genes were affected, but we did not detect any variant by WES.

Conclusion:

The identification and description of the recurrent PAX5 fusions subgroup is fundamental to improve the accuracy ofstratification and recognition of their prognostic value. Here, we identified two identical twins carrying the *PAX5::FBRLS1* fusion gene with identical breakpoint at transcriptomic level (the cloning of genomic breakpoint is ongoing). We demonstrated here for the first time that PAX5 fusion genes may have a prenatal origin and can represent a driver event in predisposition to leukemia development in childhood B-ALL. Genomic data suggested that the translocation originated in an immature cell preceding IG/TR rearrangements and further biological studies will clarify the cell of origin of PAX5 rearranged leukemia.

P 57 - Identification of the epigenetic regulator H1-0 as a mediator of ETV6::RUNX1-induced transcriptional changes

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Introduction:

The translocation t(12;21)(p13;q22) is the most common chromosomal rearrangement associated with pediatric B cell precursor acute lymphoblastic leukemia (BCP-ALL) and results in expression of the chimeric transcription factor ETV6::RUNX1. *ETV6::RUNX1* is acquired before birth and induces a distinct preleukemic state that leads to a block of early B cell differentiation. In approximately 0.2% of children carrying the fusion gene, acquiring secondary oncogenic mutations during early childhood gives rise to overt leukemia. Characterization of *ETV6::RUNX1*$^+$ preleukemia is crucial to understand transformation to full-blown leukemia and to develop preventive strategies.

Aims:

In this study, we aimed to identify novel ETV6::RUNX1-mediated cellular pathways using human induced pluripotent stem cells (hiPSCs) that exhibit physiological expression levels of ETV6::RUNX1.

Methods:

Using CRISPR/Cas9-mediated genome editing, we generated monoclonal *ETV6::RUNX1*$^+$ hiPSCs derived from two donors with stable ETV6::RUNX1 expression driven by the endogenous *ETV6* promoter. To characterize the preleukemic state induced by ETV6::RUNX1, we performed whole transcriptome sequencing (RNAseq) of *ETV6::RUNX1*$^+$ and wild-type (WT) hiPSCs. Knock-down of H1-0 was performed in *ETV6::RUNX1*$^+$ REH cells (ACC 22, DSMZ, Braunschweig, Germany) using two siRNA pools and expression changes were evaluated by RNA-seq.

Results:

RNA-seq of *ETV6::RUNX1*$^+$ hiPSCs revealed a set of 20 significantly dysregulated genes compared to the respective WT hiPSCs (up: 15, down: 5, absolute fold change > 2, $p < 0.05$). We observed consistent upregulation of H1-0, a linker histone involved in chromatin remodeling, both on RNA (2.43fold increase) and on protein level assessed by immunoblot. H1-0 upregulation was corroborated in a published dataset of *ETV6::RUNX1*$^+$ hematopoietic stem cells (HSCs), *IL7R*$^+$ progenitor and pro-B cells compared to the respective reverted control cells and healthy fetal liver cells (Böiers *et al.* 2018, Developmental Cell). In addition to *ETV6::RUNX1*$^+$ preleukemia, we observed significantly upregulated H1-0 expression in *ETV6::RUNX1*$^+$ BCP-ALL patient samples compared to other leukemia entities in published gene expression datasets of pediatric and adult leukemia patients (St. Jude PeCan database (n = 876), Jerchel *et al.* 2018, Haematologica (n = 654), MILE study (n = 1299)).

Notably, we found that H1-0 levels decrease during early B cell development in normal bone marrow using published RNA-seq data (Black *et al.* 2018, Nucleic Acids Research). In line with this, analysis of single-cell RNAseq data of early B cell precursors derived from healthy bone marrow revealed that relative numbers of *H1-0*$^+$ cells per differentiation state decreased along the B cell development trajectory. Using cell cycle gene scoring, we observed that expression of H1-0 is primarily restricted to slow-cycling or quiescent cells.

Compared to control cells, REH cells silenced for H1-0 exhibited highly significant inhibition of the ETV6::RUNX1 upstream regulator signature revealed by Ingenuity Pathway Analysis (QIAGEN, Hilden, Germany; siH1-0 pool 1: $p = 3.73 \times 10^{-16}$, siH1-0 pool 2: $p = 3.54 \times 10^{-11}$).

Conclusion:

Altogether, our study identifies linker histone H1-0 as a key mediator of transcriptional changes in *ETV6::RUNX1*$^+$ preleukemia and BCP-ALL. As revealed by single-cell RNA-seq, upregulated H1-0 expression is associated with increased stemness and slow-cycling phenotype. This might offer an explanation for the persistence of clinically silent *ETV6::RUNX1*$^+$ clones residing in the bone marrow of approximately 5% of healthy children for years.

P 58 - A novel protac-mediated degradation model of ETV6::RUNX1+ preleukemia reveals inhibition of the ATF4-induced stress response pathway

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Background:

The translocation t(12;21) is the most common structural genomic alteration of pediatric B cell precursor acute lymphoblastic leukemia (BCP-ALL) and gives rise to the chimeric transcription factor ETV6::RUNX1. ETV6::RUNX1 acts as a weak oncogene with mainly repressive functions and binds to the same DNA motif as wild-type RUNX1. The fusion gene initiates a clinically silent preleukemic state that requires further mutations for complete leukemic transformation. Gaining a deeper understanding of ETV6::RUNX1-mediated cellular processes in the preleukemic state may lay the groundwork for developing prevention approaches.

Aims:

In this study, we set out to develop a novel *ETV6::RUNX1*$^+$ cell model that enables highly selective proteolysis targeting chimera (PROTAC)-mediated degradation of the ETV6::RUNX1 fusion protein. Using this model, we aim to identify novel cellular pathways specifically altered by ETV6::RUNX1.

Methods:

We transduced the BCP-ALL cell line NALM-6 (ACC 128, DSMZ, Braunschweig, Germany) with a lentivirus expressing an ETV6::RUNX1-Halo-HiBiT (E::R-HH) construct and selected successfully transduced, monoclonal cells in methylcellulose-based medium. Degradation of E::R-HH was performed by the addition of 0.25 µM HaloPROTAC3 or entHaloPROTAC3 (negative control compound) for two weeks, and expression changes were examined by RNA sequencing (RNA-seq) and proteome analysis.

Results:

Treatment of E::R-HH$^+$ NALM-6 with HaloPROTAC3 led to reproducible degradation of tagged ETV6::RUNX1. Notably, continuous HaloPROTAC3 treatment resulted in stable fusion protein levels of < 3% compared to entHaloPROTAC3 treatment, as revealed by using the Nano-Glo HiBiT Lytic Detection System (Promega). The high efficiency of E::R-HH degradation was confirmed by immunoblot. In line with the reported function of ETV6::RUNX1 as a repressor of transcription, we observed 3.3-fold higher numbers of significantly downregulated differentially expressed genes (DEGs) in entHaloPROTAC3-treated E::R-HH$^+$ NALM-6 cells compared to HaloPROTAC3 treatment (absolute fold change > 2, $p < 0.05$; 47 upregulated DEGs, 155 downregulated DEGs).

Ingenuity Pathway Analysis (QIAGEN, Hilden, Germany) of RNA-seq data identified activating transcription factor 4 (*ATF4*), a mediator of the integrated stress response pathway, as the most strongly inhibited upstream regulator in entHaloPROTAC3-treated E::R-HH$^+$ NALM-6 cells compared to cells with HaloPROTAC3 treatment (activation z-score = 3.83, $p = 1.71 \times 10^{-12}$). *ATF4* has recently been shown to be a direct target of wildtype RUNX1 (Matsumura *et al.* 2020, Blood). Interestingly, the ATF4 target gene asparagine synthetase (*ASNS*) was downregulated both on RNA (2.54-fold) as well as on protein level (1.16-fold) in entHaloPROTAC3-treated E::R-HH$^+$ NALM-6 cells, as revealed by our RNA-seq and proteome analysis. This is in line with the previously reported higher drug sensitivity for Lasparaginase of *ETV6::RUNX1*$^+$ BCP-ALL compared to *ETV6::RUNX1*$^-$ leukemias (Ramakers-van Woerden *et al.* 2000, Blood). In addition, *ATF4* and multiple of its target genes (e.g. *ATF3*, *DDIT3*, *HERPUD1* and *WARS1*) were found to be downregulated in *ETV6::RUNX1*$^+$ BCPALL patients compared to other leukemia entities or healthy bone marrow cells in a published expression microarray dataset (MILES study, Haferlach *et al.* 2010, Journal of Clinical Oncology).

Conclusion:

Using PROTAC-mediated degradation of ETV6::RUNX1, we identified specific inhibition of the ATF4-induced stress response pathway in *ETV6::RUNX1*$^+$ preleukemia and overt BCP-ALL. As shown for Lasparaginase, ATF4 and its target genes might be promising therapeutic vulnerabilities of *ETV6::RUNX1+* BCP-ALL.

**E.K., A.F. and V.H.J. contributed equally.*

P 59 - Oncogene induced senescence in the ETV6::RUNX1 pre-leukemic phase of childhood acute lymphoblastic leukemia

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Background:

t(12;21)(p13;q22) is the most frequent chromosome translocation in pediatric B cell precursor acute lymphoblastic leukemia. This alteration causes the formation of *ETV6::RUNX1 (E::R)* fusion gene which encodes for an aberrant transcription factor with constitutive repressive function. *E::R* alone is not sufficient to cause leukemia, but induces the formation of a clinical silent pre-leukemic clone. We previously demonstrated that *E::R* expression in pro-B cells causes the slowdown of cell cycle progression, which is a hallmark of the *Oncogene Induced Senescence* (*OIS*) phenotype. This mechanism is reported to promote the tumour transformation of pre-malignant cells.

Objectives:

The aim of this project is to understand whether *OIS* is involved in sustaining the *E::R+* pre-leukemic phase and subsequent tumor transformation. This knowledge could be helpful to develop therapeutic treatments to eradicate the pre-leukemic clone.

Methods:

The murine pro-B cell line Ba/F3 was transfected to generate an inducible E::R$^+$ pre-leukemic model, a gift of Dr A.M Ford (Centre for Evolution and Cancer, The Institute of Cancer Research, London, UK). The β-galactosidase activity was detected by Senescence β-Galactosidase Staining Kit (Cell Signaling Technology), while the levels of ROS and γH2AX were analyzed by Flow Cytometry. Molecules in the supernatant of E::R$^+$ and control cells were detected by Milliplex MAP Mouse Cytokine/Chemokine panel (Merck) or by ELISA assays. P53 protein was analyzed by western-blot. Cell viability was evaluated by Flow Cytometry using AnnexinV/7-AAD staining method.

Results:

We observed that, in addition to cell cycle slowdown, Ba/F3 E::R$^+$ cells exhibited several other markers of cellular senescence, such as altered morphology, increased β-galactosidase activity (mean SA-β-gal signal: ctr = 10.1%, E::R$^+$ =34.3%; p value = 0.004), overproduction of reactive oxygen species (ROS MFI: ctr = 299, E::R$^+$ = 520, p value = 0.01) and increased level of γH2AX (MFI: ctr = 395, E::R$^+$ = 1413, p value = 0.001), a marker of DNA damage. Moreover, in the supernatant of E::R$^+$ cells we detected molecules belonging to the Senescence-Associated Secretory Phenotype (*SASP*), such as PAI1, IL4, CCL2, CXCL1, CXCL5 and GM-CSF. Interestingly, by western-blot assay, we detected in the pre-leukemic cells a higher level of p53 protein, one of the most relevant proteins involved in senescence. We investigated p53 post-transcription modifications in basal and in apoptotic conditions: in pre-leukemic cells we detected the absence of serine 392 phosphorylation, a modification that promotes its mitochondrial translocation and thus induces apoptotic cell death. In accordance with this, we found that E::R$^+$ cells were more resistant to apoptosis induced by DNA damage-inducing stimuli, such as the drug etoposide and X-rays, than wt cells. Importantly, we observed that *in vitro* treatment with the SSK1 compound, a senolytic pro-drug specifically activated by β-Galactosidase, induced cell death of E::R$^+$ pre-leukemic cells, sparing the control cells.

Conclusion:

These results suggest that *E::R* expression is able to induce a senescent phenotype in proB cells and set the stage for further studies with the goal to develop novel therapy to eradicate the pre-leukemic cells and so to avoid leukemia development and lay the basis for preventive measures.

P 60 - Post-transplant lymphoproliferative disorders in pediatric solid organ transplant recipients - A single-centre experience

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Background:

Post-transplant lymphoproliferative disorders (PTLDs) significantly contribute to morbidity and mortality of pediatric solid organ transplant recipients and represent a clinical and diagnostic challenge.

Objectives:

To evaluate the incidence, diagnostics, management, and treatment outcome in patients who developed lymphoproliferative disorder following solid organ transplantation.

Methods:

Retrospective analysis of patients up to 18 years of age with histologically confirmed PTLD after solid organ transplantation who were diagnosed and treated in Motol University Hospital from 1999 to 2022.

Results:

Thirteen children with PTLD were identified after transplantation of liver (n = 6), kidney (n = 3), heart (n = 3), and lungs (n = 1). Male to female ratio was 7:6. Median age at transplantation was 6.08 years. Median time since transplantation to PTLD development was 4.67 years with the majority of the patients presenting with late-onset (> 2 years after transplantation) disease (n = 9). The most common symptoms at presentation were fever, abdominal pain, and lymph node enlargement. Five patients were classified as monomorphic disease (DLBCL n = 3, Burkitt lymphoma n = 2), 3 patients as classical Hodgkin lymphoma type PTLD, 4 patients as polymorphic PTLD, and one patient as T gamma/delta hepatosplenic lymphoma. In 10 patients the disease was EBV positive, one patient had EBV negative disease and in 2 patients the EBV status was unknown. Ten patients with CD20 positive disease were treated with either rituximab monotherapy (n = 4) or in combination with chemotherapy (n = 6), the remaining 3 patients were treated with chemotherapy alone. Seven patients reached long-lasting remission after the first line of therapy, 2 patients relapsed and required repeated administration of rituximab to reach remission, and 3 patients died of progressive disease. Since 2002, the patients' EBV viral load in peripheral blood was regularly monitored in the post-transplant period according to the standards of their treating physicians. Interestingly, in patients with late-onset disease, the development of lymphoproliferation was not linked to an elevation of EBV viral load in peripheral blood even in case of EBV positive disease.

Conclusion:

PTLDs represent a heterogeneous group of diseases. Though their pathogenesis is complex, EBV is known to play a central role in the majority of cases. Implementation of regular monitoring of EBV viral loads followed by reduction of immunosuppression or pre-emptive rituximab in patients with high EBV load had a positive impact on the patient outcome. The number of patients with EBV negative PTLDs or late-onset PTLDs, where the link with EBV is not so clear, is therefore proportionally increasing, which is also reflected in our cohort. There is an urgent need to identify predictive biomarkers and optimal treatment strategies in these patients.

P 61 - Early administration of Pembrolizumab in therapy of Nijmegen breakage syndrome patient with Hodgkin lymphoma as an effective treatment option with superior clinical tolerance

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Introduction:

The aim of this case study was to present NBS adolescent girl with Hodgkin lymphoma in whom a complete remission was achieved by early administration of humanized monoclonal anti-PD1 antibody, after poor tolerance of upfront reduced conventional chemotherapy.

Case presentation:

Seventeen years old girl, was treated in our institution with the diagnosis of classic Hodgkin lymphoma, mixed cellularity subtype, stage IV B. The patient presented with supraclavicular and jugular lymph nodes, as well as mediastinal and left lung involvement. Initially, she received 2 courses of chemotherapy according to AHOD 1331 protocol with 50% of doxorubicin and 33% of etoposide reduction doses due to the Nijmegen breakage syndrome. The vincristine was administered in full dose in the first course, which resulted in distress of bowel motility, thus the dose was reduced to 50% in the second course. Despite the reduced treatment, the response was satisfactory, although, she suffered severe toxicity with life-threatening complications. Thus, to prevent other adverse events (AEs), we decided to continue the treatment with the application of pembrolizumab. The doses were 2 mg/kg every 3 weeks and after 4th application, the PET/MRI showed complete remission, Deauville 1. In total, the patient received 12 courses and complete metabolic remission sustained on PET/MRI done at the end of the treatment. From January 2022, the patient started to have subjectively mild joint problems, which were further closely observed as a potential AE of immunotherapy. Gradually, the issue progressed in terms of chronic arthritis of both knees, right ankle, small joints of right hand and right shoulder. This issue was concluded as a suspicious AE of the immunotherapy, and immunosuppressive treatment by rheumatologist recommendations was started. At that time, treatment with Pembrolizumab was ended. After the last F/U control, the clinical symptoms of chronic arthritis significantly regressed and patient has remained in first complete metabolic remission of Hodgkin lymphoma, OS/EFS 19 months.

Discussion:

The NBS is characterized by an exceedingly high risk of haematological malignancies development with the poor outcome. The patients have lower tolerance to conventional chemotherapy and more often suffer from severe complications. Thus, the treatment management of lymphoproliferative disease in NBS is complicated and must be balanced considering the potential excessive toxicity and the effective malignancy treatment. The Hodgkin lymphoma is known to upregulate PD-1 ligands, which resulting from alterations in chromosome 9p24.1. Thus, the goal of PD-1 blockers administered in combination with chemotherapy was to create a favourable immune environment which was conducive to effective anti-tumour response. This approach appeared as an optimal with the excellent results considering malignancy. Nevertheless, an autoimmune disorder, such as chronic arthritis, is a potential AE of the immunotherapy, and needs to be diagnosed early and appropriately managed according the severity of AEs, which is, in the most severe cases, discontinuation of immunotherapy.

Conclusion:

The PD-1 blockers are suitable for the treatment of haematological malignancies associated with NBS and may significantly increase the survival rate of these patients. Although the possible AEs can be present during treatment, usually, they are milder and better tolerated in comparison to conventional chemotherapy.

P 62 - Infant extramedullary KMT2A rearranged T/myeloid acute leukemia mimicking Langerhans cell histiocytosis

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A previously healthy 3-month-old girl was admitted to the local hospital for dyspnea and tachypnea after a short history of rhinitis and low-grade fever. Chest X-ray revealed pneumomediastinum and blood count showed a leukemoid reaction. The patient was transferred to a specialized hospital where the respiratory insufficiency quickly progressed with the necessity of intubation and inotropic support; low-dose corticosteroids were administered. CT scan showed worsening of pneumomediastinum and massive bilateral bullous lung changes. The same day patient was transferred to the pediatric resuscitation unit of the University Hospital in Motol in critical condition and bilateral chest drainage was placed. On admission, WBC was 49,000/ul with 68% of neutrophils, 13% bands, and 1.5% of blasts. Biochemical results showed metabolic acidosis with pH 6.9, potassium 6.7 mmol/l, uric acid 537 umol/l, lactate dehydrogenase 63 ucat/l, and phosphate 3.9 mmol/l. On ultrasound, only mild hepatomegaly was presented. On the following day, WBC spontaneously decreased with no further leukocytosis or detectable blasts.

Bone marrow aspiration was performed the next day without evidence of leukemic cells by morphology. By flow cytometry 6% of atypical cells of T/myeloid immunophenotype were presented with these findings: strongly positive: CD7, CD33, CD4, HLA-DR; partially positive: CD117; weakly positive: intracellular CD3; negative: iTDT, CD34, CD19, iMPO. Genetic testing from the blood for BRAF mutation was negative. Bronchoalveolar lavage was performed with PCR positivity of Pneumocystis jirovecii and rhinovirus. By flow cytometry, 15% of atypical cells with CD117+CD33+CD4dimCD1a+CD7+ were detected. No BRAF mutation was detected.

The patient's condition started to worsen, with progressive multiorgan failure. For suspicion of Langerhans cell histiocytosis, corticoids (60 mg/m2) and trametinib (0.032 mg/kg) were started. Two days later the patient died of multiorgan failure (14 days after the admission to the hospital).

The FISH from the bone marrow revealed translocation t(9;11) in 6% of the cells. The autopsy determined the massive widespread infiltration by monocytic blasts predominantly in lymph nodes, bone marrow, liver, spleen, kidneys, colon, rectum and lungs, where bilateral cystic remodeling of tissue was also found. Immunohistochemistry revealed myeloid origin with co-expression of some T-cell markers.

These findings were confirmed by high positivity of KMT2A::MLLT3 fusion gene in various tissues. Next generation sequencing for clonal Ig/TCR rearrangements was negative. Whole exome sequencing for congenital lung abnormalities was performed with negative results.

In conclusion, we present an unusual onset of extramedullary KMT2A rearranged T/myeloid acute leukemia with low blast count in the bone marrow mimicking Langerhans cell histiocytosis with lung involvement.

P 95 - Report of a novel frameshift PAX5 germline variant in two siblings with B-cell Precursor Acute Lymphoblastic Leukemia

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Background and objectives:

PAX5 somatic mutations are frequently identified in patients with sporadic B-ALL, but recently pathogenic *PAX5* germline variants have been described in families with recurrence of B-ALL.

We describe the case of two siblings both affected by B-ALL with evidence of a new familial PAX5 variant. Case A was a male, 13 years old, diagnosed with B-II ALL, with no Central Nervous System involvement and negative for recurrent fusion genes. He was enrolled into the AIEOP-BFM-ALL2009 treatment protocol and stratified as medium risk according to minimal residual disease (MRD) levels. Eight years later, his sister (14yrs, named as case B) was diagnosed with B-II ALL, CNS negative, translocations-negative and stratified in the medium risk group of AIEOP-BFMALL2017 protocol. The two patients were otherwise healthy and born from healthy unrelated parents; no significant history of hematological malignancies was reported across the pedigree.

Methods:

We first performed a custom Next Generation Sequencing ALL predisposition panel on DNA extracted from bone marrow (BM) samples of both disease onset andremission phase (defined as negativity by PCR-MRD, 10-4 sensitivity). Subsequently, whole exome sequencing was performed on germline DNA.The libraries were performed following Nextera Flex for Enrichment and sequenced on Illumina Nextseq550 platform. The variants of interest were first validated and then investigated in the family pedigree through PCR and Sanger sequencing. Digital-MLPA (MRC-Holland) testing 55 ALL hot spot genes was performed. The B-cell repertoire on peripheral blood fresh mononuclear was evaluated in the healthy carriers cells by FACS analysis.

Results:

Through the NGS analysis, we showed that both patients shared a novel germline heterozygous *PAX5* variant (c.548delG, p.Gly183AlafsTer84); moreover, the disease samples of both patients carried an additional somatic *PAX5* variant (c.239C>G, p.Pro80Arg; variant fraction: 29% in Case A and 38% in Case B). In parallel, we performed digital-MLPA analysis using the ALL panel, which excluded the presence of shared copy number alterations (CNV) and, specifically, did not identified CNV alterations involving the *PAX5* locus. The familiar segregation showed a paternal origin of the germline *PAX5* variant (patients' father and grandmother); the mother was found to be wild-type.

To investigate a possible impairment of *PAX5* function in the development of the B-cell lineage, we evaluated in both parents the B-cell repertoire on peripheral blood fresh mononuclear cells by FACS analysis. Interestingly, the healthy carriers had a significant reduction on mature B-cells.

Conclusions:

We are reporting a novel germline PAX5 p.Gly183AlafsTer84 in a family with recurrence of B-ALL. In contrast to the previous PAX5 described cases that carry an inherited missense variant resulting in a modest attenuation of PAX5 activity, our patients present a germline frameshift variant that brings to premature stop of the protein, suggesting a more destructive impact on PAX5 activity. Moreover, as in the other reported cases, our patients presented an additional somatic event on PAX5 that further compromise PAX5 function, leading to the establishment of the leukemic clone. Interestingly, the two siblings of this family share the same PAX5 p.Pro80Arg somatic variant, that has been characterized as a pathogenic variant promoting leukemogenesis.

The identification and characterization of germline PAX5 variants is of high interest and importance in the context of leukemia predisposition because of its clinical implications in terms of familial genetic counseling and because of its contribution in the understanding ALL pathogenesis.

P 96 - Results of NGS analysis in AML pediatric patients from Ukraine

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Background:

Acute myeloid leukemia (AML) is a malignancy that accounts for approximately 20% of pediatric acute leukemias. The prognosis of pediatric AML has been improved last years, but it trails that of most other types of pediatric cancer, with mortality rates of 30–40%. Currently, the newest drugs for the treatment of acute myeloid leukemia are being actively developed and used, selectively aimed at cells with specific gene changes. Also, an identification of molecular markers unique to different stages of the disease can provide valuable information about disease prognosis.

The aim of our study was to use the possibilities of next-generation sequencing technology (NGS) for children with acute myeloid leukemia and other myeloproliferative diseases (MDS, CML, UMML) first time in Ukraine.

Methods:

Bone marrow (BM) were collected from 46 AML children at the time of initial diagnosis (42 cases) or relapse (4 cases). Samples of nucleic acids (DNA and RNA) was isolated from BM using solid phase extraction methods. BM samples were sent to be analyzed with Oncomine™ Myeloid Research Assay based on a 40 key DNA genes and a broad fusion panel of 29 driver genes, in an Ion GeneStudio S5™ System.

Results:

Among 69 genes sequenced by the NGS platform, a total of 23 abnormal genes and 15 fusions were identified in 52 patients. The expression of almost all chimeric oncogenes (fusions) and some of mutation, which was detect by the NGS method, was confirmed by routine methods (PCR, real-time PCR, MLPA, fragment analysis).

The most commonly mutated gene was *CEBPA* (30.43%), followed by *NRAS* (19.56%), *FLT3-ITD* (15.22%), *FLT3-TKD* (6.5%), *PTNN11* (8.7%) and *DNMT3A* (6.5%).

The data we obtained agree with The European LeukemiaNet (ELN) risk classification system and emphasizes the role of genetic alterations in AML, for example *FLT3-ITD*, *NPM1* and *CEBPA* mutations have been shown to have a significant impact on prognostic assessment and therapeutic strategies for patients with a normal karyotype.

Conclusion/Application to practice:

As follows, the study of spectrum of genetic changes in a cohort of pediatric patients with AML is a tool that allows doctors to stratify patients by risk groups and make a prognosis.

Host genome variants and their association with treatment efficacy and treatment toxicity in childhood ALL

P 63 - Therapy of lymphoid malignancy in children with chromosomal instability and primary immunodeficiency

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Nijmegen breakage syndrome (NBS) and Ataxia-telangiectasia (A-T) are primary immunodeficiency diseases (PID) associated with chromosome instability and DNA repair defects that predispose to cancer. We retrospectively analyzed clinical characteristics and outcomes of 28 cancer cases in 14 patients with AT and 10 patients with NBS, treated in Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology.

Mature B-NHL prevailed among all tumors – 42%, of which DLBCL was most common – 91%. Other cases included T-cell ALL, B-cell ALL, Hodgkin lymphoma, NK/T-cell lymphomas, T-cell lymphoblastic lymphoma and peripheral T-lymphomas, 2 cases of T-cell prolymphocytic leukemia, 1 case of medulloblastoma and 1 case of epithelioid rhabdomyosarcoma.

In 62% of cases in patients with AT and in 100% of cases in patients with NBS the diagnosis of PID was suspected or confirmed before the start of chemotherapy. The therapy was carried out according to standard protocols with dose modifications.

Among patients with AT, dose reduction of chemotherapy drugs was carried out in 93% of cases, among patients with NBS – in 80%. The response level was quite high: 81%patients with AT and 58% patients with NBS achieved CR.

According to our data, the use of therapy regimens with reduced doses of chemotherapy drugs allows us to obtain a satisfactory toxicity profile without reducing the overall effectiveness of treatment.

P 64 - KSR2 is novel candidate oncogene regulated by miR-143-3p and promoter methylation in T-cell acute lymphoblastic leukemia

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Background:

T-cell acute lymphoblastic leukemia (T-ALL) is a heterogeneous and aggressive malignancy arising from T-cell precursors. miRNAs are implicated in negative regulation of gene expression and, when aberrantly expressed, contribute to various cancer types, including T-ALL. Previously we demonstrated the decreased level of miR-143-3p in pediatric and adolescent/young adult T-ALL patients compared to healthy controls. Here, we show that the tumor suppressor activity of this miRNA in T-ALL cell lines is mediated by *KSR2* gene, a positive regulator of RAS pathway. We also show that the expression of *KSR2* in T-ALL patients may depend on the methylation status of CpG islands in promoter region of this gene.

Methods:

2 T-ALL cell lines with low endogenous miR-143-3p expression, JURKAT and ALL-SIL, were transduced for constitutive overexpression of this miRNA. Flow cytometry-based GFP competition assay was used to assess the effect of miR-143-3p on cell growth. Ago2 RNA immunoprecipitation (RIP) followed by RNA-seq was used for miR-143-3p targetome profiling. RT-qPCR and Western Blot were used to evaluate the mRNA and protein level of KSR2. CRISPR inhibition (CRISPRi) with dead Cas9-KRAB construct was used to assess the effect of *KSR2* repression in T-ALL cell lines. RNA sequencing (RNA-seq) was performed in 48 pediatric T-ALL patients using TruSeq Stranded mRNA library protocol and IlluminaNovaSeq6000 platform, with paired-end sequencing of 150 nt reads, read depth of coverage: 150 million reads/sample. DNA methylation levels in 48 T-ALL patients were obtained using Infinium MethylationEPIC BeadChip.

Results:

We showed the loss of growth advantage in T-ALL cell lines upon overexpression of miR-143-3p, pointing to its tumor suppressor activity. Next, using a combined RIP-seq and RNA-seq approach, we unraveled the spectrum of direct miR-143-3p target genes as well as its global effect on the transcriptome of T-ALL cells. We identified *KSR2* as direct target of miR-143-3p of potential importance for T-ALL biology. KSR2 is a positive regulator of RAS pathway, which is overactivated in many cancers and related to oncogenic transformation. We confirmed the decreased KSR2 protein level in both T-ALL cell lines upon miR-143-3p overexpression. Since the oncogenic role of KSR2 has not been previously studied in T-ALL, we evaluated the effect of its CRISPRi-mediated inhibition on the survival of T-ALL cells *in vitro*. We observed that loss of *KSR2* expression leads to the loss of growth advantage of T-ALL cell lines and thus phenocopies the effect of miR-143-3p overexpression. Integrative analysis of RNA-seq and methylation array data for T-ALL patients additionally unraveled an inverse correlation between expression and methylation status of *KSR2*.

Conclusions:

miR-143-3p is downregulated in T-ALL patients and exhibits tumor suppressor activity by decreasing the growth of T-ALL cells, upon forced overexpression *in vitro*. Its effect is at least partially mediated by negative regulation of *KSR2. KSR2* is a novel candidate oncogene in T-ALL. Its inhibition leads to decreased growth of T-ALL cells *in vitro*. We propose two mechanisms of *KSR2* overexpression in T-ALL patients: by downregulation of miR-143-3p and additionally be demethylation of its promoter.

P 65 - Pharmacogenetic pilot study focused on the toxicity in AML and ALL pediatric patients

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Background / Objectives:

In pediatric cancer, 62.3% of pediatric cancer patients suffer at least one chronic adverse effect due to therapy that affects their quality of life in the short and long term. Although treatments have increased the 5-year survival rate of more than 90% of children with acute lymphoblastic leukemia over the past 20 years, chemotherapy-related toxicities can lead to underdosing, interruption or discontinuation of treatment, influencing the treatment results. In this sense, pharmacogenetics (PGx) tries to identify germline genetic variants associated with efficacy and toxicity in order to contribute to improving therapeutic decisions. The aim of this study is evaluating if the proposed genetic variants have a real impact on the chemotherapy toxicity outcomes.

Methods:

This descriptive observational study is part of a larger project where 274 patients with different types of tumor were genotyped. In this analysis, 35 patients diagnosed with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) belonging to the Pediatric Oncology Unit of Hospital La Fe were included. A PGx panel containing 63 SNPs was designed including those with relevant Clinical Annotations included in the Pharmacogenomics Knowledge Base (PharmGKB), the data sheet of the FDA and EMA agencies, recommendations from the Clinical Implementation international consortia and literature. The samples were analyzed using the AgenaBioscience MassArray platform at CEGEN-Santiago de Compostela. Clinical data were retrospectively collected form Electronic Medical Records and classified according to CTCAE v4.0 during induction and consolidation.

Results:

From the 35 patients analyzed, 91.43% presented at least one type of toxicity with a grade equal to or greater than 3 according to the common terminology criteria for adverse events (CTCAE v.4.0). Regarding analytical toxicity, 91.43% presented neutropenia; 82.85% anemia, 85.74% decreased platelet count, 57.14% increased alanine aminotransferase; 28.57% increased aspartate aminotransferase; 11.43% increased gamma-glutamil transferase and 2.86% increased blood bilirubin. Regarding infections, 65.71% presented febrile neutropenia; 31.43% infection; 11.43% sepsis and 2.86% fever or multiple organ failure. At the digestive level, 28.57% presented mucositis, 8.57% rectal mucositis and 5.71% diarrhea, nausea or vomiting. For both rash-type allergy and cardiac toxicity, the percentage was 2.86%. Finally, 5.71% of the patients presented respiratory toxicities (dyspnea and hypoxia). 100% of these patients presented at least one pharmacogenetic risk variant located in genes that participate in the metabolism and/or transport of the drugs administered for their treatment of ALL and AML. Table 1 shows the percentage of patients who have had a toxicity event and who have a pharmacogenetic risk variant associated with the treatment they have received.

Conclusion/Application to practice:

The high percentage of patients presenting risk variants in pharmacogenes associated with the treatment they receive is an indication that for those drugs, PGx may be a useful tool when defining a therapeutic regimen in pediatric patients with ALL or AML. Further multicenter studies will be necessary to confirm these results.

Table 1:

Drug	Gene	Variant	Risk genotype	Patients with risk genotype
Azathioprine,	*TPMT*	rs1142345	TC, CC	9,38%
Mercaptopurine,		rs1800460	CT, TT	9,38%
Thioguanine				
Cyclosphosphamide	*GSTP1*	rs1695	GG	75%
	SOD2	rs4880	AG, GG	71,88%
	TP53	rs1042522	CG,GG	40,63%
Cytarabine	*ABCB1*	rs1045642	GG	34,38%
Etoposide	*DYNC2H1*	rs716274	AG, GG	75%
Methotrexate	*ABCB1*	rs1045642	AG, AA	78,12%
	ATIC	rs4673993	TT	37,5%
	MTHFR	rs1801133	AA, AG	62,5%
	MTRR	rs1801394	AG, GG	81,25%
	SLCO1B1	rs11045879	CT, TT	93,75%
Prednisone	*ABCB1*	rs1045642	GG	34,38%
Peg- asparaginase	*SOD2*	rs4880	AG, GG	71,88%

P 66 - Short-term prognostic impact of disease-related gene fusions and copy number aberrations in children with acute lymphoblastic leukemia

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Background:

Despite the improved stratification and well-established protocols for the treatment of childhood acute lymphoblastic leukemia (cALL), around 15% of patients still experience a relapse with a significant decline in overall survival.

Objectives:

To evaluate the prognostic impact of specific genetic aberrations of cALL patients including genes involved in B/T-cell development, cell cycle control, and hematopoiesis with an aim to identify a subset of patients with the worst prognosis which may potentially benefit high-risk protocols.

Materials and methods:

A total of 33 patients (28 with B-cell, 5 with T-cell ALL, aged 2–11) were analyzed at diagnosis. An automatic DNA and RNA extraction were performed from bone marrow mononuclear cells on MagCore Super. All patients were tested for the most common gene fusions occurring in ALL including t12;21 (p13;q22) - ETV6-RUNX1; t1;19 (q23;p13) - E2A(TCF3)-PBX1; t9;22(q34;q11) - BCR-ABL1; t4;11 (q21;q23) - MLL-AFF1 and del(1)(p32)-SIL/TAL1, using qRT-PCR (BIOMED-1 protocol). In addition, MLPA analyses were performed on all diagnostic samples using 3 SALSA MLPA kits (MRC-Holland, Amsterdam, the Netherlands) which detect copy number aberrations in different genes or regions including iAMP21, EGR, PAR1 (CRLF2, CSF2RA, IL3RA), IKZF1, CDKN2A/2B, PAX5, ETV6, RB1, BTG1, and EBF1. All patients were treated according to the ALL-IC-BFM 2002 protocol. The median follow-up of the patients was 12 months (range 1–40).

Results:

Overall, we detected gene fusions in 8 (24%) patients, of which half with the favorable ETV6-RUNX1 fusion, 2 patients with TCF-PBX1, and 1 patient each with BCR-ABL p190 and SIL-TAL1 fusions. A gain of both chromosome X and 21 was observed in 12 (36%) patients, suggesting a possible hyperdiploidy. Three patients (9%) had a deletion of the IKZF1 gene (one of them also characterized with the TCF3-PBX1 fusion), but none had the IKZF1^{plus} genotype (additional deletions in either CDKN2A/2B, PAX5 or PAR1 genes). CDKN2A/2B deletions were found in 11 (33%) patients (6 as heterozygous and 5 as homozygous deletions), along with either PAX5, BTG, RB1, EBF1, or ETV6 deletions in 6 of them. Only 1 patient had a CDKN2A/2B with concomitant PAX5 amplifications. PAX5 deletion was observed in 4 (12%) patients and co-occurred with CDKN2A/2B deletion in 3 of them. An isolated deletion of only one of the PAR1 genes (IL3RA) was detected in 4 (12%) patients. None of the patients presented iAMP21 or ERG deletion. Relapse occurred in 3 patients with B-ALL of which one had BCR-ABL p190 fusion and CDKN2A/2B and PAX5 deletions, the second had CDKN2A/2B, EBF1, and ETV6 deletions and the third had only a possible hyperdiploidy genotype. Two patients (both with T-cell ALL, one of them having CDKN2A/2B deletion) died during induction. Two out of three patients with an IKZF1 deletion had early MRD clearance after treatment with an intermediate-risk protocol and are currently in remission after a follow-up of 25 and 29 months, while the third patient is still receiving induction therapy. All data are summarized in Figure 1.

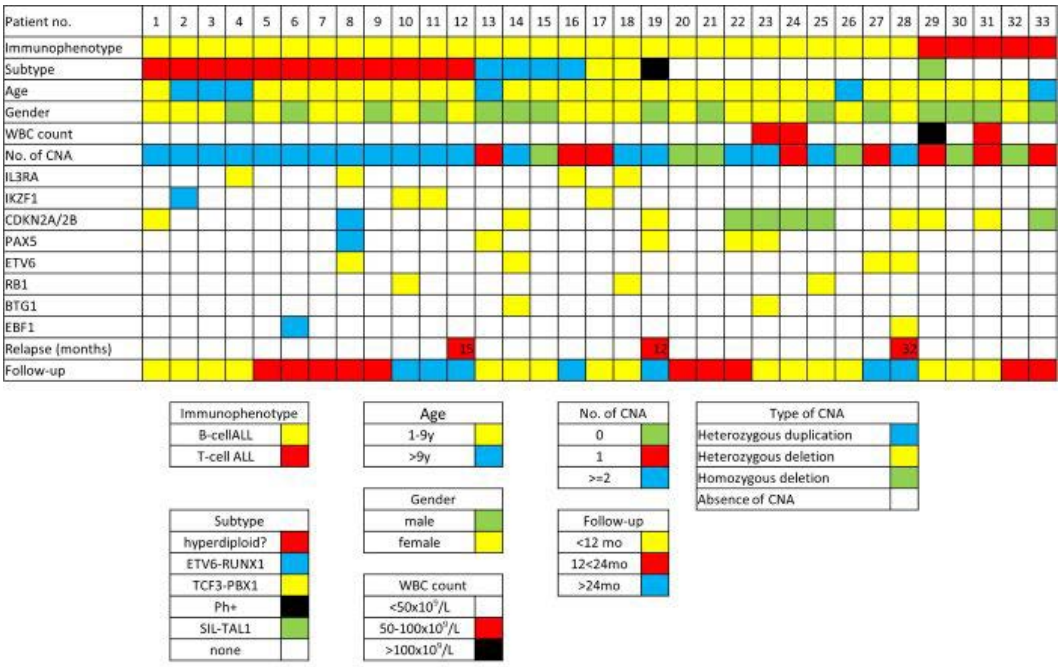

Figure 1: The heatmap of major subtypes of cALL and the associated copy number alterations (CNA).

Conclusion:

Our data indicate that IKZF1, CDKN2A/2B or PAX5 deletions are not independent prognostic factors associated with inferior outcome after a short follow-up period. The prognostic impact of these markers, as well as the influence of various combinations of the other tested markers, should be evaluated on a larger cohort of patients with a longer follow-up period.

P 67 - Thrombotic events in children with acute lymphoblastic leukemia: Experience of Italian AIEOP centers (2009–2017)

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Thrombotic events (TE) in children treated for acute lymphoblastic leukemia (ALL) are reported in 1- 37%, depending on their severity. TE impact on scheduled asparaginase treatment and may consequently have a negative effect on prognosis in children with ALL.The aim of our study was to evaluate the epidemiology and clinical relevance of TE among 2098 Italian patients enrolled in AIEOP BFM ALL 2009 protocol from October 2009 to February 2017. We included all the arterial and venous TE classified as grade 2 or more, according to Ponte di Legno Toxicity Working Group (PdLTWG) definition, confirmed by imaging (ultrasound, computed tomography or magnetic resonance). Twenty-three out of 38 Italian AIEOP centers reported about TE. Sixty-two TE were reported in total, with a 2-year cumulative incidence of 3%. The incidence varied from 0% to 6,7%, according to single center's reporting.Thirty-two (52%) were cerebral vein thrombosis (CVT), 28 (45%) were systemic deep vein thrombosis (SVT) (9 in the neck, 11 in the upper limb, 8 in the lower limb vessels) and 2 (3%) were in the right atrium. No arterial thrombosis was reported. All but one systemic thromboses were related to venous catheter.SVT were significantly more frequent in boys (OR 2.22, 95%IC: 0.99–4.97, p-value = 0.048), while no gender differences were observed in CVT. Both CVT and SVT were observed more frequently in children older than 10 years (p-value = 0.0005 and 0.0003, respectively) and in T-ALL (p-value = 0.0013 and 0.047, respectively).Meningeal involvement at diagnosis was significantly associated with risk of CVT (OR: 2.74, 95%IC: 1.21–6.17, p-value = 0.01).Obesity, mediastinal mass, blood group, initial white blood cell count and risk group were not significant risk factors.Familial thrombophilia was identified in 2 of 41 cases, no statistical association was found.TE occurred more frequently during induction phase (39 cases) (OR: 3.05, 95%IC: 1.56–7.65, p-value = 0.03).Initial treatment of TE consisted in heparin (5 unfractionated and 55 low molecular weight heparin), 2 children were treated with recombinant tissue-type plasminogen activator for symptomatic subclavian vein thrombosis. Median treatment duration was 4.5 months for SVT and 5 months for CVT. Secondary prophylaxis was administered to 55% of patients (61% of CVT and 48% of SVT).Three children experienced a second TE, two had been re-exposed to L-asparaginase and one was receiving LMWH. One patient died of subsequent cerebral hemorrhage; symptoms resolved in all other patients.Long term sequelae were observed in 8 patients (12,5%): 5 with CVT (3 patients had seizures requiring anticonvulsant therapy, 1 motor weakness, 1 cognitive impairment) and 3 with SVT (post thrombotic syndrome). Chemotherapy was modified in 21 patients (33%).

TE reported in recent Italian protocol are rare (3%) and occur more frequently in T-ALL, boys, older children and in initial meningeal involvement. SVT are mostly related to venous lines; clinical impact is significant especially in CVT. Primary prophylaxis was not suggested; it was administered only in a girl with CVT. Prophylaxis and treatment strategies should encompass new evidence from randomized studies and the use of new oral anticoagulant drugs.

P 68 - KRAS deletions are associated with fast treatment response in ETV6::RUNX1 Leukemia

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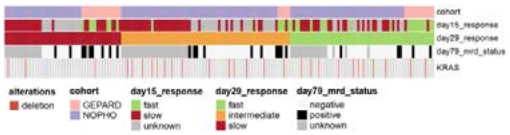
Acute lymphoblastic leukemia (ALL) is the most common cancer in children, with about 3500 children diagnosed yearly in Europe. A quarter of cases harbor the ETV6::RUNX1 (E/R) fusion gene. E/R leukemias usually have a good initial treatment response and are classified into low-risk group. However, a fraction of patients still encounter disease recurrence. The increased relapse risk has been linked to suboptimal initial therapy response measured by minimal residual disease (MRD). The aim of this study was to identify genetic differences on E/R cases stratified by MRD.

We performed whole-genome sequenced (WGS) of E/R leukemia cases in a discovery cohort of 35 patients treated according to the NOPHO ALL2008 protocol. Moreover, deep targeted sequencing was applied to validate caller accuracy and matched RNA-sequencing to analyze expression status of the mutated pathways. Expression data was complemented with single cell sequencing data from Mehtonen et al. (2020). Additional copy number analysis from the larger NOPHO cohort (N = 143) was used to validate candidate genes altered in the discovery cohort. Variant callers available in the Balsamic workflow were used to detect DNA alterations (Foroughi, 2019). Finally, target genes affecting the response to vincristine, cytarabine, methotrexate, L-asparaginase, daunorubicin and 6-mercaptopurine were retrieved from genome-wide CRISPR-screen performed in REH cell line (Oshima 2020), and compared to mutation findings in our cohort.

Overall, the number of single nucleotide variants (SNVs), indels and structural variants (SVs) were comparable between the MRD categories. Among the recurrent DNA alterations affecting driver genes, deletion of KRAS correlated with faster treatment response by day 29 MRD, and this finding was further validated using the larger Nordic cohort (p = 0.037). Association with favorable response was similarly evident on day 79, KRAS deletions correlating with negative MRD at end of consolidation (p = 0.038) (Figure 1).

Figure 1: Status of treatment response-correlating KRAS deletion in validation cohort.



Number of immunoglobulin kappa gene rearrangements correlated with treatment response with slow responders having more frequent rearrangements suggesting possible differences in cell differentiation stages by responder groups. Based on the mutation signature analysis, a significant negative correlation of MRD level at day 15 was found with Signature 2 (APOBEC), while day 29 MRD correlated positively with Signature 3 (failure of DNA double-strand repair).

Finally, multiple DNA alterations were identified in genes that were associated with drug sensitivity or resistance in REH cells, including NT5DC1 deletions sensitizing to methotrexate in 2 patients with deep response to methotrexate containing consolidation therapy. Moreover, fast responders appeared to have more vincristine sensitizing mutations compared to slow responders (p-value = 0.04).

In summary, we characterized genetic features associated with therapy response in E/R leukemia and identified several candidate genes which were linked to drug sensitivity or resistance if affected by DNA alterations. This type of data can be utilized in the design and implementation of personalized therapy in E/R ALL in future.

P 69 - Second malignant neoplasms after treatment of 1487 children and adolescents with acute lymphoblastic leukemia - Population-based analysis of the Austrian ALL-BFM Study Group

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Second malignant neoplasms (SMN) after primary childhood acute lymphoblastic leukemia (ALL) are rare. Among 1487 ALL patients diagnosed between 1981 and 2010 in Austria, the 10-year cumulative incidence of an SMN was 1.1% ± 0.3%. There was no difference in the 10-year incidence of SMNs with regard to diagnostic-, response- and therapy-related ALL characteristics except for a significantly higher incidence in patients with leukocytes ≥50.0 G/L at ALL diagnosis (2.1% ± 1.0% vs. 0% for 20.0– 50.0 G/L, and 1.0% ± 0.3% for < 20.0 G/L; p = 0.033). Notably, there was no significant difference in the incidence of SMNs between patients with or without cranial radio- therapy (1.2% ± 0.5% vs. 0.8% ± 0.3%; p = 0.295). Future strategies must decrease the incidence of SMNs, as this event still leads to death in one-third (7/19) of the patients.

	Hematologic SMN	Central nervous system tumors	"Other" SMN
Number of patients	6	9	4
Male:female ratio	4:2	3:6	2:2
Median age at ALL (years)	8.1	3.4	6.1
Range (years)	3.3–11.7	1.5–11.2	3.1–15.4
BCP-ALL	6	8	2
T-ALL	0	1	2
Median time to SMN (years)	3.5	10.2	9.6
Range (years)	2.5–4.5	3.6–22.8	3.8–20.2
Median age at SMN (years)	11.8	14.4	18.9
Range (years)	6.5–14.2	6.7–33.2	7.1–18.7

Abbreviations: BCP-ALL, B-cell precursor ALL; SMN, secondary malignant neoplasms.

Picture: Characteristics of the three secondary malignant neoplasm subgroups.

P 70 - Piperacillin pharmacokinetics in pediatric oncology patients

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Introduction:

After the chemotherapy, a common complication is the development of neutropenic fever. In these cases the extended spectrum β-lactam-β-lactamase inhibitor combination, piperacillin–tazobactam, is frequently used as empiric therapy. The efficacy of piperacillin–tazobactam is time dependent and correlate with the time the drug concentration exceeds the minimum inhibitory concentration (MIC) of the infecting pathogen (T>MIC).

Objectives:

The efficacy of the empirically used piperacillin tazobactam was investigated by measuring serum levels and performing comprehensive pharmacokinetic calculations. Three dosing regimens were compared: 100 mg/kg piperacillin infusion over 1 hour every 8 hours, 100 mg/kg infusion over 3 hours every 8 hours and 75 mg/kg infusion over 1 hour every 6 hours.

Methods:

A total of 100 piperacillin and tazobactam concentrations were obtained from 20 children. By reviewing the positive hemocultures registered in previous years, we determined the MIC of the most common pathogen in our Clinic. Antibiotic concentrations were measured by HPLC (high performance liquid chromatography) and datas were analyzed using the Pmetrics program.

Results:

In terms of bactericidal effectiveness, all three dosage forms are equally effective at a MIC value of 4 mg/L. At MIC values of 8 and 16 mg/L, on the other hand, the antibiotic given for a longer time, in a 3-hour infusion, was significantly more effective.

Conclusion:

Due to the time dependent effect, the bactericidal activity of the antibiotic may increase with increasing T> MIC, suggesting that in our investigational groups the 3x100 mg / kg / 3 hour piperacillin-tazobactam infusion has been shown to be the most effective.

P 71 - Successful treatment of T-ALL with ZBTB16-ABL1 fusion in a boy with Nijmegen breakage syndrome carrying a germline SLC22A1 gene mutation

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Background:

Apart from the *BCR-ABL1* fusion gene, *ZBTB16-ABL1* was identified as another leukemogenic driver promoting cell-cycle progression and DNA replication in T-cell acute lymphoblastic leukemia (T-ALL). Both fusion kinases showed sensitivity to tyrosine kinase inhibitor (TKI) imatinib and dasatinib. It is speculated that human organic cationic transporter 1 (hOCT1), encoded by the *SLC22A1* gene, is implicated in imatinib cellular uptake. The efficacy of imatinib influx via hOCT1 may result in inter-patient imatinib response variability. Contrarily, dasatinib achieved better *BCR-ABL1* suppression and sufficient intracellular levels even in patients with impaired hOCT function.

Objectives:

The T-ALL diagnosis based on clinical, histological, and immunological evaluation of lymph node specimens and the bone marrow, was set in a 14-year-old Nijmegen breakage syndrome patient, bearing homozygous *NBN* c.657_661del/p.K219fs mutation. Targeted RNA sequencing revealed the *ZBTB16-ABL1* gene fusion. The introduction of imatinib at the dose of 340 mg/m^2 resulted in promptly progressing imatinib-related toxicity marked by abdominalgia, myalgia, and generalized rash as MRD remained high. In order to explain extremely high plasmatic imatinib levels reaching 10 000 mg/L (ref. range. 1100 mg/L), drug interactions and toxicities, imatinib dosing error, and CYP3A4 slow metabolizer variant were considered as causative but subsequently ruled out.

As the salvage treatment options for NBS patients with T-ALL remain spare, a prompt conversion from the TKI imatinib to dasatinib was performed. Simultaneously focuse was set on the molecular basis of abnormal imatinib pharmacokinetics.

Mutational analysis of all coding regions of *SLC22A1,* the hOCT1 encoding gene, revealed two heterozygous variants, *SLC22A1* c.480G>C/p.L160F and c.1201G>A/p.G401S. The L160F variant was previously connected to imatinib resistance in patients suffering from chronic myeloid leukemia.

The conversion from imatinib to dasatinib led to the resolution of tyrosine kinase inhibitor toxicity symptoms, together with gradual complete molecular remission after dasatinib introduction based on regular RT-PCR-based MRD monitoring.

Conclusion:

Successful treatment course was based on prompt reflection on RT-PCR MRD monitoring results and on early regular drug toxicity evaluation.

The patient is alive, and in good clinical state. The ongoing maintenance treatment demands a 50% dose reduction of methotrexate and mercaptopurine in contrast to a full tolerance of dasatinib dose in the NBS T-ALL patient.

The underlying DNA double-strand repair defect seemed unlikely to cause isolated increased imatinib toxicity in parallel with excellent, TKI adverse event-free, dasatinib response.

Although the disclosed OCT1 (*SLC22A1)* heterozygous mutations' impact on imatinib pharmacokinetics remains unclear, in general the knowledge of genetic variants of cellular drug transporters could contribute to an explanation of some inter-individual variabilities in treatment responses and toxicities.

Acknowledgment:

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Therapy and outcome of acute leukemias and non-Hodgkin lymphomas in low- and middle-income countries

P 72 - A continuous PEGasparaginase dose schedule reduces inactivating hypersensitivity reactions and does not increase toxicity in children with Acute Lymphoblastic Leukemia: A DCOG ALL11 randomized trial

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Background:

Asparaginase is key in the treatment of pediatric acute lymphoblastic leukemia (ALL). Inactivating hypersensitivity reactions are one of its major side effects. The majority of these reactions occur after an asparaginase-free interval.

Objectives:

We performed a randomized study to determine whether a continuous dosing schedule (without an asparaginase-free interval) would result in less hypersensitivity reactions to PEGasparaginase compared to a non-continuous dosing schedule (with an asparaginase-free interval). Secondary endpoints were toxicity and outcome.

Methods:

All patients were treated according to the Dutch Childhood Oncology Group ALL11 protocol (EudraCT 2012–000067–25) and received three PEGasparaginase doses of 1,500 IU/m^2 intravenously at day 12, 26 (protocol IA) and 40 (protocol IB). Medium risk (MR) patients without a clinical contraindication for receiving PEGasparaginase were eligible for randomization. After randomization, patients received 14 individualized PEGasparaginase doses at the start of MR intensification (standard arm: non-continuous schedule) or received 14 doses in a continuous schedule following the third dose in protocol IB (experimental arm: continuous schedule).

Three different types of hypersensitivity were defined: 1) allergies: allergic reactions with inactivation of PEGasparaginase, 2) allergic-like reactions: symptoms of an allergic reaction without inactivation of PEGasparaginase and 3) silent inactivation: inactivation of PEGasparaginase without symptoms of an allergic reaction. Inactivating hypersensitivity is the sum of allergies and silent inactivations.

We collected other asparaginase-related toxicities (CTCAE>grade 3), like infection, pancreatitis, thromboembolic event, avascular necrosis and hypertriglyceridemia, per treatment phase. Trough asparaginase activity, anti-PEG and -*E.coli* asparaginase IgG and IgM antibodies were measured. Outcomes of both treatment arms were compared.

Results:

In induction, hypersensitivity reactions occurred in 27 out of 818 patients (3.3%) (Table 1). 157 patients were randomized to the non-continuous arm and 157 to the continuous arm. After randomization, 17 (10.8%) patients in the non-continuous treatment arm versus 4 (2.5%) in the continuous treatment arm ($p < 0.01$) had a hypersensitivity reaction, of which 13 (8.3%) versus 2 (1.3%) were inactivating hypersensitivity reactions ($P < 0.01$). 14 out of the 17 (82%) hypersensitivity reactions in the non-continuous arm occurred on the first dose after asparaginase-free interval.

The formation of total antibodies and total anti-PEG was significantly higher in the non-continuous treatment arm over time than in the continuous treatment arm ($P < 0.01$) (Figure 1). The same antibody levels were also significantly higher for patients with inactivating hypersensitivity than patients without inactivating hypersensitivity on the fourth dose (first post-randomization) ($P < 0.05$).

There were no significant differences in total number of asparaginase-associated toxicities between treatment arms. However, the timing of the toxicities was associated with the timing of the asparaginase administrations. During protocol M, there were 14 (8.9%) infections in the non-continuous arm and 29 (18.6%) infections in the continuous group. Whereas, pancreatitis (8(5.1%) non-continuous versus 1(0.6%) continuous) and thromboembolic events (13(8.4%) non-continuous versus 3(2.0%) continuous) more frequently occurred in the non-continuous arm during intensification and maintenance.

With median follow-up of 4.9 years, 5-year CIR was 4.0% (SE 1.8%) for non-continuous treatment versus 6.2% (SE 2.2%) for continuous treatment ($P = 0.526$), and 5-year EFS was 95.3% (SE 1.9%) for non-continuous treatment versus 91.3% (SE 2.5%) for continuous treatment ($P = 0.214$).

Conclusion:

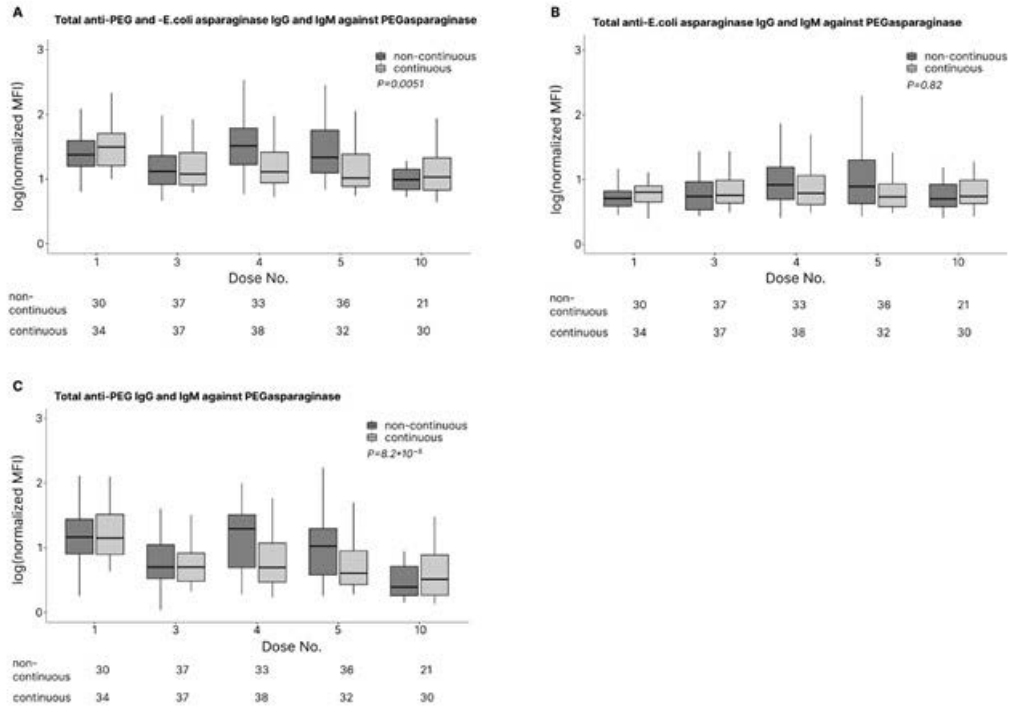
We conclude that a continuous dosing schedule of PEGasparaginase significantly reduced the inactivating hypersensitivity rate and antibody formation compared to a non-continuous schedule. The continuous schedule of asparaginase treatment did not increase toxicity but changed the timing of toxicity. Finally, the continuous schedule did not change the efficacy of the therapy.

Table 1: Proportion of hypersensitivity against PEGasparaginase per treatment arm.

	Before randomization		After randomization (post-induction)		
	Induction (n=818)		non-continuous arm (n=157)	continuous arm (n=157)	*P*
Allergies,	12 (1.4%)		8 (5.1%)	2 (1.3%)	
Dose no. 1	*6 (0.7%)*	*Dose no. 4*	*7 (4.6%)*	*1 (0.6%)*	
Dose no. 2	*0 (0.0%)*	*Dose no.5*	*1 (0.6%)*	*1 (0.6%)*	
Dose no. 3	*6 (0.7%)*	*Dose no. 6-17*	*0 (0.0%)*	*0 (0.0%)*	
Allergic-like Reaction	8 (1.0%)		4 (2.5%)	2 (1.3%)	
Dose no. 1	*6 (0.7%)*	*Dose no. 4*	*3 (1.9%)*	*0 (0.0%)*	
Dose no. 2	*0 (0.0%)*	*Dose no.5*	*0 (0.0%)*	*1 (0.6%)*	
Dose no. 3	*2 (0.2%)*	*Dose no. 6-17*	*1 (0.6%)*	*1 (0.6%)*	
Silent Inactivation	7 (0.9)		5 (3.2%)	0 (0.0%)	
Dose no. 1	*3 (0.4%)*	*Dose no. 4*	*4 (2.5%)*	*0 (0.0%)*	
Dose no. 2	*2 (0.2%)*	*Dose no.5*	*1 (0.6%)*	*0(0.0%)*	
Dose no. 3	*2 (0.2%)*	*Dose no. 6-17*	*0 (0.0%)*	*0(0.0%)*	
Total inactivating hypersensitivity	**19 (2.3%)**		**13 (8.3%)**	**2 (1.3%)***	**0.0081**
Total hypersensitivity	**27 (3.3%)**		**17 (10.8%)**	**4 (2.5%)***	**0.0067**

2-sample test for equality of proportions with continuity correction

Figure 1. Anti-drug antibody formation against PEGasparaginase in the non-continuous and continuous treatment arm over time



A linear mixed-effects model was used to assess the change in antibody formation over time between the two treatment arms

P 73 - The use of Arsenic trioxide (ATO) and trans retinoic acid (ATRA) in 9 children and adolescents with recurrent/refractory promelocytic leukemia. An xperience of a Brazilian institution

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Introduction:

Acute Promielocytic leukemia (APL) is a rare and unique subtype of acute myeloid leukemia (LMA) characterized by the presence of t (15; 17). In recent decades, the use of risk-adapted regimes, including ATRA and chemotherapy with molecular response monitoring, has obtained long-term remission rates between 90–97%. However, relapse still occurs in 17–27% and refractory disease has become a rare situation.

Materials and methods:

This retrospective, non -randomized, unicentric study was approved by the Local Ethics Committee.

Inclusion Criteria -Patients up to 21 years old to diagnosis, with APL, confirmed with positivity for PML-RARA, NPM1-RARα or NUMA-RARα mutations that received treatment according to ICC-01 (International Consortium for Childhood APL protocol) and presented recurrence or refractory disease.

Exclusion Criteria:

Refractory or recurrent patients who have not received an ATO and ATRA exclusively.

Molecular relapse, defined as the reappearance of the PML-RARa fusion transcript, detected after a previously negative reverse transcript (RT-PCR) PCR in two successive samples of repeated MO with 2 weeks break. Refractory disease was defined by evidence of APL molecular disease after the consolidation phase

According to the time of relapse patients were classified as: early relapse (<18 months from inicial diagnosis), late relapse (> 18 <36 months from inicial diagnosis), and very late relapse (> 36 months from inicial diagnosis).

Reinduction treatment included: ATO 0.15 mg/m² associated with ATRA 25mg/m² daily for 50 days.The consolidation consisted of 4 cycles of 36 days, with a two -week interval between the cycles. Each cycle included ATO 0.15mg/m²/ daily, 5 days/week for 4 weeks. Associated with ATRA 25mg/m² per day in D1-D14, D28–42.

Molecular disease control was performed every 3 months after end of therapy for a year.

Toxicities evaluated according to CTACE.5.0.

Results:

From 2009 to 2022, 82 patients with LPMA treated at the Boldrini Children's Center used the ICC-APL-01.5 protocol. Overall survival (OS) and event free survival (EFS), in 8 years, was 92.2 ± 3.1% and = 78.2 ± 7.9%, respectively. Early mortality occurred in 3.5% of patients (n = 3). Death in remission occurred in a patient.

Of a total of 82 patients, 9 were analyzed (10.9%) due to relapse, 1 early relapse, 4 late relapses, 2 very late relapse and an extramedular relapse in skin. One patient was analyzed by refractory disease after consolidation (1.2%).Only one patient, after early molecular relapse, presented refractority to the use of ATO+ATRA followed by conventional chemotherapy and died after hematological recurrence with 90,500 leukocytes /mm³.

Cardiac toxicity (grade 2) occurred in 1 patient using ATO (widening QT/QTC > 500 mseg).

Three patients during use of ATRA had dry mouth and dyspepsia (grade 2). A patient with had *pseudo tumor cerebri* toxicity (grade 3).

For 9 patients with relapse/refractority, global survival (SG), in 5 years, 88.9 ± 10.5%.The clinical characteristics and therapeutic results of the 9 patients are described in **Table 1**.

Discussion:

Our sample was small and performed retrospectively, however the results showed that combined therapy composed by ATO+ATRA (for reinduction and consolidation), was effective in offering complete long -term remission, with results comparable to literature. The results were obtained with low toxicity

Conclusion:

Our results have shown that even in a country with limited resources, good results can be obtained with lower cost and low toxicity.

Table 1: Clinical-laboratorial characteristics and results of 9 LPA patients relapsed/refractory.

Patient	Age	Sex	FAB	Initial risk	WBC/mm³	Type of relapse or refractory	MRD after induction	MRD after 1st consolidation	MRD after 2nd consolidation	MRD 3rd consolidation	MRD 4th consolidation	BMT	Outcome	Time in CR2 (years)
1	14	M	M3	HR	4.080	LATE MOLECULAR	$7,6x10^{-5}$	negative	$>10^{-6}$	$1,4x10^{-5}$	$1,79x10^{-5}$	N	CR2	8,9
2	6	F	M3	SR	2.590	LATE MOLECULAR	negative	negative	negative	negative	negative	S	CR2	9,6
3	18	M	M3	HR	2.250	EARLY MOLECULAR	$2,1 \times 10^{-3}$	$2,32 \times 10^{-3}$	$2,55 \times 10^{-2}$	NA	NA	N	Death	-
4	18	F	M3	SR	2.630	VERY LATE HEMATOLOGIC	$3,79X10^{-3}$	$1,56X10^{-4}$	negative	negative	negative	N	CR2	3,2
5	20	F	M3	SR	3.450	VERY LATE MOLECULAR	$1,1x10^{-5}$	$1,3x10^{-5}$	$5,9x10^{-6}$	negative	negative	N	CR2	4,9
6	9	M	M3	SR	3.370	SKIN ISOLATED	negative	negative	negative	negative	negative	N	CR2	4,4
7	12	M	M3	HR	3.300	LATE MOLECULAR	$5,2x10^{-5}$	negative	negative	negative	negative	N	CR2	1,8
8	16	F	M3	SR	5.810	LATE MOLECULAR	$1,19x10^{-2}$	$1,9 x10^{-4}$	negative	negative	negative	N	CR2	1,0
9	20	M	M3	SR	5.630	REFRACTORY	$6,4x10^{-4}$	negative	negative	negative	negative	N	CR2	8,9

P 74 - Comparison of diagnostic and treatment processes among pediatric and adolescents and young adults' populations suffering from acute lymphoblastic leukemia and lymphomas

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Introduction:

Acute lymphoblastic leukemia (ALL) and lymphomas affect both pediatric and adult populations, therefore, they might be treated by pediatric or adult centers. It has been proven that the prognosis among adolescents and young adults (AYA) is poorer than among children, which remains a subject of research. Many factors are suspected to affect the diagnostic and treatment processes in adolescents and young adults, one of them being the organization of the healthcare system.

The aim of the study was to compare the time intervals between different events on disease trajectory in pediatric and AYA groups suffering from ALL and lymphomas.

Methods:

We collected data on 81 patients diagnosed with ALL (50 children and 31 AYAs) and 100 patients diagnosed with lymphomas (50 children and 50 AYAs). Statistical analysis was performed in order to compare the groups.

Results:

The results confirmed the hypothesis that the duration of the diagnostic process differs significantly between groups. For patients with ALL, the analyzed time intervals were significantly shorter in the pediatric group than in the AYA group: first contact with a GP - admission to Hematology Department (2 vs. 5 days; p-value = 0.004), first contact with a GP - treatment (6 vs. 12 days, p-value = 0.001), diagnosis - treatment (1 vs. 3 days, p-value = 0.003).

In the case of patients suffering from lymphomas, the results were similar. The analyzed time intervals were significantly shorter in the pediatric group than in the AYA group: first contact with a GP- diagnosis (21 vs. 40.5 days, p-value < 0.0001), first contact with a GP - treatment (27 vs. 65 days, p-value < 0.0001). Trend analysis showed that the longer patients had presented symptoms before contacting the primary care physician, the longer they waited for the beginning of treatment both in ALL and lymphomas groups(p-values = 0.0129 and 0.0038 respectively).

Discussion:

As the diagnostic and treatment processes are longer for AYA patients, actions must be undertaken in order to ensure equality and improve the healthcare system in Poland and possibly other countries.

P 75 - Predictor value of minimal residual disease analysis by flow cytometry in children with relapsed acute lymphoblastic leukemia

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Introduction:

Relapse remains the most common cause of treatment failure in childhood acute lymphoblastic leukemia (ALL). Several prognostic factors have been analyzed, but immunophenotype, duration of first complete remission (CR) and response to treatment remain the most relevant ones.

Aims:

The primary aims of this study were to assess the minimal residual disease (MRD) by flow cytometry in children with relapsed ALL and to evaluate its prognostic impact as a risk factor at the end of the induction (EOI) therapy and prior to hematopoietic stem cell transplantation (HSCT).

Patients and Methods:

From Jan'11 to Nov´22, 290 patients were diagnosed as relapse-ALL and 164 cases were excluded from the analysis: 10 (3.4%) died during induction, 35 (12%) did not respond to second-line chemotherapy, 7 died in CR (2.4%), in 11 patients MRD was not available, 13 had been treated with Interfant Protocol as first-line therapy, 9 Down Syndrome patients, 71 were treated in other centers, 2 were lost in follow up and palliative care were offered to 6 children. The eligible cases for this study were 126 patients who achieved CR and were evaluated for MRD at least in two time-points. Of them, 120 patients corresponded to S4 and S3 and 6 patients to S1 group as defined by the Berlin-Frankfurt-Münster stratification for relapsed ALL.

MRD was analyzed by multiparametric flow-cytometry following ALLIC protocol guidelines. MRD negative was defined as less than 0.01% of blasts.

The patients were classified based on MRD level at two different time-points: EOI, before HSCT or other time-point during the follow-up in patients who did not undergo to HSCT. Patients who relapsed previous to HSCT were considered group 3. Three groups were defined: group-1: negative at both time-points (n = 52), group-2: 1 positive time-point (n = 52) and group-3: positive at both time-points (n = 22). Fifty-two patients underwent HSCT: 19 of them from group-1, 29 from group-2 and 4 patients from group-3.

The distribution of events according to receiving only chemotherapy or consolidation with HSCT was: 12 died due to transplant related mortality (TRM), 2 developed Second Malignant Neoplasms, 11 relapsed following HSCT and 33 following chemotherapy.

Results:

With a median follow-up of 54 (range: 1–126) months, overall, 3-year EFS probability (SE) was 49 (4)%. The 3-year EFSp was 73 (6)% for group 1, 46 (7)% and 5% for group 3 (p-value <0.00001). Comparing patients who did not receive HSCT versus patients who received HSCT, EFSp (SE) was 45 (7)% and 60 (7)% respectively (p-value: ns). The EFSp (SE) according to MRD groups in patients who underwent to HSCT vs patients who did not received HCST was: group-1: 53 (12)%, group-2: 48 (10)% and 25% for group-3 (p-value: 0.5) vs group-1: 48 (11)%, group-2; 26 (12)% and for group-3: 0%(0) (p-value: 0,05).

Conclusions:

MRD quantitation by flow-cytometry has demonstrated to be a significant prognostic factor. TRM and death in CR should be decreased. MRD by flow-cytometry is a relevant tool for stratifying patients with relapsed ALL in order to achieve a better selection of patients who need to intensify the treatment.

P 76 - BFM-based treatment in lymphoblastic lymphomas in children

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Background:

Survival rates in non-Hodgkin lymphoma (NHL) have increased significantly in the last decades. This study aims to assess the demographic data and treatment outcome of children with lymphoblastic NHL (LNHL) treated in a single institution in a middle income country.

Methods:

152 children (105 male, 47 female) diagnosed and treated with NHL in Istanbul University, Oncology Institute, between Jan 1990 - Feb 2022 were evaluated retrospectively. Eighteen patients treated with BFM protocols.

Results:

The median age of 18 patients (10 male, 8 female) with LNHL was 7 (2–16) years. 13 had T-cell and 5 had precursor B-cell origin. The primary site was mediastinum in 8, neck (cervical lymphadenopathy) in 5, skin 2, bone in 3. Fourteen had advanced stage (St Jude stage III and IV), four had early stage disease (St Jude stage I and II). There were two deaths. One developed CNS relapse during re-induction treatment, a routine lumbar puncture revealed blasts in the cerebrospinal fluid (CSF). The patient developed neurological signs and symptoms consequently. Despite complete response after craniospinal radiotherapy and second line chemotherapy, the patient experienced CNS relapse again and expired due to disease progression. One with widespread disease at diagnosis, had a complete response died at six months due to sepsis. All others are alive with no evidence of disease. One is continuing maintenance treatment. Median follow-up was 137 (8–375) months. The 5 year survival is 88.5%.

Conclusions:

Childhood LNHL are treated successfully with similar protocols as used in ALL. BFM based protocols have been successfully used in middle income countries. Supportive care is important since treatment related toxicity is still an important cause of mortality.

P 77 - ALL-BFM treatment protocol day 33 evaluation: Why are we late?

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Acute lymphoblastic leukemia (ALL), which is characterized by the proliferation of immature lymphoid cells, is the most common malignant disease among children. Survival rate in children is 80–90% with modern, current treatment protocols. One of the most known is Berlin-Frankfurt-München (BFM) therapy regimen which is used in ALL for over 40 years. According to the protocol, bone marrow aspiration (BMA) is performed and minimum residual disease (MRD) is implemented during and after induction therapy (days 15, 33) to establish the risk groups and to assess treatment response. Although one of the main time point for the evaluation of response to treatment is on day 33, depending on the regeneration state of the bone marrow, this procedure cannot always be accomplished exactly on the scheduled day. This study shows the details and outcomes of delayed day 33 BMA with BFM protocol in a single center. Although the original BFM regimen is used since 1995 in our center only files of 110 patients, who were treated between 2007 and 2023 were retrospectively reviewed for the study. We aimed to assess both the exact time of delayed BMA, which is due on day 33 as per protocol and the causes and the consequences of these postponements. Of the 110 patients 64 (58.2%) were male. They were between 9 months to 17.3 years of age (mean age: 7.1 ± 4.9 years, median age 5.5 years). Seventy-one of them (64.5%) were identified as precursor B-ALL (Pre-B ALL), 13 (11.8%) had B-ALL, 12 (10.9%) had T-ALL, ten (9.1%) had biphenotypic leukemia, three (2.7%) had common ALL, and one (0.9 %) had infant leukemia. There were 45 (40.9%) standard risk (SR), 49 (44.5%) intermediate risk (IR) and 16 (14.5%) high risk patients. Although day 33 BMA was performed in all patients as planned, only in 20 (21.2%) of the patients it was normocellular and conclusive. In 90 patients (81.8%), the day 33 BMA evaluation was suspended for 3–39 days (mean 10.5± 7.8 days, median 8 days) due to various reasons like the lack of bone marrow regeneration (59.4%), neutropenic fever (27%), sepsis (10.8%), and veno-occlusive disease (2.7%). There was a significant delay in the T-ALL subgroup ($p < 0.05$). Only eight patients in the SR and IR groups had a relapse. Following induction, the day 33 BMA examination before the consolidation therapy has a paramount importance in leukemia therapy. However, this scheduled procedure is sometimes delayed due to poor bone marrow recovery and other factors. As this delay also lead to the postponement of the chemotherapy protocol, it causes great anxiety among physicians treating leukemia. To our knowledge, this issue was not studied in the pediatric leukemia patients before. Yet larger studies are warranted to identify the underlying reasons.

P 78 - Native E.coli asparaginase and pegaspargase toxicity in children treated for acute lymphoblastic leukemia – Single center experience

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Background:

L-asparaginase is well known as of one the most important chemotherapy drugs used in the treatment of childhood acute lymphoblastic leukemia (ALL). The medication breaks down the amino acid asparagine, necessary for the growth and survival of leukemia cells. By depriving these cells of asparagine, L-asparaginase reduces the disease burden and significantly improves outcome for children with ALL. However, it also has side effects, and careful monitoring and management are necessary to ensure the best possible outcomes for pediatric patients.

Objectives:

To present the toxicity profile of asparaginase treatment in patients with ALL in a single center.

Methods:

The retrospective study enrolled 166 consecutive patients with *de novo* ALL, diagnosed and treated at the Department of hematology and oncology of University Children's Hospital, in Belgrade, Serbia, from April 2010 until March 2022. Laboratory and clinical data regarding adverse events (AE) of asparaginase treatment were collected and analysed by descriptive statistics.

Results:

Gender distribution showed slight predominance of male patients (58%), age ranged from 9 days to 18.2 years (median 4.9 years). Native E. coli L-asparaginase (L-ASP) was exclusively administered in 133 patients, 24 patients were switched to pegaspargase (PEG-ASP) due to withdrawal of L-ASP from the market (May 2020) and nine patients were treated with PEG-ASP from the outset. The AEs were noted in 39% of the patients (total of 106 AEs in 2297 drug administrations, thus having an average rate of about 1 AE per 21 doses). They were most frequent in the induction phase (68%) and of all toxic episodes, coagulation system was affected in 74% and hepatotoxicity occured in 9%. Acute pancreatitis, with subsequent treatment interruption, was seen in 2.4% of the patients. Severe allergic reactions occured in 12% of the patients, triggering switch to Erwinase, with addition of one patient who switched due to silent inactivation. Deep vein thrombosis was diagnosed in 4% of the patients. Comparing the toxicity between L-ASP and PEG-ASP indicated that allergic reactions and vascular events occured more frequently with native form, while other toxicities (coagulation and hepatic disturbancies) were predominant in patients treated with PEG-ASP.

Conclusion:

L-asparaginase is an indispensable drug in treating childhood ALL with an acceptable toxicity profile. The toxic manifestations can usually be overcome with substitution treatment. In a small number of patients, potentially fatal manifestations can occur, including anaphylactic reactions, acute pancreatitis and/or vascular accidents. Medical awareness, careful clinical and laboratory follow up and monitoring of the drug activity can mitigate the unfavorable toxicities of this important antileukemic medication.

P 79 - Epidemiology, etiology, and factors affecting the incidence of severe complications and mortality of febrile neutropenia in children with acute leukemia

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Background:

Febrile neutropenia (FN) is an important and common complication that causes high morbidity and mortality in patients with malignancy. Epidemiological data and agents causing infections in FN can change in each region and over time, which affects the management and outcomes of the patients.

Objective:

We aimed to investigate the etiology, epidemiological distribution and its change over the years, clinical course, and outcomes of FN in children with acute leukemia.

Methods:

We retrospectively analyzed the demographic data, foci of infection, etiological pathogens, laboratory parameters, treatments, severe complications, and mortality of patients with acute leukemia under the age of 18 who were diagnosed with FN between January 2010 and December 2020. FNs occurring in bone marrow transplant recipients were excluded.

Results:

In 153 patients, a total of 450 FNEs occurred, 84 (54.9%) were male and the median age of the patients at the first FNE was 6.5 years (3–12.2). 127 patients (83%) were diagnosed with ALL and 26 patients (17%) with AML. The median number of FNE per patient was 2 (1–11). The median duration of fever was 2 days (1–5), and the median duration of post-fever neutropenia was 7.5 days (5–13). Fever focus was found in approximately half of the patients, and etiology was found in 38.7% of the patients. The most common focus of fever was bloodstream infection (n = 74, 16.5%), upper respiratory tract infection (n = 43, 9.6%), low respiratory tract infection (n = 34, 7.6%), and urinary tract infection (n = 25, 5.6%). In etiology, bacterial infection was identified in 22.7% (n = 102), viral infection in 13.3% (n = 60), and fungal infection in 6.7% (n = 30) of the episodes. More than one etiologic pathogen was detected in 18 (4%) FNEs. Of the 112 bacteria identified, 72 (64.3%) were gram-negative pathogens. While 26 (23.2%) of a total of 112 bacteria were antibiotic resistant, 33.3% of gram-negative bacteria were found to be resistant. Monotherapy was used in the empirical treatment of 182 (40.4%) of FNEs. The median treatment duration of FNEs was 10 (7–17) days. The severe complication rate was 7.8% (n = 35) and the mortality rate was 2% (n = 9). Prolonged duration of fever and prolonged neutropenia after fever, high CRP level, and having refractory/relapsed malignancy increased the frequency of serious complications and mortality. In logistic regression analysis, refractory/relapsed malignancies and high CRP at first admission were found to be the most important independent risk factors for mortality.

Conclusion / Application to practice:

We showed that the most common etiologic cause was bacterial infections due to gram-negative pathogens and the most common focus of FN was bloodstream infections in our center in the last ten years. Prolonged duration of fever, relapsed/refractory malignancies, presence of fever focus, and high CRP level were significant poor risk factors on clinical course and outcome. Evaluating the etiological and epidemiological data and outcomes of patients regularly is important to better manage FNE, improve quality of life, and reduce complication and death rates in children with acute leukemia. For this purpose, each region should closely monitor its etiological distribution, epidemiological characteristics, and changes over time.

P 80 - Anaplastic large cell lymphoma (ALCL) in pediatric patients from Northern Greece

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Introduction:

Anaplastic large cell lymphoma (ALCL) represents the most common pediatric peripheral T- cell lymphoma, that accounts for 10–15 % of NHLs in children (1.2 / 10^6 children). It has a better prognosis than other NHLs, despite the high 2-year recurrence rate of 30%. Overexpression of anaplastic lymphoma kinase (ALK) is seen in > 95 % of cases. The responsible molecular finding is the t (2:5) (p23:q35) translocation of ALK with the nucleophosmin (NPM) gene. It is a retrospective cohort study of children with ALCL from the 2 pediatric oncology departments of Northern Greece.

Methods:

The data of patients treated for ALCL between 1999 and 2021 in 2 Pediatric Oncology Departments of Northern Greece were reviewed and analyzed for age, sex, histology, primary site, stage, radiotherapy, and survival outcomes.

Results:

Eleven patients (8 males, 72.7 %) were diagnosed with ALCL from approximately 1500 pediatric cancer cases. The median age at diagnosis was 12 years (range 8–15 years). The main symptoms were lymphadenopathy, fever, and weight loss, and the most common primary sites were trunk (10), abdomen (4), head and neck (6). Two patients had extra-nodal bone disease involving the sacrum, pleura, and vertebra. In all patients the diagnosis established after biopsy. The predominant type was initiatives of T cellular origin (9/11), while two patients were assigned a null cell phenotype. The diagnosis was confirmed by molecular studies. Overexpression of ALK (ALK positive) had 5 patients. All patients received chemotherapy according to EICNHL ALCL-99, NHL BFM (NHLBFM 90, NHL BFM 2012) study protocols, but one received LSA2-L2 protocol. There were 4 stage II, 5 stage III, and 2 stage IV cases. A significant response to chemotherapy was recorded in the majority of cases. Radiotherapy (RT) was administered to 2 patients, one of whom had a small intranasal mass. One patient with bone disease underwent HSCT. There was 1 recurrence 15 months after diagnosis, in a patient without serious risk factors for recurrence such as B symptoms or bone marrow involvement. This patient was stage III at initial diagnosis, and died due to ileus with unresponsive disease. At last follow-up (median 17 years, range: 1- 22 years) 10 patients were alive tumor-free in CR1, overall survival (OS) in our cohort was 90,9 %. One patient has chronic side effects as long-term consequences of treatment: osteoporosis, hypogonadism, and nodular goiter.

Conclusions:

ALCL is a group of rare tumors in the pediatric population. Survival is influenced by stage, high-risk histology and bulk disease. The molecular studies will provide an opportunity for future targeted therapies with ALK inhibitors and anti-CD30 antibody drug conjugates.

P 81 - QoL-Blina protocol of AIEOP Study Group: Preliminary data

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Background:

Immunotherapy with monoclonal antibodies represents a new therapeutic approach in pediatric Acute Lymphoblastic Leukemia (ALL) both in front-line treatment and in relapse. Previous researches have demonstrated the efficacy of blinatumomab, but no specific studies have been performed on Quality of Life (QoL) in pediatric patients during this treatment. A single phase 3 clinical trial (Topp et al., 2018) conducted in adult patients demonstrated an improved QoL in subjects treated with blinatumomab.

Based on this findings, the first observational multicenter study in pediatric hemato-oncology area, comparing the QoL of patients treated with blinatumomab (Blina) versus the standard chemotherapy, is in progress.

Objectives:

The aim of the study is to analyze the perceived QoL and the psychosocial risk in pediatric patients affected by ALL-B, recruited in the AIEOP-BFM-ALL-2017 protocol (intermediate/high risk) and treated, after randomisation, with standard chemiotherapy (Group 1) or blinatumomab immunotherapy (Group 2), compared with their caregivers.

Methods:

The study, lasting 24 months, includes a sample of about 200 patients (aged 2–17 years at diagnosis of ALL) in 26 AIEOP Centers. Patients and their caregivers will be administered the following questionnaries:

- PAT (Psychosocial Social Support; Child Problems; Sibling Problems; Family Problems; Parent Stress Reactions; Family Beliefs) and a general psycosocial risk score (universal, targeted and clinical), Assessment Tools, Kazak, 2006) that measures seven subscales (Family Structure and Resources);
- PedsQoL (Pediatric Quality of Life Inventory 3.0) cancer module consisted of 27-item divided into 8 scales: 1) pain and hurt (2 items), 2) nausea (5 items), 3) procedural anxiety (3 items), 4) treatment anxiety (3 items), 5) worry (3 items), 6) cognitive problems (5 items), 7) perceived physical appearance (3 items), and 8) communication (3 items). The format includes a General Score, with higher scores indicating better QOL.

In this phase of the study, descriptive analyses, with SPSS software, have been run to explore the levels of psycosocial risk and perceived QoL.

Results:

From February 2022 to March 2023, 23 patients have been recruited in 2 of 6 AIEOP Center activated. Preliminary data on 17 patients and 23 caregivers show that:

- considering the levels of psycosocial risk (PAT score) on 23 caregivers, N = 8 reported universal/low risk, N = 11 targeted/specific risk and N = 4 clinical/high risk;
- regarding the levels of perceived QoL (General QoL Score) on 23 caregivers, N = 8 reported low level of perceived QoL, N = 13 intermediate level, N = 3 high level;
- considering the levels of QoL (General QoL Score) on 17 patients, N = 2 revealed low level of perceived QoL, N = 12 intermediate level, N = 3 high level.

Conclusion / Application to practice:

This preliminary results show that the perceived QoL in patients/caregivers is influenced much more by presence/absence of psychosocial risk and is independent from the administered therapy (Blina or standard chemotherapy) and from any reported physical symptoms. Further patients and caregiver are needed to confirm the data. Therefore, we suggest to focusing on the individual and family psychosocial aspects, using personalized clinical interventions to provide adequate coping strategies and support the critical psychological issues related to disease.

P 82 - Post-consolidation MRD is the strongest prognostic factor of relapsed T-cell Acute Lymphoblastic Leukemia

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Background:

T-cell Acute Lymphoblastic Leukemia (T-ALL) outcome is lower than B-cell ALL, due to higher relapse rates. At relapse, the prognosis is ominous, without improvements in the last decades. Initial characteristics, prednisone and MRD response as molecular abnormalities were associated with an increased risk of relapse.

Objectives:

We aimed to identify prognostic factors and analyze the outcome of relapsed T-ALL patients treated with first-line ALLIC-BFM-2009 protocol.

Methods:

Retrospective observational study. Included T-ALL patients enrolled in the ALLIC-BFM-2009 protocol from October-2009 to December-2022. Biological features and treatment responses were evaluated as possible relapse risk factors. Relapsed/refractory T-ALL outcome was analyzed according to initial characteristics.

Results:

During this period, 947 ALL patients were admitted and 122 (12.8%) were T-ALL. 5.7% (n = 7) of T-ALL patients died during the induction phase. Of 115 patients who achieved Complete Remission (CR), 9.8% (n = 12) died in CR: 7 post-HSCT, 4 due to sepsis, and 1 to pancreatitis. Three patients presented second malignancies and three were late responders. 24 (20.9%) patients relapsed, with a 5-year cumulative Incidence of relapse of 24.5 (SE 4.6)%.

Comparative analysis between relapsed and non-relapsed T-ALL patients without treatment-related mortality was performed. The relapsed group presented significantly more patients with initial WBC >200,000/mm^3 (58.3% vs 35.4%, p = .046), aged between 1–10 years old (79.2% vs 54.4%, p = .03) and positive MRD >0.05% at end-of-consolidation (EOC) (20.8 vs 7.6%, p = .037). No difference was detected in extramedullary compromise, ETP-phenotype, complex karyotype, *CDKN2A/B* deletions, poor prednisone response (PPR), and MRD on Day-15 and Day-33 of treatment.

Univariate and multivariate analysis of prognosis factors of relapse is shown in the table.

	Univariate Analysis				Cox regression model	
Total Patients (n = 103)	Patients	Events	5-years DFS (Standard Error)	**P value**	Estimated HR (95% CI)	**P value**
1–10 years old	**62**	**19**	**65.7 (6.7)%**	**.0328**	2.36 (.77–7.22)	.13
Initial WBC >200,000/ mm^3	**42**	**14**	**66.5 (7.7)%**	**.0619**	**2.49 (1.01–6.11)**	**.047**
Extramedullary-compromise	66	24	69.4 (6.4)%	.174	--	
CNS3	8	3	62.5 (17.1)%	.268	--	
Early-T	10	3	68.6 (15.1)%	.707	--	
CDKN2A/B deletion	64	16	78.6 (5.6)%	.902	--	
PPR	38	10	70.4 (7.9)%	.551	.92 (.39–2.15)	.851
MRD-D15 <0.1% 0.1–10% >10%	16 54 32	1 16 7	93.3 (6.4)% 67.4 (7.1)% 77 (7.6)%	.371	--	

MRD-D33						
>0.05%	41	10	75.2 (7.3)%	.873	--	
>0.1%	38	9	77.2 (7.1)%	.896		
>1%	11	4	60.6 (15.4)%	.626		
EOC-MRD						
>0,1%	**10**	**6**	**41.6 (15.6)%**	**.0002**	**5.6 (2.04–15.37)**	**.001**
>0.05%	**12**	**6**	**41.6 (15.6)%**	**.0089**	**--**	

When analyzed same risk factors for treatment-related mortality: PPR ($p < .0001$), MRD at EOI >1% ($p = .0086$), EOC-MRD > 0.05% ($p = .0002$), and HSCT in first-CR ($p = .0002$) were associated with increased deaths-in-CR.

The median time of relapse was 13 (1–62) months. Sites of relapse were: bone marrow (n = 15), combined (n = 3), isolated extramedullary (n = 5), and 1 unknown. Fourteen patients achieved a second CR, but only three (12.5%) are still alive in a second CR. The 5-year OS since relapse was 10.43 (SE 6.8)% with a median survival time of 3 (0–120) months. A longer duration of the first CR (t-test (-12.4)=-2.13, $p = .047$) and testicular isolated/combined with BM site ($p = 0.036$) were favorable prognostic factors for achieving a second CR.

Conclusions:

Post-consolidation MRD is not only the most potent prognostic factor of relapse but also treatment-related mortality (principally related to HSCT) in T-cell ALL. Intensification of first-line treatment, without intensive chemotherapy/HSCT and minimal toxicity, is essential in order to achieve a long-term and deeper response.

P 83 - Safety of arsenic trioxide in a patient with acute promyelocytic leukemia on hemodialysis

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Arsenic trioxide (ATO) is efficacious and safe and leads to high cure rates in patients with acute promyelocytic leukemia (APL). ATO is eliminated via renal excretion, but paucity of data exists regarding ATO pharmacokinetics, dosing and timing in patients with renal failure. No such data is available for children and young adults.

Objectives and Methods:

In this report, we describe the successful treatment with ATO in young adult patient dependent on hemodialysis due to acute renal failure. We carefully monitored ATO levels and achieved molecular remission after induction therapy without significant ATO-related side effects.

Results:

A twenty-year-old female with Down syndrome was admitted with newly diagnosed APL. She received one dose of G-CSF and broad spectrum antibiotics due to presumed sepsis in a district hospital before the diagnosis of APL. Her initial blood counts were as follows: WBC 11.3 x 10^9/L, hemoglobin 116 g/L, platelets 40 x 10^9/L. Upon admisson she was anuric. She developed respiratory failure, was admited to intensive care unit and started treatment with hemodialysis. Her baseline EKG showed QTc < 460 and ECHO revealed normal LVEF. We initiated treatment with ATRA (all- trans retinoic acid) and the next day with ATO.

During the induction treatment, we initiate ATO three times a week (0.15 mg/kg) in two hours infusion after hemodialysis. During the treatment, we monitored the concentration of arsenic in the plasma before and after the hemodialysis. We observed that hemodialysis following the second dose of ATO removed approximately 38% of arsenic from the blood, which is in agreement with previous reports in elderly patients (figure 1).

EKGs were obtained before each ATO dose. Manual calculation of QTc interval using Bazett's formula was used as a guide in adjusting the interval of ATO administration. Upon three doses of ATO, the patient's EKG reading indicated a critical QTc interval >520 ms, and we omited two of the ATO doses accordingly.

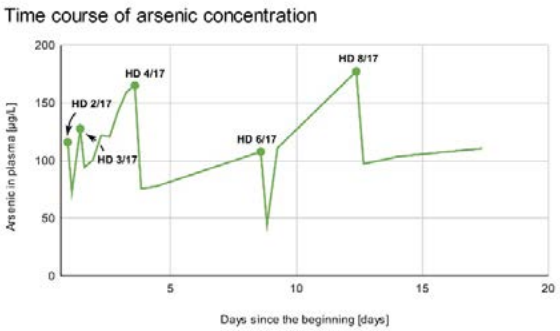
The patient was weaned off mechanical ventilation on 34th day. Also, her kidney disease improved to the extent that only diuretic treatment sufficed (estimated GFR = 20–24 mL/min). We confirmed molecular remission after induction course with 19 doses of ATO (after six weeks of therapy).

The patient had stable kidney disease with GFR 20–30 ml/min during the consolidation. She received ATO twice a week; in the first week we administered ATO in reduced dose (0.075 mg/kg) and took samples for the ATO concentration assessment. Prior to the consolidation arsenic plasma concentration was still 54.1 µg/L. After the first consolidation dose of ATO arsenic plasma concentrations were drawn immediately post-dose (63.5 µg/L), at 6 hours (59.2 µg/L), at 24 hours (57.3 µg/L) and 56 hours (65.5 µg/L). This demonstrated a previously described infusional peak with distribution of arsenic into the tissues and redistribution of arsenic back into the blood.

Conclusion:

There is limited data about ATO safety and eficacy in APL patients with renal dysfunction. Our case supports that ATO can be administered safely and effectively to a hemodialysis-dependent patient.

Based on a previous reports in adult patients we report a strategy of a dosing frequency of two to three times weekly in a standard dose (0.15 mg/kg) after the hemodialysis in paralel with EKG, potassium and magnesium monitoring.


Time course of arsenic concentration
Arsenic in plasma [µg/L]
200
150
100
50
0
HD 2/17
HD 3/17
HD 4/17
HD 6/17
HD 8/17
5
10
15
20
Days since the beginning [days]

P 84 - Evaluation of CD20 expression in B Acute Lymphoblastic Leukemias in pediatric patients at public hospital

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Introduction:

Acute Lymphoblastic Leukemias are a heterogeneous group of diseases whose diagnosis is based on morphological, immunophenotypic, cytogenetic and molecular studies. Immunophenotyping by flow cytometry allows assigning B or T lineage to pathological cells by studying surface and intracytoplasmic antigens at diagnosis. Monitoring of the therapeutic response at day 15 is based on the quantification of blasts by flow cytometry. The expression of cellular antigens such as CD20 allows the possible use of immunotherapies in patients with B Acute Lymphoblastic Leukemia (B-ALL).

Objective:

To compare the expression of CD20 antigen in pediatric patients with ALL-B at diagnosis (Dx) and on day 15 (D15) of their treatment.

Materials and methods:

Retrospective observational study, case analysis. The immunophenotypic study by multiparametric flow cytometry was performed in EDTA-anticoagulated bone marrow of 97 pediatric patients from January 2017 to November 2022, treated according to the ALLIC BFM/GATLA 2010 guidelines. Euroflow standardized protocols with panels of eight fluorescences were used. In all cases, the clone used to evaluate the expression of CD20 antigen was L27 conjugated with V450 fluorochrome. The acquisition and analysis of the results was performed in a FACSCanto II flow cytometer using the FacsDIVA and Infinicyt software. The Dx and D15 files were re-analyzed, the expression of CD20 values were recorded in percentage (%), mean fluorescence intensity (xMIF) and median fluorescence intensity (MeMIF), both in blasts and normal B lymphocytes (LB). The relationship between xMIF and MeMIF of blasts and LB were calculated, establishing the normalized xMIF and MeMIF. A descriptive statistical approach was carried out, the comparisons were made using the Student›s t test for normal distributions and the Mann-Whitney test as a non-parametric method, establishing statistical significance with $p < 0.05$.

Results:

Of the 97 patients evaluated, 19 of them were not evaluated due to the total absence of pathological cells. Five patients did not express CD20 on Dx and on D15. In 57/73 patients, an increase in the % expression of CD20 was observed in the blast cells present on D15 compared to Dx, with statistically significant differences ($p < 0.00001$). 37% (27/73) patients changed their status from Negative at Dx (<10%) to positive for CD20 on D15. Both the comparison of the xMIF and MeMIF parameters at Dx and D15, as well as their normalized values, showed statistically significant differences.

Conclusions:

In this study it was observed that the expression of CD20 on day 15 of the treatment in patients with ALL-B was increased versus its value at the moment of diagnosis. This was reflected in the expression percentage values, in the comparison of means and medians of fluorescence intensity and in the normalized fluorescence ratio of CD20 (clone L27) conjugated with V450. It was evidenced that 37% of the patients studied had positive expression of CD20 at D15, and could become candidates for immunotherapy. The analysis of the data shows the importance of evaluating the intensity and reporting the % expression of CD20 in the pathological cells at D15.

P 85 - Diverting end-colostomy for severe perianal wound infection in two pediatric patients with acute lymphoblastic leukemia. A single center experience

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Background:

Perianal wounds may be life-threatening complication in pediatric patients suffering from acute lymphoblastic leukemia (ALL) especially during prolonged neutropenia. Local perianal wounds may progress into deeper layers and cause invasive sepsis, which can be fatal. (1) The immunocompromised host state coupled with the importance of avoiding treatment delays make management challenging. Conservative treatment is not always effective. Data regarding surgical treatment in this population is scarce. (2,3) We report here our experience with two pediatric patients who underwent diverting colostomy, which facilitated the recovery of perianal wounds and allowed for prompt renewal of therapy.

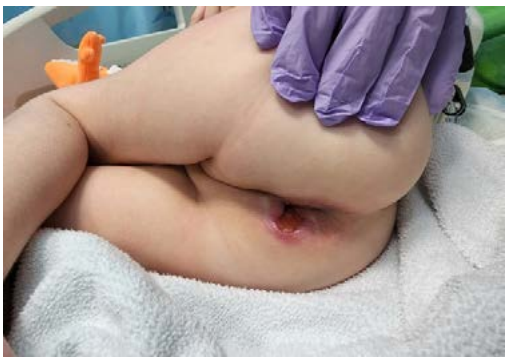
Case study:

Between September 2018 and February 2023 two female patients suffering from ALL developed severe perianal sepsis. The first was diagnosed during induction and the second was diagnosed after receiving high risk protocol. Both suffered from fever and prolonged severe neutropenia (less than 500/mm neutrophils for ≥ 7 days). The first patient developed septic shock which required temporary hemodynamic support. Blood cultures demonstrated gram negative bacteremia - Pseudomonas aeruginosa and Morganella morganii. They received conservative treatment consisted of broad spectrum antibiotics according to bacterial sensitivity and local dressing therapy, but unfortunately, even after recovery of their blood counts the local improvement was insufficient as was demonstrated by physical examination under sedation. Both patients underwent open diverting colostomy without intraoperative complications. Therapy was initiated in both patients a week after surgery. One patient completed chemotherapy according to the initial protocol and the colostomy was closed during maintenance, and the second patient is receiving blinatumomab until complete healing of the perianal lesion, then we plan to renew chemotherapy.

Conclusions:

Deep perianal wound infection in pediatric patients with ALL may be life-threatening. Early initiation of broad spectrum antibiotics and local dressing therapy is crucial. Temporary diverting end-colostomy is a safe and effective procedure that should be considered in failure of conservative treatment in order to avoid severe complications and delayed chemotherapy treatment.

1. Gastrointestinal and liver infections in children undergoing antineoplastic chemotherapy in the years 2000. Castagnola et al, World J Gastroenterol 2016; 22(25):5853–5866
2. Temporary diverting end-colostomy in critically Ill children with severe perianal wound infection. Gun et al, WCET Journal 2021;41(3):38–43
3. Early diverting colostomy for perianal sepsis in children with acute leukemia. Prato et al journal of Pediatric Surgery, 2012;47(10):23–27



Patient	protocol	Phase of protocol	Duration of neutropenia (<500) prior infection	Symptoms	surgery	Therapy delay	Therapy after surgery
1	AIEOP BFM ALL 2009	Induction day +25	9 days	Septic shock followed by perianal lesion with necrosis	open surgery – diverting end-co-lostomy abdominal washout	7d	Cont. AIEOP-BFM ALL
2	AIEOP BFM ALL 2017	After protocol high risk 1 (day+13 from start)	7 days	Neutropenic fever, bacteremia, perianal lesion without necrosis	open surgery – diverting end-colos-tomy	7d	Blinatumomab course

P 86 - Copy number variation analysis in pediatric T-cell precursor Acute Lymphoblastic Leukemia: Report from the SAHOP group

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T-cell precursor ALL (TCP-ALL) is a genetically heterogeneous disease and comprises multiple genomic alterations involving different pathways. The prognostic impact of copy number variation (CNV) is controversial based on different reports, most of them involving adults or showing a small sample size. In addition, the characterization of *NUP214::ABL1* is important due to its therapeutic potential. The description of genetic alterations with prognostic relevance in pediatric TCP-ALL could contribute to the development of new adapted therapy strategies.

Aims:

1-to describe the CNV profile of pediatric TCP-ALL patients from Argentina. 2-to describe the co-occurrence of genetic deletions. 3-to assess the prognostic impact of these alterations in our population.

Patients and Methods:

A total of 126 patients diagnosed with TCP-ALL in Sociedad Argentina de Hemato-Oncología Pediátrica (SAHOP) institutions and treated with ALLIC-BFM-2009 protocol, from July-2011 to September-2022, were analyzed. Of them, 49.2% were stratified as intermediate risk (IR) and 50.8% as high risk (HR). The median age was 8 [range:1–17] years and the male to female ratio was 3:1. MLPA Probemix P383-T-ALL, P335-ALL-IKZF1 and RT-PCR for fusion transcripts were performed.

Results:

Deletions were detected in 88.9% of all cases and duplications in 46.8%. The most frequent deletions were: *CDKN2A* 81.7% (n = 103) (88.3% homozygous deletions), *CDKN2B* 62.7% (n = 79) (84.8% homozygous deletions); *MTAP* 47.6% (n = 60), *MLLT3* 16.7% (n = 21), *CASP8AP2* 14.3% (n = 18); and duplications: *EZH2* 23.0% (n = 29), *LMO1* 15.1% (n = 19), *MYB* 10.3% (n = 13), *AHI* 9.5% (n = 12). The incidences of *STIL::TAL*; *NUP214::ABL1* and *LMO2::RAG2* fusions were 23.8% (n = 30), 4.0% (n = 5), 3.2% (n = 4), respectively. In HR patients, the incidence of *CASP8AP2*-deleted was 23.4% (n = 11) (p = 0.0029). Co-occurrences were observed between: *STIL::TAL1* fusion with *CASP8AP2* (p < 0.00001) and *PTEN* (p < 0.00001) gene deletions; *PTEN* and *LEF* deletions (p = 0.0144); *LMO1* and *EZH2* deletions (p < 0.00001). EFS (SE) for total TCP-ALL was 66.1 (4.6)% and for *CDKN2A/B*-deleted with *MTAP*-deleted 54.1 (7.3)% vs *MTAP*-non-deleted 79.9 (6.4)% (p = 0.0416). The genotype *MTAP*-deleted with deletion of *PTEN* and/or *PTPN2* showed a EFS (SE) of 36.0 (14.7)% vs 70.1 (4.6)% in remaining patients (p = 0.0314). In addition, this subgroup of patients was associated with negative minimal residual disease (MRD) (≤0.05%) on day 33 of treatment (p = 0.0500). EFS (SE) of *AHI*-duplicated was 81.8 (11.6)% (p = 0.2457).

Conclusions:

The incidence of *CDKN2A/B*, *MTAP* and *PTEN* deletions was higher than reported data. Most cyclin deletions were in homozygous status. A higher incidence of *CASP8AP2* deletion was found in HR patients compared with IR. Statistically significant co-occurrences were found between: *STIL::TAL1* fusion and *CASP8AP2* deletion; *STIL::TAL1* and *PTEN* deletion; *PTEN* and *LEF* deletions; *LMO1* and *EZH2* deletions. In cases where *CDKN2A/B* deletion included *MTAP* gene on 9p21.3 region, an adverse prognosis was observed compared to deletion of the *CDKN2A/B* genes only. A similar outcome was found for the genotype *MTAP*-deleted with deletion of *PTEN* and/or *PTPN2*, allowing the identification of a potential group of patient's candidates for treatment intensification or eventual target therapies. MRD on day 33 does not seem to be a predictive marker for this subgroup of patients. More patients should be studied to support our findings.

P 87 - Analysis of the impact of flow cytometry MRD at end of induction and end of consolidation within SAHOP (Sociedad Argentina Hemato-Oncología Pediátrica) considering the stratification of ALLIC-BFM-2022

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Background:

The ALLIC-BFM-2022 study has new criteria for risk group (RG) assignment based on genetic features, flow cytometry MRD at end of induction (FC-MRD-EOI) and end of consolidation (FC-MRD-EOC). Besides, complete remission (CR) status is defined by FC-MRD-EOI <1%.

The main differences regarding FC-MRD reassignment are that for B-ALL, FC-MRD-EOI ≥0.05% will reallocate patients to High RG (HRG), while for T-ALL FC-MRD-EOC ≥0.05%. Only patients with FC-MRD-D15 <0.1% will stay within Standard RG (SRG) if *ETV6::RUNX1* or hyperdiploidy are detected. In addition, neither age/WBC will be considered nor will poor prednisone response (PPR) for B-ALL.

No T-ALL will be assigned to SRG.

Aims:

Our aim was to analyze the impact of this new stratification on SAHOP cases from the ALLIC-BFM-2009 and ALLIC-BFM-2022-Pilot study. Given the importance of FC-MRD-EOI and EOC, we also reviewed any FC-MRD-EOC higher than FC-MRD-EOI or FC-MRD-D15, in order to detect and rule out false positive results.

Methods:

We retrospectively analyzed the results from 1199 eligible patients diagnosed with ALL (July´11-Dec´22), treated according to ALLIC-BFM-2009 protocol, with a complete molecular, cytogenetic and immunophenotype work-out. FC-MRD was performed based on ALLIC-BFM-SOPs. Molecular (MLPA, RT-PCR) and cytogenetic (G-banding, FISH) studies were performed following standard techniques.

Results:

When comparing RG assignment of ALLIC-BFM-2009 patients applying ALLIC-BFM-2022 definitions, we observed that SRG would shift from 9.9% to 12.3%, IRG from 61.5% to 55.7% and HRG from 28.6% to 32.0%.

Among patients with FC-MRD-D15 < 0.1%, the main factor for relocating SRG to IRG was the absence of *ETV6::RUNX1* or hyperdiploidy.

Only 1/131 T-ALL would have been reassigned from SRG to IRG as FC-MRD-D15 was <10.0%.

For B-ALL, IRG to HRG relocation was mostly and equally due to *IKZF1*^{plus} status with FC-MRD-D15 ≥0.1% and by FC-MRD-EOI ≥0.05%. Six B-ALL cases with PPR and *ETV6::RUNX1* or hyperdiploidy with FC-MRD-D15 <0.1% would have been allocated to SRG instead of HRG.

PPR among B-ALL with FC-MRD-D15 ≥0.1 < 10% was the main parameter for relocating HRG to IRG.

The new criteria for CR status, FC-MRD-EOI ≥1.0% was detected in 78 patients. Of them, 72 were already in HRG based on different risk factors and 6 would have been reassigned to HRG due to not achieving CR.

We identified 10 cases with FC-MRD-EOI > FC-MRD-D15 and 15 with FC-MRD-EOC > FC-MRD-EOI, mainly in the first years of the study. When reanalyzed, 13 cases were corrected. The main causes of error were to consider a population that did not represent a clear cluster, misinterpretation of evaluability in the first time point, and underestimation of blast percentage due to partial expression of an aberrant marker.

Conclusions:

Based on our results, we would expect in the upcoming ALLIC-BFM-2022 protocol an increase in SRG due to the presence of *ETV6::RUNX1* or hyperdiploidy with FC-MRD-D15 <0.1%, either in patients from HRG with PPR or from IRG due to their age and/or WBC at diagnosis. An increase in HRG would be expected, mainly due to IKZF1^{plus} or IKZF1^{del} status of patients with FC-MRD-D15 ≥0.1% or due to FC-MRD-EOI ≥0.05%. We do not expect an increase in HRG based only on not achieving CR status by FC-MRD-EOI >1.0%.

The revision of cases emphazises the importance of expertise achievement in MRD analysis.

This new stratification will help to a better individualized therapy based on genetics and MRD response.

P 88 - Causes of death during induction therapy for acute lymphoblastic leukemia in children - a nationwide experience

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Background:

Outcome of childhood acute lymphoblastic leukemia (ALL) has been successful during the last decades due to improvements in intensive combination chemotherapy, new drugs and supportive measures. However, the important cause of first-line therapy failure are serious complications of chemotherapy. The incidence of treatment-related deaths (TRDs) in ALL children ranges from 2% to 3%.

Objectives:

The aim of this study was to evaluate causes of treatment-related deaths in ALL children during the induction phase of therapy residing in Poland. Part of the data on the clinical outcome of patients treated on this respective protocol has been reported elsewhere, but not in the context of early treatment-related death.

Patients and Methods:

A total of 1376 patients with newly diagnosed ALL, treated according to the ALL IC-BFM 2009 (International Berlin-Frankfurt-Munster Study Group) protocol in Polish pediatric hematology centers from November 2011 to September 2018, were included in the study. The analyzed group included 597 (43.4%) girls and 779 (56.6%) boys, aged 1–18 years. Standard risk group (SRG) included 192 (14%) patients, intermediate (IRG) - 868 (63.1%), high (HRG) - 316 (23%) ones. Cumulative death curves were estimated based on the Kaplan–Meier method with 95% confidence intervals. Tests were two-tailed with a level of significance of P = 0.05.

Results:

A total of 88 (14.3%) deaths were noted in the entire group, including 2 (2.3%) deaths in the SR group, 40 (4.5%) in the IR group, and 46 (52.3%) in the HR group. The cumulative death risk at 5 years was estimated to be 1.0% (SE 0.007) in the SR group, 6.1% (SE 0.014) in the IR group, and 18% (SE 0.026) in the HR group. In 14 (16%) cases, deaths happened during induction therapy before complete remission (CR) was achieved, and the estimated death rate during induction therapy was 1.02% (0% for the SR group, 0.65% for the IR group, and 0.37% for the HR group). This group included 7 girls and 7 boys, range aged 3–15 years (average 6.58 years). Characteristic features of this group was as following: white blood cells (WBC) at diagnosis > 20 000/μL - 6 patients; CNS infiltration – 3; *BCR::ABL* positive – 1; immunophenotype: T-cell ALL – 5, precursor B-cell ALL – 9; steroids poor response – 3 ones. In most patients (86%), the main cause of death was microbiologically documented infection (bacterial-5; combined bacterial and fungal -3; bacterial and viral -4). Other causes of death were central nervous system (CNS) hemorrhage (one child) and leukostasis CNS (one child). Deaths occurred between 8–58 days after diagnosis.

Conclusions:

The mortality rate evaluated before CR in our material was 1.02%. The results of this study present that infection, mainly bacterial and infection-related mortality remain a concern in pediatric ALL, despite improvement of supportive care. Currently, there are limited literature data about antibiotic prophylaxis in children during the induction phase of therapy.

P 93 - Pediatric acute lymphoblastic leukemia treatment related toxicity and impact on outcome

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Background:

Treatment results of protocols for children with Acute Lymphoblastic Leukemia (ALL) have improved through more accurate definitions of risk of relapse and thus personalized modalities of treatment, in an attempt to spare toxicity while maintaining high survival rates. Dana Farber Cancer Institute (DFCI) 05-01 and 11-011 protocols report, respectively, an estimated overall survival (OS) of 93% and 94,9% and event-free survival (EFS) of 87% and 86.5%. However, its toxicity is not negligible. Here we characterized toxicity in children under DFCI 05–01 and 11–011 protocols in order to understand if treatment modifications due to toxicity had an impact on patients outcome.

Objectives:

To describe treatment related toxicity and corresponding impact in protocol compliance and event-free survival in children and adolescents with ALL treated with DFCI 05–01 e 11–011.

Methods:

Retrospective, unicentric, observational study in pediatric population treated between 2015–2019. The following toxicities were considered: hematologic, infectious, stomatitis, hypersensibility to asparaginase, diabetes, thromboembolism, pancreatitis, osteonecrosis, pathologic bone fracture, seizure and posterior reversible encephalopathy syndrome (PRES). We defined as impact on treatment compliance when there was a treatment delay of ≥1 week or dose reduction due to treatment toxicity. EFS was calculated by STATA program, v13. A global EFS analysis and comparison between patients with and without toxicity was done.

Results:

121 patients were included, median age at diagnosis was 4 years (mínimum 1, maximum 16 years), 83% B phenotype ALL, 66% stratified in standard risk group, 27% high risk, 7% very high risk. Median number of days with neutrophils <500/uL was higher in Induction (19) and Consolidation II (10), average febrile neutropenia (FN) episodes per patient were 1.45, and non programmed hospitalizations (NPH) were 2.42 ±1.3. Infectious causes predominated as reason for hospitalization in every phase. 117 toxicity episodes were identified, with stomatitis being the most frequent (71), prevailing on Induction phase. 13 episodes of Asparaginase infusion reaction were observed, in which there was switching of PEGaspargase to Erwinase in 4 patients. Diabetes appeared on Induction (7) and Consolidation II (2). Thromboembolism episodes (7) occurred in every phase except Consolidation I. There were 3 osteonecrosis episodes and 2 of pathologic bone fractures. Every case of pancreatitis (3), seizure (3) and PRES (6) motivated changes in treatment.

With an average follow-up of 4 years and 9 months, 7 patients relapsed and 10 died (8 from infectious cause, 2 due to progression). 5-year EFS was 90% (CI 95%,0.83–0.94). We did not confirm statistically significant EFS difference between patients without toxicity and with toxicity impacting therapeutic course, HR 2.03 [95% CI 0.91–4.5], p = 0.08.

Conclusions:

Therapeutic toxicity episodes were seen in 61% patients, 54% of them suffered treatment alterations. Induction was the phase with more toxicity (44 episodes), prevailing stomatitis. Many toxicities were observed in Consolidation II mainly FN and NPH. Toxicity impact in treatment contributed to inferior EFS compared with the group of patients without toxicity. In this population, the 5-year EFS was comparable to what was previously described in literature.

Allo-HSCT and other cellular therapies of acute leukemias

P 89 - CAR-T in paediatric refractory / relapsed acute lymphoblastic leukaemia – Fundeni Clinical Institute Experience

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Background:

Outcomes in paediatric patients with acute lymphoblastic leukaemia (ALL) are constantly improving. Current treatment options provide survival rates ranging from 75 to 90% depending on various risk factors. However, some of the patients will present refractory disease or will relapse. To improve outcome in patients with refractory/ relapsed (r/r) disease, immunotherapies targeting specific B cell antigens have been developed. Tisagenlecleucel is an anti-CD19 chimeric antigen receptor therapy (CAR-T) approved for patients with r/r disease.

Objectives. Methods:

We performed a retrospective study to analyse the risk factors, complications and outcome of paediatric patients with B-cell ALL, included in the CAR-T program, from January 2022 to January 2023, in Fundeni Clinical Institute, Bucharest, Romania.

Results:

7 patients were included in the CAR-T program, in 5/7 patients we managed to harvest T-cells and only 4/5 have received treatment (Tisagenleucel, nr = 4). Patients were with relapse after HSCT (1/4), ≥2nd relapse (1/4) or r/r disease (2/4). All patients (4/4) received chemotherapy ≥ 3 lines (max.6) prior to CAR-T infusion. Median time from T-cell harvest to infusion was 68.5 days (range 49–217). In 3/4 patients tumour burden prior to CAR-T was <1%, only in 1/4 patient bone marrow blast % was very high (90%). 3/4 patients received lymphodepleting regimens, 2/3 standard with Flu-Cy, and 1/3 with additional etoposide, cytarabine, dexamethasone. All 4/4 patients received a number of cells ranging from 1.2 x 10^6 to 6 x 10^8. All patients (4/4) developed CRS, 2/4 had grade 1 CRS and 2/4 had grades 3, 4. One patient developed severe HLH/MAS, another one had ICANS grade 1, and one patient had hypertriglyceridemia that was linked to CAR-T. One patient had CRS grade 4 from day+3, required ICU measures for 14 days, intubated and ventilated, and was discharged on day +34, with full recovery. All patients (4/4) achieved haematological response, 3/4 achieving CR, 1/4 responded to CAR-T but died following HLH/MAS. One patient relapsed 3 months after CAR-T, following infection with SARS-COV2. Patient received 1 cycle of Inotuzumab and proceeded to HSCT, patient is still alive, in CR.

3/4 patients have good clinical condition and maintain CR.

Table 1: Patients demographics.

variable		Patient 1	Patient 2	Patient 3	Patient 4
patient sex		male	male	male	male
age at diagnosis		5y	11y	7y	7y
age at infusion		11y	14y	9y	9y
no. of previous lines of treatment before CAR-T		5	3	6	3
bridging therapy		VCR, Carfilzomib, doxorubicin, Peg-Asp, dexamethasone	VCR, BTZ, doxo-rubicin, Peg-Asp, dexamethasone	VCR, BTZ, doxo-rubicin, Peg-Asp, dexamethasone, VP16, 6-MP, AraC	Mitoxantrone, VCR, dexamethasone, Peg-Asp, Imatinib
time from T-cell harvest to infusion		63 days	49 days	74 days	217 days
BM blast % prior to CAR-T		0.08%	< 1%	90%	0.06%
lymphodepletion		Flu-Cy	no lymphodepletion	Flu-Cy-VP16, AraC, Dexamethasone	Flu-Cy
complications	CRS, grade	4	1	3	1
HLH/MAS	-	-	severe	-	
ICANS	1	-	-	-	

other	-	-	-	hypertriglyceri-demia	
response		CR4, after CAR-T, 100 % donor chimerism	CR4, after HSCT	death (HLH/MAS)	CR1, after CAR-T

Conclusion/ Application to practice:

CAR-T infusion represents a promising treatment option for paediatric patients with ALL and r/r disease or patients who relapse after HSCT. Future role as bridging therapy to HSCT is to be investigated. Overall survival in our group was 75% (3/4 patients), all with CR achieved following CAR-T infusion.

P 90 - Genomic characterization of relapsed/refractory B-cell Acute Lymphoblastic Leukemia pediatric patients undergoing CAR-T treatment in a single center

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Background:

Chimeric Antigen Receptor T cells (CAR-T) have revolutionized the treatment of pediatric patients with relapsed/refractory B-cell acute lymphoblastic leukemia (R/R-ALL), with a significant improvement in survival. However, a significant proportion of patients relapse after CAR-T. Little is known regarding the factors associated with failure to CAR-T therapy. The identification of such prognostic factors may help to better select patients who will benefit from this therapy. Further genetic characterization at the time of indication of CAR T-cell therapy might help as a predictive factor to identify patients at higher risk of CAR-T cell failure.

Aims:

We aimed to identify genomic alterations with prognostic relevance at CAR-T cell therapy indication in R/R-ALL pediatric patients in a single center.

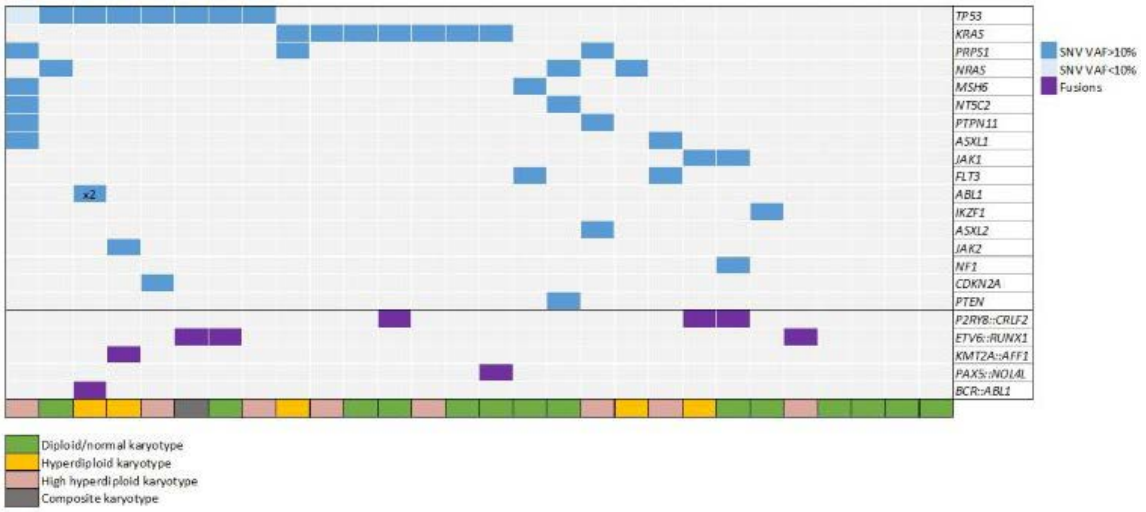
Methods:

The study included 28 R/R-ALL pediatric patients treated with CAR-T at Hospital Sant Joan de Déu from 2016 to 2022. To obtain the karyotype G-banding technique was performed. For identification of fusion-genes and mutations, we sequenced bone marrow samples before CAR-T therapy using the AmpliSeq™ for Illumina® Childhood Cancer Panel (Illumina, San Diego, CA). Only mutations with VAF>10% were reported, except for *TP53,* as high- and low-VAF mutations in this gene have been previously reported to be associated with prognosis in CAR-T cell patients in lymphoma and leukemia.

Results:

We studied twenty-eight pediatric R/R-ALL patients treated with CAR-T in a single center. Globally, at the time of CAR-T cell indication, considering karyotype, DNA mutations and fusion genes, 85% of patients (24/28) showed at least one genetic alteration (Fig 1). Regarding karyotype, 8 patients were classified as high hyperdiploid karyotype (51 to 67 chromosomes), 5 as hyperdiploid (47–50 chromosomes), 1 as composite karyotype, and 14 presented a normal karyotype. Regarding fusions, we observed five different rearrangements in 9/28 patients, all of them previously described and with potential clinical impact: *ETV6::RUNX1* (n = 3), *P2RY8::CRLF2* (n = 3), and *KMT2A::AFF1* (n = 1), *BCR::ABL1* (n = 1), and *PAX5::NOL4L* (n = 1) rearrangement. Twenty-three patients (82%) had at least one mutation. The mean number of mutations per patient was 1.7 (range 1 to 6 mutations per sample). The most commonly mutated genes were *TP53* (8/23, 34.8%) and *KRAS* (7/23, 30.4%). One of the patients with a *TP53* mutation had a VAF < 10%. *NRAS* and *PRPS1* were mutated in three patients; *MSH6*, *NT5C2*, *PTPN11*, *ASXL1*, *JAK1,* and *FLT3* were mutated in two patients, and *ABL1*, *IKZF1*, *ASXL2*, *JAK2*, *NF1*, *CDKN2A,* and *PTEN* were mutated in one patient each. Interestingly, the patient with mutated *ABL1* presented two different mutations in this gene with a VAF >10%. Overall, a high rate of pathogenic variants in genes involved in signaling pathways and driver genes were observed, all of them involving hotspot regions and previously reported in pediatric acute leukemia.

Figure 1: Genetic abnormalities found at the time of indication of CAR-T cell therapy: mutations (blue), fusion genes (purple) and the main findings in the karyotype (green, yellow, pink, grey) are depicted.



Summary / Conclusions:

Our results showed a heterogeneous genomic landscape in those patients with R/R-ALL at the time of CAR-T cell therapy indication. Most alterations involved apoptosis and cell cycle regulation, kinase activation, and cell proliferation, and have been described as therapeutic targets. A thorough biological characterization of R/R-ALL patients may help understand the impact of genetic alterations in the era of immunotherapy, and predict the risk of relapse after CAR-T cell therapy.

P 91 - Management of a pediatric patient with Ph+ acute lymphoblastic leukemia with TKI intolerance and fast relapse after hematopoietic stem cell transplantation – Case report

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Background:

Allogeneic haematopoietic stem cell transplant (HSCT) represents a curative approach for refractory/relapsed Ph+ ALL (acute lymphoblastic leukaemia). The role of tyrosine kinase inhibitors (TKI) after HSCT is still unclear. Disease relapse following HSCT is associated with poor prognosis and survival, therefore carefully post-HSCT monitoring is required.

Objectives. Methods:

We report the case of a 5y old girl with Ph+ ALL. She was treated using ALL BFM-2017 protocol and Imatinib 340 mg/m^2. Evaluation at day15, day 3 and day78 were performed, also NGS analysis was performed, and chimerism monitoring following HSCT. NGS was performed using TruSight Oncology 500, chimerism analysis was performed using Mentype Chimera.

Results:

Patient was diagnosed with Ph+ ALL in February 2022. Initial evaluation showed: WBC = 11.18 x 10^9/L, ANC = 0.75 x 10^9/L, Haemoglobin = 11.10 g/dl; PLT = 29 x 10^9/L, 76% blasts on peripheral smear. Bone marrow evaluation showed 90% L1 lymphoblasts, immunophenotyping: 87% blast cells, common B phenotype. Cytogenetic analysis revelead complex karyotype : 44~46, X, -X, t(9;22)(q34;q11), -2, 3, 4, -9, -14, 2mar, inc[cp20]; Ph+ celss 70%, and molecular biology showed presence of BCR-ABL p190.

Patient received chemotherapy according to EsPhALL 2017 protocol. Evaluation at day15 showed 8.8% blasts, at day 33 0.01% with % BCR-ABL IS = 0.0052%, and at day 78 0.02%. Patient was proposed for HSCT, and matched family donor was identified.

In April 2022 patient completed induction treatment and two high-risk (HR) courses. For the 2 HR courses the patient was switched to Dasatinib. Pre-HSCT evaluation showed 0.08% MRD. Conditioning regimen used was Fludarabine-Thiotepa-Busulfan, as TBI (total body irradiation) was not available in our country, with Cyclosporine and short course of Methotrexate for graft-vs-host-disease prophylaxis. Patient received a 5 x 10^6 CD34/kg PBSC graft, with neutrophil and platelet engraftment in day+12, 98% donor chimerism in day+13. On day+12 patient developed engraftment syndrome and required intensive care measures, high-dose corticosteroids and hemodiafiltration with cytokine filters, with full recovery on day+22. On day+23 patient developed severe gastro-intestinal bleeding, with prolonged recovery, severe malnutrition and 1 month stay in surgery department. On day+69 TKI treatment was initiated. Evaluation at day+75 showed 100% donor chimerism and MRD 0.15%. On day+97 patient presented with Flu + SARS COV2 infection, and 40% blast cells on peripheral smear, CNS relapse, 48% donor chimerism. Patient was included in the CAR T program, but died on day+122 due to progressive disease.

NGS analysis performed at relapse revealed the presence of BCR-ABL1 fusion transcript with missense mutation in ABL1 p.E255K (E274K), associating also ETV6 p.Y346C c.1037A>G, ESR1 p.R269C c.805C>T. The missense mutation in ABL1 p.E255K (E274K) has been demonstrated to confer drug resistance. Also, point mutations in the BCR-ABL1 kinase domain are involved in secondary resistance to TKI therapy, thus associating with poor prognosis and an increased risk of disease progression. Pre-existing mutations found in the BCR-ABL1 kinase domain, which have been associated with resistance to treatment, have been found in many patients who relapse after HSCT.

Conclusion / Application to practice:

Ph + ALL patients may present with unfavourable outcomes. Our patient had many complications following chemotherapy, including resistance to TKI's, not being able to achieve CR at any point. HSCT though performed rapidly was also associated with severe complications, and relapse appeared before day +100. NGS analysis performed at relapse was consistent with our patient's evolution.

P 92 - Very poor prognosis in paediatric and adult patients with relapsed/refractory cortical (CD1a+) T-cell acute lymphoblastic leukaemia (ALL). The Spanish experience from the SEHOP and PETHEMA ALL groups

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Background:

T-cell acute lymphoblastic leukaemia (T-ALL) accounts for 15% and 25% of ALL diagnosed in children and adults, respectively. Prognosis after first-line therapy is better in children than in adults, with a 5-year probability of overall survival (pOS) of 85% and 50%, respectively. In refractory or relapsed patients (R/R-T- ALL) the prognosis is very poor, with 5-year pOS of less than 25%. Therefore, new therapeutic strategies, such as immunotherapy, are required. CD1a is a potential target CAR-T therapy being tested in one clinical trial (EudraCT:2021–002333–42).

Our aim is to describe the epidemiological characteristics and survival of paediatric and adult patients in R/R CD1a+ T-ALL in order to have a historical control for comparison with patients treated with CAR T-cells targeting CD1a.

Patients and methods:

Descriptive and retrospective study in paediatric and adult patients diagnosed with R/R-T CD1a+ ALL between January 2005 and November 2021 in Spain. All patients were treated in first-line according to the treatment protocols SEHOP-PETHEMA 2013 (paediatric patients) and PETHEMA LAL-AR-03 & LAL-AR-11 (adults).

Results:

Forty-three patients were included, 15 paediatric and 28 adult (Table). Three patients were primarily refractory and 40 relapsed. One patient died at diagnosis of relapse and another due to toxicity to the first salvage therapy. Seventeen patients achieved a 2nd complete remission (CR) and 24 were refractory to first salvage treatment.

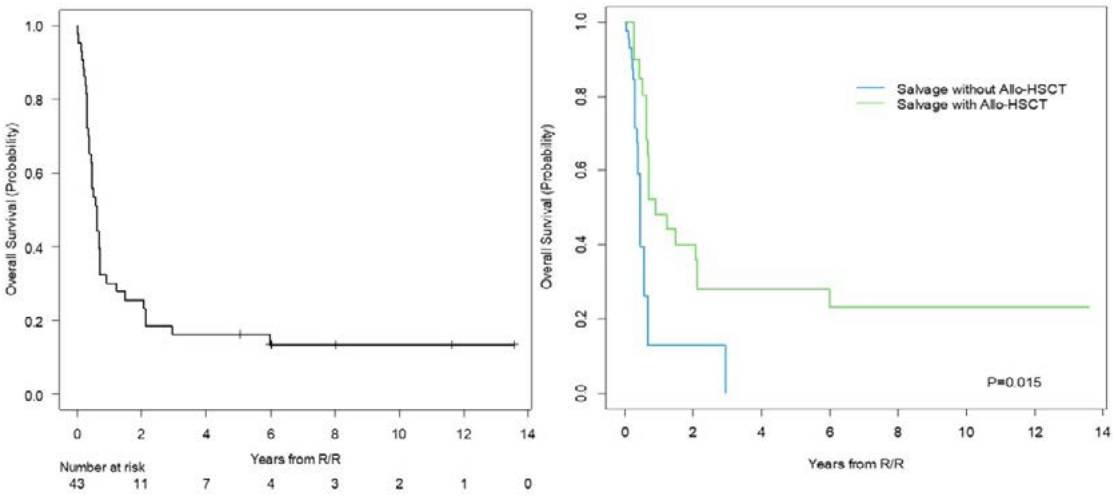
Among the patients who achieved a 2nd CR, 12 undewent allogenic haematopoietic stem cell transplantation (allo-HSCT) and 4 were still alive and in remission at data cut-off. Among the remaining 24 patients, 10 were bridged to allo-HSCT (sequentially or in subsequent CR): 2 were alive in CR.

Overall, out of 42 evaluable R/R-T-ALL patients, 23(55%) received an allo-HSCT: 11 (48%) relapsed, 6 (25%) died from transplant-related mortality and 6 (26%) were still alive and in remission, at data cut-off.

With a median follow-up of 7 years (range, 5.1–13.6), 5yr-pOS was 16% (95%CI, 7–29). Among patients who received an allo-HSCT, 2-year pOS was 40% vs. 13% in those not transplanted (p = 0.015) (figure).

Table: Characteristics of patients with R/RCD1a+ T-ALL.

Characteristics of patients	n = 43
Age at diagnosis, median (range)	24 (4–56)
Female, n(%)	8 (19)
Leukocytes x10e9/L, median (range)	75 (1.4–456.4)
CNS involvement, n(%) at diagnoses/relapse	10 (23) / 18 (45)
Mediastinal mass, n(%)	21 (49)
Extramedullary disease, isolated or combined, n(%) at diagnoses/relapse	5 (12) / 26(65)
Median time to relapse, months(range)	12 (1–32)
Median therapy lines, (range)	2 (1–5)



Conclusions:

The prognosis of patients with relapsed/refractory CD1a+ T-ALL is dismal, with only 16% being long-term survivors. The only curative option is allo-HSCT. The development of new strategies such as CD1a directed CAR-T cells might offer a therapeutic alternative.On behalf the leukemia group of SEHOP and PETHEMA.

Authors' Index