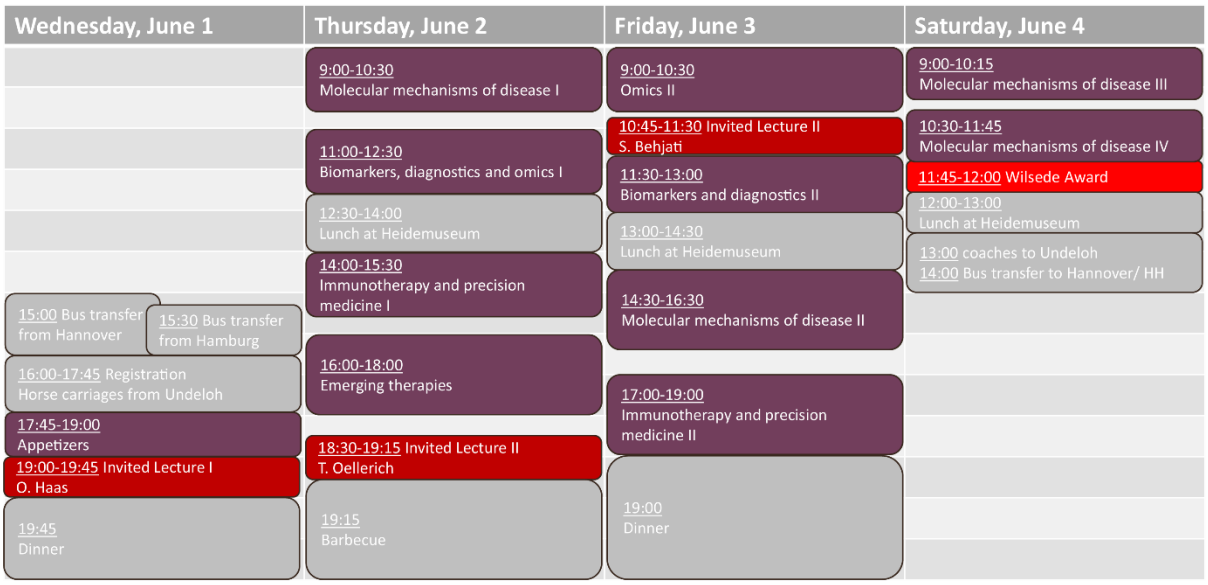
ABSTRACT BOOK
JUNE 1-4, 2022



WEDNESDAY, JUNE 1

17.45 – 18.00 **Welcome by Rolf Marschalek**

18.00 – 18.45 **Appetizers** *Chair: Evelyn Ullrich*

Beyond GD2 – Glycolipids as immunotherapeutic targets in neuroblastoma

Rekowski L [1,2], Bley I [1,2], Schattling B [1,2], Möller YS [1,2], Hunczek A [1,2], Cornils K [1,2], Müller I [1,2]
[1]Clinic of Pediatric Hematology and Oncology, Division of Pediatric Stem Cell Transplantation and Immunology, University Medical Center Hamburg-Eppendorf
[2]Research Institute Children's Cancer Center Hamburg

Neuroblastoma (NB) is the most common extracranial solid tumor in children and accounts for 6-10% of all pediatric cancers. Although antibody-based immunotherapy with Dinutuximab improved the outcome for high-risk patients, the 5-year survival rate is below 60%. To improve the prognosis, the development of new therapeutic options is urgently needed. As researchers found natural IgM antibodies in 3% of the healthy population, which effectively lysed neuroblastoma cells, we aimed to identify the target. By systematic biochemical modifications of the glycocalyx of neuroblastoma cell lines we identified new tumor-associated glycolipids, which showed high density on neuroblastoma cells. We further generated recombinant antibodies using different available B cell receptor sequences against these targets. Our antibodies showed potent and specific complement-dependent lysis of neuroblastoma cells *in vitro*. Also, natural killer (NK) cells expressing target-specific chimeric antigen receptors (CARs) revealed effective eradication of tumor cells and highlight the discovered glycolipids as promising targets for the treatment of neuroblastoma patients.

The immune checkpoint ICOSLG is a relapse-predicting biomarker and therapeutic target in infant t(4;11) ALL

Külp M[1], Larghero P[1], Dietz A[1], Cario G[2], Eckert C[3], Caye-Eude A[4], Cazzaniga G[5], Bonig H[6], Meyer C[1], Marschalek R[1]
[1]Institute of Pharmaceutical Biology/DCAL, Goethe-University, Frankfurt am Main, Germany
[2]Department of Pediatrics, University Medical Center Schleswig-Holstein, Campus Kiel, Germany
[3]Department of Pediatric Hematology and Oncology, Charité-Universitätsmedizin Berlin, Germany
[4]Département de Génétique, Hôpital Robert Debré, Assistance Publique des Hôpitaux de Paris (AP-HP), Paris, France
[5]Centro Ricerca Tettamanti, Pediatrics, University of Milan-Bicocca, Fondazione Monza e Brianza per il Bambino e la sua Mamma (MBBM)/San Gerardo Hospital, Monza, Italy
[6]Institute for Transfusion Medicine and Immunohematology, Goethe University, Frankfurt am Main, Germany

Infant proB ALL is mainly caused by the chromosomal translocation t(4;11), generating the fusion oncogenes KMT2A::AFF1 and AFF1::KMT2A. Generally, KMT2A-rearranged iALL is characterized by dismal outcome due to high relapse incidences and relapse-associated mor-tality. Relapse appears despite continuous chemotherapy but without selection of secondary mutations. Therefore, the mechanism of therapy failure and relapse formation remains to be elucidated. Here, we present first evidence that ALL cells overexpressing ICOSLG are strongly correlated with relapse formation and poor event-free survival (EFS). ICOSLG ex-pression is transcriptionally regulated by the IRX1-EGR3 axis, and co-culture of ICOSLG-expressing t(4;11) ALL cells with primary T-lymphocytes led to an increase of regulatory T-cells (Tregs). A commercial α-ICOSLG antibody is available that impairs this mechanism. We propose that these Tregs potentially protect leukemia initiating cells in their bone marrow niche. In summary, our data point to ICOSLG as a relapse-predicting biomarker and therapeutic target, involved in an immune evasion relapse-mechanism in infant t(4;11) proB-ALL.

Mutational impact of chemotherapy on hematopoietic cells and evolution towards therapy-related pediatric AML

Bertrums E J M[1,2,3], de Kanter J K[1,2], Rosendahl-Huber A K M[1,2], Zwaan C M[1,3], van den Heuvel-Eibrink M M[1,4], Goemans B F[1], van Boxtel R[1,2]
[1]Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands
[2]Oncode Institute, Utrecht, the Netherlands
[3]Department of Pediatric Oncology, Erasmus Medical Center Hospital, Rotterdam, the Netherlands

[4]Utrecht University, Utrecht, the Netherlands

The long-term survival rate of childhood cancer has increased to over 80%, largely due to chemotherapy as a major treatment modality. Chemotherapy acts predominantly by fatally damaging DNA of malignant cells; however, the mutational effects in normal tissues remain unknown. Here, we studied mutation accumulation in hematopoietic stem and progenitor cells (HSPCs) of 23 pediatric cancer patients, of which 18 developed therapy-related myeloid neoplasms later in life. Phylogenetic inference demonstrated that during chemotherapy exposure mutant HSPC clones are induced and selected, which may ultimately contribute to therapy-related acute myeloid leukemia (t-AML). Post-treatment HSPCs showed a significantly increased mutation load compared to treatment-naïve cells. Mutational signature extraction revealed that only few chemotherapeutic drugs, such as platinum drugs and thiopurines, caused direct mutagenic effects. While cisplatin affected all exposed cells, thiopurine affected leukemic blasts and MLL-rearranged HSPCs. Intriguingly, increased mutagenesis in most patients was caused by processes similar to those underlying clock-like signatures also present during normal aging.

A single-cell expression atlas of human AML-LSCs unravels the contribution of HIF pathway and its therapeutic potential

Velasco-Hernandez T[1], Trincado J L[1], Vinyoles M[1], Closa A[2], Molina O[1], Velten L[3], Bueno C[1], Eyras E[2], Heyn H[4], Menendez P[1]

[1]Josep Carreras Leukemia Research Institute, Barcelona, Spain
[2]The Australian National University, Camberra, Australia
[3]Centre for Genomic Regulation, Barcelona, Spain
[4]CNAG-CRG, Barcelona, Spain

Relapse remains a major challenge in the clinical management of acute myeloid leukemia (AML) and is driven by rare therapy-resistant leukemia-initiating stem cells (LSCs) that reside in specific bone marrow niches. Hypoxia signaling keeps cells in a quiescent and metabolically relaxed state, desensitizing them to chemotherapy. This suggests the hypothesis that hypoxia contributes to AML-LSC function and chemoresistance and is a therapeutic target to sensitize AML-LSCs to chemotherapy. Here, we provide a comprehensive single-cell expression atlas (119,000 cells) of AML cells and AML-LSCs in paired diagnostic-relapse samples from risk-stratified pediatric and young adult patients with AML. The HIF/hypoxia pathway is attenuated in AML-LSCs compared with differentiated AML cells, but is enhanced when compared with healthy hematopoietic cells. Accordingly, chemical inhibition cooperates with standard-of-care chemotherapy to impair leukemogenesis, substantially eliminating AML-LSCs. These findings support the HIF pathway as a stem cell regulator in human AML, and reveal avenues for combinatorial targeted and chemotherapy-based approaches to specifically eliminate AML-LSCs.

THURSDAY, JUNE 2

9.00 – 10.30 **Molecular mechanisms of disease** *Chair: Irmela Jeremias*

Deciphering the molecular mechanism of NUP98-KDM5A chromosomal translocation in pediatric non-DS-AMKL

Cifarelli L N[1], Issa H[2], Schuschel K[2], Menge K[1], Gack L[1], Klusmann JH[2]*, Heckl D[1]*
[1]Dep. of Pediatric Hematology and Oncology, Martin-Luther-University Halle-Wittenberg, Halle, Germany
[2]Dep. of Pediatrics, Goethe-University Frankfurt, Frankfurt am Main, Germany
*Authors contributed equally to the work

The chromosomal translocation NUP98-KDM5A (NK5A) occurs in 15% of pediatric non-Down Syndrome-Acute Megakaryoblastic Leukemia (non-DS-AMKL) and is linked to poor prognosis. To address the poorly understood molecular mechanisms of NK5A-driven leukemogenesis we overexpressed NK5A in murine fetal liver cells (mFL) Interestingly, a biphasic NK5A-mediated leukemic transformation was observed with an initial and immediate strong depletion of NK5A positive cells upon expression of the fusion oncogene. NK5A almost exclusively occurs in pediatric AMKL. Speculating on the involvement of fetal gene signatures in the NK5A-mediated transformation, we next performed a fetal signature focused CRISPR-Cas9 screening. Complementing these data with RNAseq at different time points of NK5A-mediated transformation, we were able to identify 36 high confidence candidate genes regulated by NK5A, which we will further refine with ongoing CUT&Tag Histone profiling and ATACseq. Overall, our study identified new molecular mechanisms of a biphasic NK5A-mediated leukemic transformation and will provide us new insights into potential therapeutic strategies for the treatment of high-risk pediatric AMKL.

Identification of direct target genes of NUP98-KDM5A reveals regulatory gene networks in Acute Myeloid Leukemia

Troester S[1], Schmoellerl J[2], Eder T[1], Manhart G[1], Winter G[3], Zuber J[2], Grebien F[1]
[1]University of Veterinary Medicine Vienna, Austria
[2]Research Institute of Molecular Pathology (IMP), Vienna, Austria
[3]Research Center for Molecular Medicine of the Austrian Academy of Sciences (CeMM), Vienna, Austria

Oncogenic Nucleoporin 98 (NUP98) fusion proteins are recurrently found in pediatric acute myeloid leukemia (AML) with poor prognosis, but the molecular mechanisms of NUP98-fusion-driven leukemogenesis are unclear. We aimed to characterize transcriptional programs that govern the development and maintenance of NUP98-KDM5A-driven AML. A genome-scale CRISPR/Cas9 loss-of-function screen identified 4105 genes that were essential in NUP98-KDM5A-driven murine AML cells. To study direct transcriptional effects of NUP98-fusion-dependent gene regulation we developed a model for ligand-induced degradation of NUP98-KDM5A. Nascent RNA-seq upon fusion protein degradation identified 45 direct NUP98-KDM5A target genes, of which 12 were classified as essential for NUP98-KDM5A AML growth. RNA-seq analysis upon RNAi-induced knockdown revealed that a small subset of the 12 NUP98-KDM5A target genes was able to recapitulate global patterns of NUP98-KDM5A-induced gene deregulation. Further investigation of the interplay between core members of the NUP98-KDM5A effector network will lead to a better understanding of aberrant gene expression in NUP98-fusion AML and might identify novel therapeutic targets.

Designing specific chromosomal translocations of the MLL/KMT2A gene

Benz T[1], Kowarz E[1], Marschalek R[1]
[1]Institute of Pharmaceutical Biology/DCAL, Goethe-University, Frankfurt, Germany

Chromosomal translocations are the major cause of hemato-malignant cancers. Many of them have been shown to be necessary and sufficient for the onset of leukemias or lymphomas. For some of them, experimental data are still controversial, e.g. the chromosomal translocation t(4;11). Experiments using only the MLL-AF4 fusion protein did never result in cancer development,

while the combination of MLL-AF4 and AF4-MLL result in leukemia (see Schneidawind et al. 2018). Here we present a novel CRISPR/CAS9-mediated experimental system to induce specific chromosomal translocations in primary cells or cell lines, by using a "chromosomal homology mediated end joining" (HMEJ) approach. In our case, we used genomic fragments of MLL/KMT2A and AF4/AFF1 to induce specifically a t(4;11) translocation which was subsequently positively (Puromycin) and negatively (Ganciclovir) selected. We present our initial data on this novel and universal system that allow to induce specific genome rearrangements known to cause the oncogenic conversion of normal cells into malignant cells.

MLL-r fusion transcripts in healthy individuals by induced gene proximity

Streb P[1], Kowarz E[1], Marschalek R[1]
[1]Institute of Pharmaceutical Biology/DCAL, Goethe-University, Frankfurt, Germany

Chromosomal rearrangements are a major cause of hemato-malignant diseases. With about 10% of the ALL and 5% of the AML cases, MLL-rearrangements play a key role for the onset of leukemia. The specific chromosomal translocation t(4;11) has been identified in 50% of infant ALL, 44% of pediatric ALL and 80% of adult ALL cases. Here, we asked the question why the AF4 gene is such a prominent fusion partner in MLL-r leukemia. Different investigations have already shown that chimeric fusion RNAs are produced in white blood cells of healthy donors (e.g. BCR-ABL, MLL-AF4). We have shown recently that these fusion transcripts are formed by trans-splicing events which may be caused by "early terminated transcripts". Now we present the formal prove that an experimental induction of "gene proximity" in the interphase nucleus is sufficient to allow the formation of MLL-AF4 fusion transcripts in the absence of any t(4;11) translocation (missing AF4-MLL fusion transcript). Thus, "gene proximity" in combination with certain "transcriptional features" (termination of primary transcripts in the bcr of genes) are likely to be the cause for the onset of chromosomal translocations.

Modelling congenital neutropenia, a pre-leukemic bone marrow failure syndrome in zebrafish

Aghaallaei N[1], Doll L[1], Skokowa J[1], Walte K[1], Bajoghli B[1]
[1]University Hospital Tübingen, Germany

Defects in zebrafish hematopoiesis reliably copy human blood diseases, making it a highly attractive model to develop therapeutic strategies. We study severe congenital neutropenia (CN), a blood disorder characterized by disrupted neutrophil development. Mutations in more than 20 genes are associated with CN, including HAX1, JAGN1, and CSF3R. Around 20% of CN patients develop leukemia. However, the underlying mechanisms are poorly understood. We use zebrafish to model CN and leukemogenic transformation in CN. Zebrafish harboring mutations in the HAX1, JAGN1, and CSF3R genes were generated using the CRISPR/Cas9 technology. Akin to CN patients, zebrafish deficient for HAX1 and JAGN1 displayed reduced neutrophil development, which could be rescued by G-CSF. Intriguingly, the introduction of truncated mutations in the CSR3R gene mimicking somatic mutations seen in CN/AML patients led to dysplastic hematopoiesis and high zebrafish lethality. Taken together, our established in vivo models enable studying CN and the leukemogenesis associated with CN. They can also serve as in vivo platform to perform drug screening and identify new therapeutic strategies for CN and leukemia development.

Identification of RBMS1 in the amplified region 2q24 as a major driver of cellular growth in childhood hepatoblastoma

Rodemann M[1], Dreschmann V[1], Dörner E[1], von Schweinitz D[2], Vokuhl C[3], Pietsch T[1]
[1]Department of Neuropathology, University of Bonn Medical Center, Bonn, Germany
[2]Department of Pediatric Surgery, LMU University of Munich Medical Center, Munich, Germany
[3]Pediatric Pathology, Department of Pathology, University of Bonn Medical Center, Bonn, Germany

In the framework of the clinical hepatoblastoma study of the GPOH, we had the opportunity to study samples of 76 hepatoblastoma patients. Using molecular inversion probe (MIP) array technology we generated quantitative chromosomal copy number profiles that uncovered

chromosomal alterations, in particular gain of chromosome 2q (44.7%) and in some cases amplification of the region 2q24 (11.8%). This suggests the presence of a so far unidentified oncogene in this chromosomal region. The RBMS1 gene located within the amplicon on chromosome 2q24 encodes a single-stranded DNA/RNA binding protein and showed significant RNA overexpression in 2q24 amplified tumors. This overexpression was validated by immunohistochemical studies at the protein level. RBMS1 knockdown by specific siRNA transfection resulted in a significantly reduced proliferation and marked reduction of the WNT pathway activity in hepatoblastoma cell lines. We identified RBMS1 as a potential oncogenic driver in hepatoblastoma which may exert this function by interaction with the WNT signaling pathway that is pathologically activated in hepatoblastoma.

Contribution of aneuploidy to the initiation and progression of childhood B-cell acute lymphoblastic leukemia

Thampi N[1], Bueno C[1], Rodríguez-Cortez V[1], Martínez A[1], Vinyoles M[1], Camps J[2], Menendez P[1], Molina O[1]

[1]Josep Carreras Leukemia Research Institute, Barcelona, Spain

[2]Institut D'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

Aneuploidy pose deleterious effects on cellular fitness due to important consequences on transcriptome and proteome, leading to reduced proliferation and activation of immune surveillance mechanism. Despite this, aneuploidy is the most frequent genetic abnormality observed in childhood B-cell acute lymphoblastic leukemia (B-ALL) and is the primary oncogenic event arising in utero in hematopoietic precursors. However, the precise role of aneuploidy on leukemia initiation and progression is currently unknown. In here, we aim to study the mechanisms involved on cellular tolerance to aneuploidy in human CD34+ hematopoietic stem/progenitor cells (HSPC), to decipher its contribution to B-ALL initiation. We treated cells with either Reversin or Cytochalasin D, to model random abnormal mitoses or tetraploid intermediates, respectively. HSPCs were followed in vitro and in vivo to assess proliferation, aneuploidy rates, differentiation and karyotype evolution. Our initial results show that aneuploidy is highly detrimental to HSPC and is ultimately lost in competition with their euploid counterparts. In vivo experiments are ongoing to assess whether aneuploidy is tolerated in their own niche.

11.00 – 12.30 **Biomarkers, diagnostics and omics I** *Chair: Markus Metzler*

Genetic landscape of large cell/anaplastic medulloblastoma – more than one disease

Krause K[1], Goschzik T[1], Dörner E[1], Pietsch T[1]

[1]University of Bonn Medical Center, Dept. of Neuropathology, Bonn, Germany

Medulloblastom is the most common malignant pediatric brain cancer. The large cell/anaplastic type of MB is characterized by poor survival. We collected FFPE samples of 155 LC/A MB with a median age of 9 years, and performed a systematic histological, immunohistochemical and genetic analysis by next-generation panel sequencing (NGS), 450k/850k methylation bead-array hybridization and molecular inversion probe array. Methylation-based subtyping and NGS panel sequencing showed that the cohort of LC/A MB represent different biological entities: 8% WNT-MB, 18% SHH-MB to be divided in 58% SHH TP53-activated and 42% SHH TP53-wildtype and 69% non-WNT/non-SHH-MB (68% Grp3 and 32% Grp4). Copy profiling by MIP showed MYC amplification, mostly in Grp3 MB; 82% of them in methylation subtype II of Grp3 MB. Focal gains of OTX2 were present in 54 % of cases (all subgroups) and i17q in 86% of non-WNT/non-SHH. By NGS we detected recurrent mutations in 37 MB-related genes; most frequent were TP53 (16%; mostly in SHH-TP53mut and WNT), KMT2D (9%; all types), CTNNB1 (9%; only WNT), and PIK3CA (9%, mostly in SHH-TP53wt and WNT). Our data demonstrate that LC/A MB represent different biological entities.

The extended potential of optical genome mapping (OGM) in pediatric AML compared to classical cytogenetics

Suttorp J[1], Lühmann J L[2], Steinemann D[2], Reinhardt D[1], von Neuhoff N[1], Schneider M[1]
[1]University Hospital Essen, Clinic of Pediatrics III, Essen, Germany
[2]Department of Human Genetics, Hannover Medical School, Hanover, Germany

Pediatric AML is characterized by numerous genetic aberrations (GA) impacting its classification for risk of treatment failure. GA are described by classical procedures (karyotyping, FISH, RNA-fusion transcripts) which harbor limitations. OGM is an emerging chip-based DNA technique with high resolution (~500 bp, for details see: https://bionanogenomics.com/products/saphyr/).
In 24 pediatric AML patients OGM results were compared to classical procedures. Discrepancies were detected in 17/24 cases including 32 previously unknown GA called by OGM only. One newly detected deletion and 2 translocations were validated by primer walking, breakpoint-spanning PCR, and DNA-sequencing. Additionally, in 2 cases OGM identified a new minimal residual disease (MRD) marker. Comparing impact on risk stratification, 19/20 de-novo AML cases had concordant results while OGM unraveled another high-risk aberration.
In conclusion, OGM expands the methodological spectrum to optimize the diagnosis of pediatric AML by identification of new GA. Results will contribute to a better understanding of leukemogenesis in pediatric AML. In addition, GA identified by OGM may provide markers for MRD monitoring.

IKZF1plus under investigation: Optical Genome Mapping to detect additional alterations

Lühmann J L[1], Kater J[1], Wendeburg L[1], Möricke A[2], Cario G[2], Schrappe M[2], Schlegelberger B[1], Stanulla M[3], Steinemann D[1]
[1]Department of Human Genetics, Hannover Medical School, 30625 Hannover, Germany
[2]Department of Pediatrics I, ALL-BFM Study Group, Christian-Albrechts University Kiel and University Medical Center Schleswig-Holstein, 24105 Kiel, Germany
[3]Pediatric Hematology and Oncology, Hannover Medical School, 30625 Hannover, Germany

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer. Leukemic cells are characterized by structural and numeric genomic aberrations, which strongly correlate with prognosis and clinical outcome. A proportion of ALLs cannot be assigned to a distinct subgroup and are classified as B-other. Within this group the IKZF1plus-profile was described as a copy-number based and MRD-dependent stratification profile with very poor prognosis. It is unknown so far, if this profile presents as a surrogate for other unidentified underlying variants. Using optical genome mapping (OGM), a method to detect all types structural variants, we retrospectively investigated IKZF1del/plus-ALL samples and searched for genomic aberrations driving or contributing to the poor prognosis for these patients. Applying OGM we detected several recurrent stratification markers associated with poor prognosis such as a ETV6::ABL1 fusion (ins(12;9)), a PAX5::JAK2 fusion (inv(9)), a EBF1::PDGFRB fusion (t(5;5)). Additionally, we identified a novel NPAT::JAK2 fusion resulting from a t(9;11). Our preliminary data show that IKZF1del/plus-ALL carry additional markers that might be the drivers of the disease.

Unravelling the recombinome of IKZF1 deletions in B-ALL

Lopes B[1], Meyer C[2], Maciel A L[1], Barbosa T[1], Venn N C[3], Sutton R[3], Fazio G[4], Cazzaniga G[4], Marschalek R[2], Emerenciano M[1]
[1]Molecular Cancer Study Group, Division of Clinical Research, Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA), Rio de Janeiro, Brazil
[2]Institute of Pharmaceutical Biology/DCAL, Goethe-University, Frankfurt/Main, Germany
[3]Children's Cancer Institute, Lowy Cancer Research Centre UNSW, Sydney, New South Wales, Australia
[4]Centro Ricerca Tettamanti, Clinica Pediatrica, Dipartimento di Medicina e Chirurgia, Università degli Studi di Milano-Bicocca, Fondazione MBBM, Monza, Italy

IKZF1 deletions (ΔIKZF1) are associated with an increased risk of relapse in B-ALL patients. Although a multiplex PCR (M-PCR) has been designed for the detection of recurrent ΔIKZF1 (Δ2-3, Δ2-7, Δ2-8, Δ4-7, Δ4-8), we still lack information regarding the breakpoints of non-recurrent ΔIKZF1 to provide an accurate diagnosis. Thus, we explored sequence features in pediatric and adult B-ALL

patient with ΔIKZF1, and evaluated CNAs within IKZF1 locus by using MLPA. Non-recurrent ΔIKZF1 were then sequenced by targeted NGS. We also compiled DNA-seq data from literature, and mapped the breakpoint sequences for the identification of clusters and motifs associated with breakpoints. A total of 1,474 B-ALL samples were included of which 16% had ΔIKZF1. We compiled 935 breakpoint sequences of ΔIKZF1, and 24 clusters were identified. Non-recurrent ΔIKZF1 had three exclusive clusters. Heptamer-like sequences for RAG recombination were identified near those clusters, also in rare ΔIKZF1. Therefore, we provide the basis for updating M-PCR and MLPA design to facilitate the detection of almost all ΔIKZF1, and show that illegitimate RAG activity could potentially promote rare types of ΔIKZF1.

Diagnostic and functional role of extracellular vesicles (EV) from blood and CSF in pediatric medulloblastoma (MB)

Reetz L[1], Ghanam J[1], Chetty V K[1], Reinhardt D[1], Thakur B K[1]
[1]Department of Pediatrics III, University Hospital Essen, Essen, Germany

Magnetic resonance imaging (MRI) and invasive surgery are being performed to examine the tumor tissue to detect and verify MB at diagnosis or relapse. Although some risk factors are established for a bad prognosis in MB, it remains unclear which MB patients will suffer from relapse after treatment. Thus, there is a great scope for the minimal invasive tool for detecting MB residual disease or relapse. Tumor-derived EVs were found to carry DNA, RNA, and proteins cargos with the same mutational status, suggesting their use in MB as an early biomarker. Therefore, we aimed to establish a liquid biopsy approach to characterize the EV-associated DNA-protein complex in MB patients' blood and CSF. EVs were isolated using Size Exclusion Chromatography and Ultrafiltration from pediatric MB patients' blood plasma and CSF. EVs were characterized using NTA, TEM, beads-based flow cytometry. Next, EV-DNA derived from blood and CSF of MB patients will be analyzed using the MB-NGS panel for early biomarker application. Functional EV-protein targets will be identified using LC-MS proteomics and EV interaction with the tumor microenvironment will be performed using cell and molecular biology tools.

Different isolation techniques for isolation of extracellular vesicles

Schultheis L[1], Stückle J[1], Rajab H[1], Fuchs J[1], Warmann S W[1], Schmid E[1]
[1]Department of Pediatric Surgery and Pediatric Urology, University Children`s Hospital Tübingen, Germany

Despite reports on several techniques there is currently no consensus for the optimal extracellular vesicle (EV) isolation method with respect to yield, quality, purity, and small patient plasma volumes. We compared four different techniques (precipitation (PP), size-exclusion (SEC), membrane affinity (MA), and ultracentrifugation (UCF)) for EV isolation from plasma. EV characterization included electron microscopy and nanoparticle tracking analysis. EV preparation quality and purity was assessed using protein quantification methods, flow cytometry, and Western Blotting. Purification was performed via ultrafiltration and Albumin out kit. Each tested method resulted in approximately the same particle size (115-147nm) with a similar amount of time. PP and MA showed the highest protein purity. SEC showed higher purity on electron microscopy and the highest values of exosome markers CD9 (99%), CD63 (82%), and CD81 (97%). Purification techniques did not further improve purity levels. In conclusion, SEC seems to be the most promising method for EV isolation. Nevertheless, certain applications require higher purity, so efforts should be made to improve isolation purity.

Back to the roots – multi-omics as a road atlas to the cell-of-origin in rare childhood leukemia

Hartmann, M[1,2]
[1]Section of Translational Cancer Epigenomics, Division of Translational Medical Oncology, German Cancer Research Center (DKFZ) & National Center for Tumor Diseases (NCT), Heidelberg, Germany
[2]Department of Pediatric Hematology & Oncology, University of Freiburg

JMML is a disease with divergent clinical characteristics. A small set of known genetic drivers cannot explain the clinical heterogeneity. We have recently described that distinct DNA methylation patterns characterise three different subgroups that correlate with prognosis. We hypothesise that different cellular origins determine the phenotypic heterogeneity of those subgroups. Therefore, we established an elaborate multi-omics approach for characterising the molecular and cellular heterogeneity underlying the pathogenesis of JMML. Epigenetic profiling reveals that differential methylation already exists in hematopoietic stem cells (HSCs), suggesting leukemia initiating cells on top of the hematopoietic hierarchy. Single-cell transcriptomics confirm the distinct nature of HSCs across subgroups, however, reasons for the clinical heterogeneity remain elusive. Strikingly, comparative DNA methylome analyses reveal distinct developmental signatures across patients, suggesting that the course of disease is significantly affected by the developmental origin of JMML. As a result, we are expanding our studies by generating a developmental DNA methylome atlas of healthy foetal to adult HSCs.

14.00 – 15.30 **Immunotherapy & precision medicine I** *Chair: Martin Sauer*

Primary CD33-targeting CAR-NK cells for the treatment of acute myeloid leukemia

Albinger N[1], Pfeifer R[2], Kreyenberg H[3], Schubert R[3], Schneider D[2], Kühn M W M[4], Penack O[5], Zhang C[2], Möker N[2], Ullrich E[1]

[1]Childrens Hospital, Experimental Immunology, Johann Wolfgang Goethe University, Germany
[2]Miltenyi Biotec, Bergisch Gladbach, Germany
[3]University Hospital Frankfurt, Germany
[4]University Hospital Mainz, Germany
[5]Charite, University Hospital Berlin, Germany

Clinical application of CD33-CAR-T cells remains challenging due to potential side effects and its restriction to autologous products. We report on the generation of primary CD33-CAR-NK cells, which are highly effective in combating AML in vitro and in AML xenograft models. Transgene integration by BaEV-LV resulted in 35-60% CAR-expression and CD33-CAR-NK cells displayed unimpeded ex vivo-expansion as well as increased cytotoxicity against CD33+ OCI-AML2 cells compared to untransduced (UTD)-NK cells in vitro. Using an OCI-AML2 NSG-SGM3 xenograft mouse model (n=7/group) a significant reduction of leukemic burden by day 21 could be observed following weekly injections (three in total) of 10e7 CAR-NK cells i.v. BLI-analysis of femur, tibia and spleen on day 22 revealed impeded AML engraftment in CAR-NK cell-treated mice which was confirmed by flow cytometry analysis of isolated BM and spleen. Furthermore, NK cell infiltration in the BM and spleen was significantly increased in mice which received CAR-NK cells and the majority were identified as CAR+ (>73%). Chimerism analysis of peripheral blood revealed higher persistence of NK cells and absence of AML cells in CAR-NK-treated mice.

Drug repositioning for MLL-rearranged B-cell acute lymphoblastic leukaemia

Che N[1], Cantilena S[1], Looi-Somoye R[1], de Boer J[1], Williams O[1]

[1]UCL Great Ormond Street Institute of Child Health, London, United Kingdom

Despite extensive research on therapeutic susceptibilities of different B-cell acute lymphoblastic leukaemia (ALL) subtypes, outcomes for MLL-rearranged ALL remain extremely poor. MLL rearrangements account for 70–80% of infant ALL cases and are associated with poor prognosis, less than 50% surviving current treatment regimens. Our group has developed a bioluminescence platform to screen for drugs which induce the degradation of MLL-fusion proteins. This platform was used to screen the Prestwick Chemical Library, which contains 1520 FDA-approved & EMA-approved drugs. We identified Disulfiram and Niclosamide as potential candidates which targeted MLL-fusion proteins for degradation. Interestingly, we found that neither drug was able to sensitise ALL cells to current conventional chemotherapies. Instead, they had an additive/synergistic inhibitory effect on RS4;11 (ZIP=4.5), SEMK2 (ZIP=3.7) and BEL-1 (ZIP=7.8) ALL cells. We further demonstrated that the combination of these 2 drugs downregulated MLL fusion target genes and

induced apoptosis in ALL cells due to the degradation of MLL-fusion proteins. In this study, we provided a novel treatment regimen in MLL-rearranged ALL.

ErbB2-CAR mediated immunotherapy for the treatment of high-risk rhabdomyosarcoma

Heim C[1], Moser L M[1], Merker M[1], Wels W S[2], Ivics Z[3], Bönig H[4], Ullrich E[5], Klusmann J-H[1], Bader P[1], Rettinger E[1]

[1]Division for Stem Cell Transplantation, Immunology, and Intensive Care Medicine, Department for Children and Adolescents, University Hospital Frankfurt, Goethe University Frankfurt, Frankfurt, Germany
[2]Mental Therapy, Frankfurt, Germany
[3]Division of Medical Biotechnology, Paul Ehrlich Institute, Langen, Germany
[4]Department of Cellular Therapeutics/Cell Processing (Good Manufacturing Practice, GMP), Institute for Transfusion Medicine and Immunotherapy, Goethe University, Frankfurt, Germany
[5]Experimental Immunology, Department for Children and Adolescents, University Hospital Frankfurt, Goethe University, Frankfurt, Germany

Metastatic rhabdomyosarcoma (RMS) is a tumor entity with unmet need for novel treatment strategies. Applying the chimeric antigen receptor (CAR) technology to innate immune cells may translate into effective, safe and affordable therapies that contribute to cancer immune surveillance not only by their intrinsic cytotoxicity, but also by modulating antitumor immune responses.

CAR-engineered natural killer cell line NK-92/5.28.z directed against ErbB2 on RMS displayed effective cytotoxicity against primary RMS in vitro. Even if kept under hypoxic conditions of tumor spheroids, NK-92/5.28.z was able to overcome effects of the inhibitory tumor microenvironment. In mice carrying a patient's tumor-derived RMS xenografts, serial infusions of NK-92/5.28.z delayed tumor engraftment, whereas one systemic application of ex vivo activated ErbB2-CAR-engineered cytokine induced killer (CIK) cells inhibited tumor growth, suggesting that the selection of the immune effector cell is critical for CAR therapy. For clinical application, we are now continuing with early treatment attempts in vitro using the novel Sleeping Beauty transposon-based system (SBTS) for CAR gene transfer in CIK cells.

Improving NK-cell immunotherapy against rhabdomyosarcoma

Reindl L M[1], Grèze V[1], Wendel P[1], Särchen V[2], Wels W S[3], Vogler M[2], Ullrich E[1]

[1]University Hospital Frankfurt, Department for Children and Adolescents Medicine, Experimental Immunology, Goethe University, Frankfurt, Germany
[2]Institute for Experimental Cancer Research in Pediatrics, Goethe University, Frankfurt, Germany
[3]Georg-Speyer-Haus, Institute for Tumor Biology and Experimental Therapy, Frankfurt, Germany

Rhabdomyosarcoma (RMS) is the most common soft tissue cancer in children and although the survival rate has increased over the last decades the outcome of patients with metastatic and relapsed disease is still poor.

Natural killer (NK) cells are known for their high intrinsic cytotoxic capacity and can be safely applied as 'off-the-shelf' third party donor product. Chimeric antigen receptor (CAR)-expressing NK cells showed promise for the treatment of B-cell malignancies, while so far only limited data exist for solid tumor therapy.

We addressed relevant pathways in the peripheral blood derived NK (pNK)-RMS interaction by RNA sequencing analysis, RT-qPCR and CRISPR/Cas9-knockouts in NK or RMS cells. pNK cells that were rechallenged with RMS spheroids after RMS contact showed reduced cytotoxicity, indicating that the NK-RMS interaction impaired pNK antitumor activity.

To enable more specific targeting and enhance NK-cell cytotoxicity, we generated pNK cells equipped with a CAR targeting the epidermal growth factor receptor (EGFR). These EGFR-CAR NK cells showed increased cytotoxicity against various RMS cell lines. In vivo analysis in an RMS mouse xenograft model is ongoing.

High antileukemic efficiency of CD19-CAR NK cells engineered with *Sleeping Beauty* transposon vectors

Bexte T[1,2,3], Botezatu L[4], Miskey C[4], Reindl L M[1], Campe J[1], Mertlitz S[5], Gebel V[1,2,3], Schubert R[6], Cremer A[7], Rettinger E[8], Prommersberger S[9], Penack O[5], Wels W S[2,10,11], Hudecek M[9], Ivics Z[4], Ullrich E[1,2,3,11]

[1] Experimental Immunology, Department for Children and Adolescents Medicine, Hospital of the Goethe University Frankfurt, 60590, Germany

[2] Frankfurt Cancer Institute, Goethe University, Frankfurt am Main, 60590, Germany

[3] University Cancer Center (UCT) Frankfurt-Marburg

[4] Division of Medical Biotechnology, Paul Ehrlich Institute, Langen, 63225, Germany

[5] Charité Universitätsmedizin Berlin, Berlin, 10117, Germany

[6] Department for Children and Adolescents, University Hospital, Goethe-University, Frankfurt, Germany

[7] Department of Hematology and Oncology, Johann Wolfgang Goethe University, Frankfurt, Germany

[8]Division of Stem Cell Transplantation and Immunology, Department of Children and Adolescent Medicine, Hospital of the Goethe University Frankfurt, 60590, Germany

[9] Department of Internal Medicine II, University Hospital Würzburg, Würzburg, 97080, Germany

[10] Georg-Speyer-Haus, Institute for Tumor Biology and Experimental Therapy, Frankfurt am Main,60596, Germany

[11] German Cancer Consortium (DKTK) partner site Frankfurt/Mainz, Frankfurt am Main, 60590, Germany

Expression of chimeric antigen receptors (CAR) constitutes a promising approach to enhance therapeutic efficacy of NK cells. Here, we demonstrate that primary CAR NK cells can be generated using the non-viral *Sleeping Beauty* (SB) transposon/transposase.

SB transposons vectorized as minicircles, encoding a CD19-CAR were transfected together with the hyperactive SB100X transposase. SB-modified NK cells displayed long-lasting CD19-CAR expression during *ex-vivo* expansion. Assessment of vector integration revealed a significantly higher frequency of insertion into genomic safe harbors for SB-CAR-NK cells compared to lentivirally-transduced CAR-NK cells. SB-CD19-CAR NK cells demonstrated significantly higher cytotoxicity compared to non-modified NK cells *in vitro*. Enhanced antitumor potential was confirmed in a systemic CD19-expressing leukemia xenograft model (NSG-NALM-6/Luc) *in vivo*. In addition, enhanced SB-CD19-CAR NK cell-mediated killing capacity was also observed against primary patient-derived B-ALL blasts.

The *Sleeping Beauty* transposon system represents a safe and cost-effective platform for CAR NK cells generation and can be suitable also for other cancer immunotherapies.

Efficient Expansion of Immature Acute Myeloid Leukaemia Cells in an Ex Vivo Co-culture System

Tuk D[1], Nelson R[1], Heidenreich O[1,2], Krippner-Heidenreich A[1]

[1]Princess Maxima Center for Pediatric Oncology, Utrecht, the Netherlands

[2]Wolfson Childhood Cancer Research Centre, Newcastle University, United Kingdom

Acute myeloid leukaemia-initiating cells (LICs) comprise immature cells driving leukemogenesis. Viable ex vivo AML culture systems are hampered by poor viability and high differentiation of AML cells. We have designed a mesenchymal stromal cell (MSC)/AML co-culture system that enables expansion of primary AML cells including immature AML cell populations for more than two weeks. Both primary AML and PDX samples comprising different karyotypes were tested to expand ex vivo using healthy MSCs as feeder cells in different cell culture conditions. Cell number, viability and the differentiation status of AML cells were assessed by flow cytometry using a nine colour antibody panel. All primary AMLs expanded at high viability (majority >80%) with up to 50-fold or 350-fold expansion of LIC-like populations for up to 14 or 28 days, respectively. Fusion gene expression was maintained for up to 2.5 months. Thus, this co-culture system will provide the basis for functional assessments of different signalling pathways, AML-niche interactions and therapeutic approaches including the use of epigenetic modulators.

Flow Cytometry to detect Dasatinib sensitive T-ALL

Poll AA[1], Shi Y[1], Bell H[1], Irving J[1], van Delft F[1]

[1]Centre for Cancer, Newcastle University, Newcastle upon Tyne, United Kingdom

Outcomes for relapsed/ refractory T-ALL patients are extremely poor and represent a major unmet clinical need, with resistance to dexamethasone (DEX) a major driver of treatment failure. LCK is a tyrosine kinase in the preTCR pathway, which directs T-cell development. Dasatinib (DAS) inhibits LCK; we have previously demonstrated that combining DAS with DEX can overcome DEX resistance,

resulting in synergistic cell death. However only a subset of patients (approx. 40%) are sensitive to DAS. Identifying patients who will benefit from DAS treatment is crucial to allocating patients to the correct treatment. Currently, this relies on in vitro testing of each sample to ascertain DAS sensitivity. In cell lines, we have shown that DAS sensitivity correlates with preTCR activation, as measured by phosphoLCK (pLCK) abundance. We show here that preTCR activation can be measured by flow cytometry, using antibodies against pLCK and pCD3ζ, and that samples can be reliably categorised as sensitive or resistant on this basis. This offers a readily implementable method to identify patients who will benefit from DAS treatment, which can be integ…

16.00 – 18.00 **Emerging therapies** *Chair: Owen Williams*

Targeting metabolism effectively complements tyrosine kinase inhibitor treatment of chronic myeloid leukemia

Häselbarth L[1], Saul D[2], Krumbholz M[1], Mougiakaos D[3], Metzler M[1], Karow A[1]
[1]Department of Pediatrics and Adolescent Medicine, Friedrich–Alexander University (FAU) Erlangen–Nuremberg, Germany
[2]Department of Internal Medicine 5, Hematology and Oncology, University Hospital Erlangen, Germany
[3]Department of Hematology and Oncology, University Hospital Magdeburg, Germany

Tyrosine kinase inhibitors (TKI), targeting the BCR/ABL1 fusion protein, like imatinib, dasatinib, nilotinib and the first-in-class myristoyl pocket targeting TKI asciminib have revolutionized the treatment options of chronic myeloid leukemia (CML). However, resistance to therapy, relapse after treatment termination and side effects are problems of long-term TKI-treatment. Therefore, it is important to optimize TKI therapy and to investigate additional therapeutic approaches. Here, we tested how different TKI affect the metabolism of CML cells and whether the synergistic lethality can be increased by inhibition of different checkpoints such as the respiratory chain and the unfolded protein response (UPR). We found that glucose metabolism was strongly impaired by TKI and that an additional inhibition of complex I (rotenone) or V (oligomycin) of the respiratory chain caused an increased cell death. Similar results were obtained when generating ER-stress by thapsigargin in TKI-treated CML cells, which might be due to a TKI-induced UPR-inhibition. All in all, we hypothesize that TKI treatment is more effective by additional inhibition of glucose metabolism and by ER-stress induction.

Novel therapeutic avenues for MLL-AF4+ pro-B ALL patients based on a unique microRNA expression signature

Malouf C[1], Duguid A[1], Ottersbach K[1]
[1]Centre for Regenerative Medicine, The University of Edinburgh, United Kingdom

MLL-AF4+ infant leukemia patients have a unique microRNA expression signature that can be used to understand leukemogenesis and to identify novel therapeutic avenues. We have previously shown that miR-128a and miR-130b, which are upregulated in patients, act as essential lineage-specific co-drivers of MLL-AF4+ leukemia (PMID: 34111240). Our most recent and unpublished work now focuses on three additional microRNAs that are downregulated in MLL-AF4+ pro-B ALL patients: miR-194, miR-99b and miR-125a-5p. The overexpression of all three microRNAs decreased the survival of MLL-AF4+ leukemic blasts and impaired the maintenance of MLL-AF4+ pro-B ALL in mice, indicating that they act as tumour suppressors. Downstream of these, we identified CA5B (miR-194 target), PPP3CA (miR-99b target) and PPP2R5C (miR-125a-5p target) that are upregulated in patients and targeted by Diamox, FK506 and LB-100, respectively. Importantly, in vivo treatment with these drugs reduced the leukemia burden of MLL-AF4+ pro-B ALL mice. Future studies will focus on assessing the drug repurposing potential of Diamox, FK506 and LB-100 for the treatment of MLL-AF4+ pro-B ALL patients.

Targeting the MLL/Menin interaction in NUP98-rearranged AML

Rasouli M[1], Blair H[2], Szoltysek K[1], Cameron R[1], Krippner-Heidenreich A[1], Zwaan CM[1], McGeehan G[3], Heidenreich O[1,2]
[1]Princess Maxima Center for Pediatric Oncology, Utrecht, the Netherlands

[2]Centre for Cancer, Newcastle University, Newcastle upon Tyne, United Kingdom
[3]Syndax Pharmaceuticals, Waltham, United States

The *NUP98*-rearrangement (*NUP98r*) is a common translocation (3-5%) in pediatric acute myeloid leukemia (AML). The expression of NUP98 fusion target genes is mainly dependent on the MLL-menin complex and we hypothesized that this interaction can be targeted by the SNDX-5613. Treatment of NUP98r AML cells resulted in a profound dose-dependent inhibition of proliferation with IC50 values between 2 and 120 nM. The growth-inhibitory effect was associated with a reduction in the clonogenic potential of *NUP98/NSD1* cells. Furthermore, SNDX-5613 treatment induced myeloid differentiation in AML blasts as demonstrated by diminished expression of CD34 and CD45RA and concomitant elevated expression of CD11b, CD14, and MNDA. Transcriptional profiling upon SNDX-5613 treatment revealed specific changes in leukemogenic gene expression with downregulation of NUP98 fusion and MLL target genes, including *HOXA7-10, MEIS1, MEF2C, FLT3*, *PBX3*, and *IGF2BP2*. This study highlights the pharmacologic inhibition Menin-MLL interaction as a promising therapeutic option for NUP98 fusion-driven leukemia.

Silencing the leukaemic fusion gene RUNX1/ETO by siRNA-loaded lipid nanoparticles restores myeloid differentiation

Swart L[1], van Oort A[1], Ashtiani M[1], Krippner-Heidenreich A[1], Kooijmans S[2], Tuk D[1], Koekman A[2], Seinen C[2], Issa H[3], Blair H[4], Schiffelers R[2], Heidenreich O[1,4]
[1]Princess Maxima Centrum for Pediatric Oncology, Utrecht, The Netherlands
[2]Clinical Chemistry and Haematology, University Medical Center Utrecht, Heidelberglaan 100, Utrecht 3584 CX, The Netherlands
[3]Department of Pediatrics, University Hospital Frankfurt, Goethe-University Frankfurt, Frankfurt (Main), Germany
[4]Wolfson Childhood Cancer Research Centre, Newcastle University, Newcastle upon Tyne, United Kingdom

Leukeamias are often driven by the expression of leukaemic-specific fusion genes. Exclusive targeting using RNA interference is therefore an attractive therapeutic concept, but lacks so far suitable delivery systems. Here, we use targeted lipid nanoparticles (LNPs) containing siRNA (siRE) to reduce RUNX1/ETO protein expression in patient-derived AML cells.
We generated LNPs using microfluidic mixing followed by decoration with a modified short LDV peptide that has high affinity towards the very late antigen-4 (VLA-4). Compared to non-targeted LNPs, LDV-LNPs showed an enhanced uptake in RUNX1/ETO positive cell lines and patient-derived cells within the first 8 hours. In addition, a single dose of LDV-LNPsiRE resulted in significant knockdown of RUNX1/ETO transcripts. Sequential treatment prolonged and enhanced this effect. Multiparameter flow cytometry analysis of patient cells show the restoration of myeloid differentiation upon RUNX1/ETO depletion. Currently, in vivo biodistribution and efficacy studies using orthotopic mouse models are ongoing.
Thus, targeted LNP-mediated delivery of siRNAs might be a promising new approach for the treatment of fusion gene-driven leukaemias.

Epigenetic perturbation by BMI-1 inhibitors as a novel therapeutic approach for hepatoblastoma

Demir S[1], Bentrop M[1], Cairo S[2], Kappler R[1]
[1]Department of Pediatric Surgery, Dr. von Hauner Children's Hospital, LMU Munich, Germany
[2]XenTech, Evry, France

Treatment of hepatoblastoma (HB) has drastically improved by refinements of surgical procedures and clinical risk stratification. However, identifying novel drug and molecular targets is still crucial due to the resistance towards conventional chemotherapy and its toxicity. Since the mutational frequency is extremely low in HB, targeting strategies based on genetic alterations is challenging, addressing the involvement of epigenetic modifications. In this project, we tested 11 compounds directed against epigenetic regulators on 10 HB cell lines and compared their efficacy to standard of care chemotherapeutics. Consequent viability assays revealed that the polycomb complex protein BMI-1 inhibitors PTC209 and PTC596 reduced HB cell growth in a dose-dependent manner. Moreover, reduction of colony formation capability, decrease of migration potential, induction of apoptotis, retardation of proliferation and 3-dimensional spheroid growth were observed in HB cells upon BMI-1 inhibition. Furthermore, the combination of BMI-1 inhibitors and cisplatin

revealed a strong synergistic effect, suggesting BMI-1 inhibition as a potential target for HB therapy.

Mebendazole inhibits growth of hepatoblastoma cells by cell cycle arrest

Li Q[1], Demir S[1], Bao X[2], Wagner A[1], Fan Y[3], Cairo S[4], Kappler R[1]
[1]Department of Pediatric Surgery, Dr. von Hauner Children's Hospital, LMU Munich, Germany
[2]Department of Medical Oncology, The First Affiliated Hospital, School of Medicine, Zhejiang University, People's Republic of China
[3]Department of Pediatrics, Dr. von Hauner Children's Hospital, LMU Munich, Germany
[4]XenTech, Evry, France

Although survival of hepatoblastoma patients has dramatically increased by combining preoperative chemotherapy and surgical tumor resection, drug resistance remains a huge challenge in the clinical management. Here, we integrated gene expression data of five responders and two non-responders into the pharmacologic perturbation prediction tool Connectivity Map and identified the anthelmintic mebendazole as a putative drug to circumvent chemoresistance in hepatoblastoma. Mebendazole treatment of cell lines grown from patient-derived xenografts resulted in a potent reduction of tumor cell growth in a dose-dependent manner. Moreover, mebendazole treatment resulted in a reduced colony formation capability, induction of apoptosis, and cell cycle arrest in G2/M phase, which was associated with blockage of microtubule formation. Consequently, RNA sequencing analyses confirmed the transcriptional downregulation of tubulins. Using a subcutaneous patient-derived xenograft transplantation model we found that mebendazole significantly reduced tumor growth in vivo. In conclusion, our results strongly support the clinical use of mebendazole in the treatment of chemoresistant hepatoblastoma.

De novo design of growth factor inhibiting proteins

Maksymenko K[1], Skokowa J[2], Lupas A N[1], Aghaallaei N[2], Müller P[3], ElGamacy M[1]
[1]Max Planck Institute for Biology, Tübingen, Germany
[2]University Hospital Tübingen, Tübingen, Germany
[3]University of Konstanz, Germany

Growth factors are signaling molecules coordinating the complex functionality of multicellular organisms during development and homeostasis. Since aberrant expression of growth factors can cause diverse disorders, growth factors and their receptors are central targets for therapeutic modulation. Here, we present two different strategies of computational protein design to obtain inhibitors against growth factors which contribute to tumor progression. Adopting a re-engineering approach, we designed inhibitors of epidermal growth factor (EGF) using a single domain of EGF receptor as a template. Experimental evaluation of two designed candidates revealed that both of them bind EGF with nanomolar affinities and inhibit EGF-induced proliferation of epidermoid carcinoma cell line. Using a de novo design strategy, we designed inhibitors of vascular endothelial growth factor (VEGF). The best designs showed the ability to inhibit proliferation of VEGF-dependent cells in vitro and in zebrafish assays. Thus, our results demonstrate the feasibility of computational protein design approaches to create therapeutic leads in a time- and cost-effective manner.

De novo design of cytokines, antikines, and novokines

ElGamacy M[1], Ullrich T[1], Maksymenko K[1], Lupas A N[1], Hernandez B[1], Skokowa J[2]
[1]Max Planck Institute for Biology, Tübingen, Germany
[2]University Hospital Tübingen, Tübingen, Germany

Cytokines are key signaling molecules critical for multiple aspects of health and disease. This motivated the development of modulators of cytokine signaling as part of the course of treatment for several types of malignancies. Developing protein-based modulators of these pathways has nevertheless been restricted to classical approaches such as recombinant cytokines (i.e. agonists) and monoclonal antibodies (i.e. antagonists). Our work deploys cutting-edge de novo protein design techniques to create and develop novel proteins with superior pharmaceutical properties compared to existing classes. These designed mini-proteins can bind cytokine receptors to either

activate or inhibit their native signaling. Moreover, we could create single-domain proteins that encode two distinct receptor binding sites (novokines). These novokines can bind and dimerise non-native combinations of cytokine receptors, and depending on the context, can play novel pharmacological roles. Taking the granulocyte-colony stimulating factor receptor (G-CSFR) as an example, we will discuss our ongoing work that could achieve potent activators, inhibitors, and novel modulators.

Selinexor, a selective inhibitor of XPO-1, shows antitumor activity in rhabdomyosarcoma cell lines

Hoefler L, Fuchs J[1], Schmid E[1], Ellerkamp V[1]
[1]Department of Pediatric Surgery and Pediatric Urology, University Children`s Hospital Tübingen, Germany

Rhabdomyosarcoma (RMS) are treated with neoadjuvant chemotherapy and local therapy. In order to reduce long-term effects and to improve the prognostic parameters for high-risk RMS, alternative and individualized therapy methods are desirable. We could show that the nuclear export protein XPO-1 is overexpressed in RMS. Elevated XPO-1 prevents many tumor suppressors from performing their function. The present study investigates the effect of the XPO-1 Inhibitor Selinexor (SEL) in combination with Doxorubicin (DOX) on rhabdomyosarcoma cells.
Two RMS cell lines (RD and RH30) were used. Reduction in viability was assessed by MTT, cell cycle by flow cytometry, migration by transwell - and scratch assay, and protein expression of cell cycle proteins via Western blot.
The combination therapy results in a significant viability inhibition on RD cells compared to monotherapy with DOX. In addition, significantly decreased migration was seen in RH30 cells. Observations of the cell cycle showed G1 phase arrest upon treatment with SEL. Western blots showed upregulation of cyclin D1 under treatment with SEL.
In summary, our study shows that SEL supports the effect of DOX.

A selection free ex vivo gene therapy approach to congenital neutropenia causing HAX1 mutations

Ritter M U[1], Nasri M[1], Dannenmann B[1], Kaufmann M M[2], Zeidler K A[3], Zeidler C[4], Klimiankou M[1], Cathomen T[2], Welte K[3], Skokowa J[1]
[1]Innere Medizin 2, Universitätsklinikum Tübingen, Germany
[2]ITG, Universitätsklinikum Freiburg, Germany
[3]Allgemeine Pädiatrie, Hämatologie, Onkologie, Universitätsklinikum Tübingen, Germany
[4]Klinik für Pädiatrische Hämatologie und Onkologie, Medizinische Hochschule Hannover, Germany

Severe congenital neutropenia (CN) patients lack mature neutrophils (< 500/µl) and have a 20 % risk to develop MDS or AML. Approximately 10 % of patients carry a homozygous loss of function mutation in the HAX1 gene. With these prospects, the clinical need for a genetic cure of CN is imminent.
We developed a selection-free approach to correct the most common HAX1 mutation p.W44X, in hematopoietic stem cells (HSCs) of CN patients ex vivo. Through electroporation, HiFiCas9 and a guide RNA are delivered as RNP into the HSCs. Then a correction template is delivered by a recombinant adeno associated virus 6 (rAAV6) vector. In patient HSCs we observed 65,8 % (± 7,12 %) correction efficiency and total editing (TE) of 84,4 % (± 4,2 %) (n=5), which led to re-expression of HAX1 protein in corrected cells. The HAX1 correction rescued the maturation arrest of granulopoiesis, as assessed by in vitro differentiation of HSCs to neutrophils. We are currently assessing in vivo engraftment capacity of gene edited cells. GUIDE and CAST-Seq show a good off-target profile of our sgRNA. Our results imply that ex vivo CRISPR-based gene-editing might be a feasible and safe approach for HAX1-CN patients.

FRIDAY JUNE 3

9.00 – 10.30 **Omics II** *Chair: Meinolf Suttorp*

RUNX1/ETO effects on the interactions with t(8;21) AML with bone marrow niche: lessons from scRNAseq

Derevyanko P K[1], Swart L E[1], Blair H J[2], Heidenreich O[1,2]
[1]Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands
[2]Wolfson Childhood Cancer Research Centre, Newcastle University, United Kingdom

To study the role of the RUNX1/ETO fusion in interactions of AML cells with the bone marrow niche, we co-cultured a t(8;21) AML PDX with mesenchymal stromal cells (MSC) and performed a RUNX1/ETO knockdown (KD) using a lipid nanoparticle siRNA delivery system.
Single cell RNA-seq followed by gene set enrichment analysis showed that co-culture with AML cells interfere with cell cycle-related pathways in MSCs. Knockdown of RUNX1/ETO not only restored these pathways in MSCs, but also affected multiple pathways in AML and MSCs in opposite direction. In addition to cell cycle, metabolic pathways such as glycolysis, fatty acid metabolism and amino acid metabolism were downregulated in AML cells and upregulated in MSCs. These data indicate that targeted interference with a central driver of leukaemia can have profound consequences for the transcriptional programmes of surrounding niche cells and suggest a direct role for fusion genes such as RUNX1/ETO in the dysregulation of the leukaemic niche.

Altered extrinsic and intrinsic factors synergistically accelerate the thymic T-ALL development

Dick A M[1], Tsingos E[2], Aghaallaei N[1], Bajoghli B[1]
[1]Department of Hematology, Oncology, Immunology, and Rheumatology, University Hospital of Tübingen, Otfried-Müller-Strasse 10, 72076 Tübingen, Germany
[2]Mathematical Institute, Leiden University, Niels Bohrweg 1, 2333 CA Leiden, Netherlands

The etiology of T cell acute lymphoblastic leukemia (T-ALL), an aggressive blood malignancy derived from developing T cells (thymocytes) in the thymus, is unknown. In humans and mice, T-ALL is diagnosed when malignant T cells are identifiable in the peripheral blood, i.e., at a stage when the initiation of disease is completed. Therefore, we do not know to what extent thymic microenvironments are affected and whether their alterations are the cause or the consequence of T-ALL development. Here, we report the development of two complementary models. We first took advantage of our recently developed “virtual thymus”, a cell-based computational model (Aghaallaei et al., Science Advances, 2021) to predict the outcome of various scenarios, in which we tested the effect of IL-7 and Notch signaling on proliferation and clonal expansion. Our model predicted a condition in which a single clone can transform into malignancy and proliferate massively. We used our in vivo model, the medaka fish, to confirm our simulations. Our results reveal that an excess of IL-7 in the niche combined with mutations in thymocytes can result in a rapid T-ALL development within five days.

Identification of functional defects promoting leukemogenesis in GATA2-deficient individuals

Yigit B I[1,2], Fernandez-Orth J[1], Weiss J M[1], Molnar C[1], Andrieux G[3], Gonzalez-Mendez I[4], Borries M[3], Niemeyer C[1], Teichert-von Lüttichau I[2], Erlacher M[1]

[1]University Medical Center Freiburg, Center for Pediatrics, Department of Pediatric Hematology and Oncology, Freiburg, Germany
[2]Kinderklinik Muenchen Schwabing, Department of Pediatrics and Children's Cancer Research Center, TUM School of Medicine, Technical University of Munich, Munich, Germany
[3]University Medical Center Freiburg, Institute of Medical Bioinformatics und Systems Medicine IBSM, Freiburg, Germany
[4]University of Tübingen, Institute of Pathology and Neuropathology, Tübingen, Germany

Hematopoiesis is the process in which blood cells are produced by the proliferation of hematopoietic stem and progenitor cells (HSPCs) and their differentiation into the various blood cell subsets. This process is tightly regulated by different co- and transcription factors. Amongst them, GATA2 plays a pivotal role in the proliferation and balance of hematopoietic stem cells (HSCs). Thus, germline GATA2 mutations can disrupt hematopoiesis leading to cytopenia, immunodeficiency or myeloid neoplasia, for instance myelodysplastic syndrome (MDS) or leukemia. Using transgenic mice who are heterozygous in GATA2 (Gata2+/-) in the hematopoietic system, we have previously demonstrated that bone marrow (BM) failure and secondary leukemia can be induced by transplanting Gata2+/- HSPCs into lethally irradiated mice. Interestingly, two weeks after transplantation, HSPC numbers are significantly reduced in the BM of mice who were transplanted with Gata2+/- HSPCs. At later time points, Gata2+/- HSPCs reconstitute the BM of recipients before reaching exhaustion. We now investigate whether, after transplantation, Gata2+/- HSPC numbers decrease due to increased apoptosis or reduced proliferation.

Characterization of cooperating mechanisms in GATA2 syndrome

Hagenbourger F[1,2], Fernandez-Orth J[1], Bohler S[1], Weiss J M[1], Andrieux G[3], Börries M[3], Niemeyer C M[1], Erlacher M[1]

[1]University Medical Center Freiburg, Center for Pediatrics and Adolescent Medicine, Department of Pediatric Hematology and Oncology, Freiburg, Germany
[2]MOTI-VATE Graduate School, Faculty of Medicine, University of Freiburg, Freiburg, Germany
[3]University Medical Center Freiburg, Institute of Medical Bioinformatics und Systems Medicine IBSM, Freiburg, Germany

Hematopoiesis is regulated by different transcription factors and cofactors that ensure a proper proliferation, differentiation and survival of immature hematopoietic stem and progenitor cells (HSPCs). Germline mutations in these genes can affect both their expression and their activity. GATA2 is a transcription factor where more than 400 germline pathogenic variants have been identified leading to variable hematological and non-hematological phenotypes with a high risk of developing MDS or AML. However, the underlying mechanisms of this malignant transformation remain unknown.

Hence, we aim to identify possible tumor suppressor genes promoting leukemogenesis in mice with GATA2 haploinsufficient hematopoietic system. Because of the association of GATA2 deficiency with monosomy 7, we are focusing on putative tumor suppressors located on human chromosome 7. First, we downregulate IKZF1 and EZH2 by transducing shRNA-expressing lentiviruses into Gata2+/- / WT HSPCs. Outcome parameters are the capacity to proliferate, differentiate and undergo apoptosis. These data will shed light on cooperating mechanisms in GATA2 haploinsufficiency for the predisposition to MDS/AML.

Characterizing alternative splicing landscape by RUNX1/ETO reveals novel vulnerabilities in t(8;21) leukemia

Barneh F[1], Grinev V V[2], van Oort A[1], Heidenreich O[1,3]

[1]Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands
[2]Department of Genetics, Faculty of Biology, Belarusian State University, 220030 Minsk, Republic of Belarus
[3]Wolfson Childhood Cancer Research Centre, Newcastle University, United Kingdom

t(8;21) is one of the most frequent types of pediatric leukemia driven by fusion transcription factor RUNX1/ETO (RE) and secondary mutations. RE affects gene expression by recruiting epigenetic repressors to its binding sites blocks differentiation while enhances self-renewal of hematopoietic stem cells. Beside gene-level effects, we were interested to know whether RE also regulates

alternative splicing as another complexity layer. We generated an in silico pipeline of different bioinformatics sources to identify alternatively spliced genes in a corresponding cell line and patient samples with knockdown of RE. We identified 378 alternatively spliced genes, 75% of which were predicted to be potentially protein coding, while the rest were non-coding RNAs. We found 2 mechanisms for regulating alternative splicing by RE:1)regulation of non-canonical transcription start sites,2) affecting the expression level or function of 42 RNA binding proteins and splicing factors. These novel transcripts, beside having functional roles, may serve as reservoir of immunogenic peptides for immunotherapy of t(8;21).

Identification of novel fusion transcripts in Acute Myeloid Leukaemia

Konstantin Schuschel[1], R Bhayadia[1], Jan-Henning Klusmann[1]
[1]Goethe-University Frankfurt, Germany

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder, caused by genetic aberrations that enhance self-renewal and proliferation, block differentiation, and inhibit apoptosis. Fusion genes (FGs) are main drivers of leukemogenesis, but their variety and occurrence in rare pediatric AML subgroups is yet incompletely defined. Whole transcriptome sequencing, followed by bioinformatical processing provides a powerful tool for analyzing FGs.
Here we are utilizing a fusion detection pipeline to study and evaluate 279 human blood samples of pediatric AML patients and healthy donors. We are able to confirm the cytogenetic assessment of leukemic samples in 83% of the cases, classify 33% of not further specified samples (MLL, AMKL, M5, M7) and define new undescribed fusions. Novel fusions will be further assessed by molecular genetic assays to evaluate their leukemogenesis and reveal new underlying mechanisms.

11.30 – 13.00 **Biomarkers and diagnostics II** *Chair: Katrin Ottersbach*

A multifunctional tracking approach to study clonal heterogeneity in leukemia by flow cytometry and scRNA-seq

Steding H[1], Mätzig T[1]
[1]Hannover Medical School, Hannover, Germany

Acute myeloid leukemia (AML) is characterized by clonal heterogeneity, the main cause of treatment failure and relapse. Sequencing is commonly used for studying clonal composition; however, this impairs the functional validation of new findings. Therefore, we envisioned a system that combines scRNA-seq with flow cytometric clonal tracking and additionally enables the re-isolation of viable clones for further characterization. We developed a lentiviral gene marking strategy that labels leukemic cells with color codes and transcribed DNA-barcodes compatible with single-cell sequencing. Here, we could show the reliable detection of up to 48 color codes by flow cytometry. Populations of different color codes were tracked in vitro allowing the visualization of competitive growth behaviors. Furthermore, we could prove the specificity of our barcode detection by scRNA-seq. This project aims to delineate clonal competition under naïve conditions and upon therapeutic pressure in vivo. By correlating clonal behavior to transcriptional and (epi-) genetic profiles, we envision the identification of disease-relevant features and their functional validation for improved intervention strategies.

Comprehensive bone marrow analysis integrating deep learning-based pattern discovery (BMDeep)

Pontones M[1], Höfener H[2], Kock F[2], Schwen L[2], Westphal M[2], Dickel N[3], Kunz M[3], Metzler M[1]
[1]Universitätsklinikum Erlangen, Germany
[2]Fraunhofer MEVIS, Germany
[3]FAU Erlangen, Germany

Bone marrow morphology forms the basis for the assessment of hematopoiesis. The currently established approach is dependent on manual microscopic counting of a limited number of cells by specially trained personnel, which is inherently associated with substantial intra- and inter-

individual variability. The aim of our project is to automatize and improve the evaluation of bone marrow smears and to identify pathological patterns in pediatric leukemia.
Bone marrow smears from acute lymphoblastic, acute myeloid and chronic myeloid leukemia were scanned with high resolution after identification of hard- and software requirements for the scanning process by evaluating ten different scanner models. We developed an annotation tool for large scale classification of hematopoietic cells. We then designed a supervised Deep-learning model that is currently trained using the leukemia dataset. Next steps are the implementation of clinical data to enhance the capabilities of our model and allow for multi-dimensional pattern recognition. These patterns might identify new biomarkers to further enhance our understanding of pediatric leukemia.

Prognostic significance of minimal residual disease prior to reinduction in intermediate risk patients with ALL

Dzajic E[1], Kaupat-Bleckmann[1], Alten J[1], Zimmermann M[1], Möricke A[1], Schrappe M[1], Cario G[1]
[1]Department of Pediatrics, University Medical Center Schleswig-Holstein, Campus Kiel, Germany

Occurrence of relapse still remains one of the major therapeutic challenges in the treatment of ALL. The evidence of MRD in the early phase of therapy has shown to be highly predictive for the risk of relapse and has been used for risk stratification for the last two decades. This also concerns the large group of patients with intermediate-risk ALL (MR), where we still observe the highest number of relapses in absolute terms despite favorable overall outcome. The aim of this study was to evaluate the prognostic significance of MRD positivity prior to reinduction therapy in MR patients.A number of 66 MR patients who were treated according to the AIEOP-BFM ALL 2000 protocol were included in this analysis. Bone marrow samples obtained prior to reinduction were analyzed by RQ-PCR of clone-specific junctional regions of immunoglobulin and T-cell receptor gene rearrangements identified at time of diagnosis. MRD-positivity prior to reinduction was associated with significantly higher risk of relapse and reduced overall survival compared to MRD-negative patients. The measurement of MRD prior to reinduction could contribute decisively to the identification of MR patients at risk of relapse.

Prognostic relevance of persisting minimal residual disease in children with ALL and slow early response to chemotherapy

Nofcz L[1], Alten J[1], Zimmermann M[2], Koehler R[3], Möricke A[1], Schrappe M[1], Cario G[1]
[1]Department of Pediatrics, University Hospital Schleswig-Holstein, Kiel, Germany
[2]Department of Pediatric Hematology and Oncology, Hannover Medical School, Hannover, Germany
[3]Institute of Human Genetics, University Hospital Heidelberg, Germany

Detection of minimal residual disease (MRD) at early treatment time points is widely used to stratify children with acute lymphoblastic leukemia into risk-directed treatments. The role of MRD during the late treatment course is still unclear. Internal laboratory studies in a limited number of cases had indicated that detectable MRD before reinduction therapy (TP3) was highly associated with relapse in children of the medium-risk (MR) group with slow-early-response (SER) to therapy. In this project, it was investigated whether the importance of MRD at TP3 in SER could be confirmed in a larger cohort. RQ-PCR of immunoglobulin/T-cell receptor gene rearrangements was used to determine MRD in cryopreserved bone marrow samples from TP3 of 74 patients with SER of the ALL-BFM 2000 study. The results were classified as positive/quantifiable, positive/not quantifiable and negative. Children with quantifiable MRD positivity at TP3 had significantly poorer 8-year EFS (9 %) compared to positive/not quantifiable (58 %) and negative (63 %) MRD. Persisting MRD at TP3 led to high relapse risk, but negative MRD at TP3 could not prevent relapse in SER patients treated with an MR chemotherapy.

Application of mediator probe PCR chemistry in multiplex target assays for monitoring MRD of high-risk neuroblastoma

Schroeer A[1], Frietsch A L[2], Fillies M[1], Winkler A[1], Egger A[1], Henssen A G[1], Lehnert M[3], Schulte J[1], Szymansky A[1], Eckert C[1]

[1]Department of Pediatric Hematology, Oncology and Stem Cell Transplantation, Charité – Universitätsmedizin Berlin, Berlin, Germany
[2]Faculty of Medical and Life Sciences, Furtwangen University, Furtwangen, Germany
[3]Hahn-Schickard-Gesellschaft für angewandte Forschung e.V., Freiburg, Germany

Patients with high-risk neuroblastoma show in about 85% a bone marrow infiltration. Even though most of them achieve remission, minimal residual disease (MRD) frequently persists on submicroscopic level and causes a relapse. Our aim was to detect MRD cells in bone marrow aspirates taken during treatment, by employing a novel sensitive multiplex PCR chemistry, the mediator probe PCR (MP-PCR), which consists of a target-specific, unlabeled mediator probe and sequence-universal reporter conjugated with a fluorescent dye. Previously, the MP-PCR was successfully applied by Kipf et al. to determine MRD levels of acute lymphoblastic leukemia. Comparably to leukemia, high-risk neuroblastoma harbor multiple genetic alterations, which in addition appear on extrachromosomal DNA, including MYCN breakpoints, TERT rearrangements and ALK mutations. Our patient cohort comprises 18 patients with MYCN amplified neuroblastoma with multiple MYCN breakpoints and other mutations. To capture this tumor heterogeneity, we combined up to four MRD markers in one multiplex assay. Sensitive quantification of MRD benefits for the reconstruction of the clonal biology and personalization of neuroblastoma therapy.

CtDNA release mechanisms in a therapeutic Ewing Sarcoma mouse model

Eiblwieser J[1], Krumbholz M[1], Semper S[1], Frey B[2], Nagel L[3], Bäuerle T[3], Metzler M[1]

[1]Department of Pediatrics and Adolescent Medicine, University Hospital Erlangen, Germany
[2]Department of Radiation Oncology, University Hospital Erlangen, Germany
[3]Department of Radiology, University Hospital Erlangen, Germany

EWSR1-FLI1 fusion sequence levels in circulating tumor DNA (ctDNA) have been shown to be a valuable biomarker in Ewing Sarcoma (EwS) patients, which can be used for prediction of treatment response and risk stratification. However, it remains unclear, under which circumstances ctDNA is released into the blood stream during tumor development and therapeutic intervention.
Different human EwS cell lines were injected intrafemorally in NSG mice. Tumors were treated locally by radiotherapy. Fusion sequences were quantified in mouse plasma throughout the whole experiment and tissue response was determined through histologic analysis, MRI, and PET-CT.
Quantified plasma levels were correlated to tumor activity, proliferation, mitosis, apoptosis and angiogenesis. Evaluation of plasma ctDNA levels, imaging data and histologic analysis revealed different responses of EwS tumors to radiotherapy.
A better understanding of ctDNA release contributes to the improved establishment of ctDNA as a non-invasive biomarker in EwS patients enabling therapy monitoring and risk stratification in clinical practice.

Identification and isolation of homogenous AML derived extracellular vesicles for MRD detection in pediatric AML

Lesch M J[1], Giebel B[1], Reinhardt D[1], Thakur B K[1]
[1]University Hospital Essen, Essen, Germany

AML specific mutations were previously detected by our group in EV-DNA employing ultracentrifugation (UC) based EV isolation either at diagnosis or during therapy. However, it is challenging to detect minimal residual disease (MRD) status in these samples because the UC method provides heterogeneous EVs containing DNA from leukemic and non-leukemic immune and stromal cells, leading to a massive decline in mutational information after remission during MRD stages. Therefore, there is an urgent need for novel strategies to be developed to allow homogenous EV preparation with high purity.
To obtain homogenous EVs with biomarker potential, we have established a single EV identification and capturing platform using antigen panels specific for immunocapturing AML-cell-derived EVs. Using the cohort of patient samples at diagnosis (n=30) and three different time points of therapy (n=120), we aimed to perform EV isolation using AML-EVs surface antibody bead capturing approach. Next, after the isolation of EV-DNA, MRD detection will be performed utilizing pediatric AML NGS panel to establish EV biomarker potential in leukemia diagnostics.

14.30 – 16.30 **Molecular mechanisms of disease II** *Chair: Torsten Pietsch*

SOD2 Promotes Acute Leukemia Adaptation to Amino Acid Starvation Through the N-Degron Pathway

Ibrahim N K[1], Schreek S[1], Cinar B[1], Loxha L[1], Bourquin J-P[2], Bornhauser B[2], Forster M[3], Stanulla M[1], Gutierrez A[4], Hinze L[1]
[1]Hannover Medical School, Hannover, Germany
[2]University Children's Hospital, Zurich, Switzerland
[3]Institute of Clinical Molecular Biology, Kiel, Germany
[4]Boston Children's Hospital, Boston, USA

Amino acid availability is crucial for leukemia cell survival. However, mechanisms of adaptation to nutrient starvation remain poorly understood. Using a genome-wide CRISPR/Cas9 screen in T-ALL cells, we identified superoxide dismutase 2 (SOD2) as a regulator of the amino acid starvation response. Indeed, culturing SOD2-inhibited T-ALL cells in the absence of essential amino acids (EAA) or non-EAA, induced a significant decrease in cell viability by increasing levels of apoptosis. Importantly, sensitization appeared independent from known SOD2-associated pathways such as ROS signaling and glutamine anaplerosis. Using the Bioplex database (Huttlin et al., 2020), we identified UBR2, an E3 ubiquitin ligase in the N-degron pathway, as a unique binding partner of SOD2. Indeed, SOD2 and UBR2 co-immunoprecipitated, and SOD2 inhibition significantly decreased ubiquitin levels. These data collectively suggest that a complex formation with SOD2 positively regulates N-degron-mediated protein catabolism. The interaction of SOD2 and the N-degron pathway thus represents a previously unknown molecular adaptation of cancer cells in response to amino acid starvation.

Understanding the role of ontogeny for the development of MLL-AF9 infant leukaemia

Antunes E[1], Schwaller J[2], Ottersbach K[1]
[1]University of Edinburgh, United Kingdom
[2]University of Basel, Switzerland

MLL fusions disproportionately cause infant leukaemia, where MLL-AF9 generates AMKL, AML and B-ALL, despite causing only AML in adults. We used a doxycycline-inducible mouse model of MLL-AF9 expression to show that following in utero induction from E12.5, mice develop AML from birth to 3 weeks of age; a faithful human-like latency. Transplantation of E14.5 foetal liver (FL) HSPCs into adult recipients showed AML that is most aggressive in HSC/MPP and least in GMP. Transplantation of LMPP in neonates gave an expansion of B220+ CD19+ blasts that were eventually outcompeted by an expanding CD11b+ population that caused AML, demonstrating microenvironment influence in leukaemia. Parallel experiments in methylcellulose assays with FL

and adult BM HSPCs found that in myeloid conditions, FL HSC/MPP were most able to generate CD41+ c-Kit+ blasts, suggesting a foetal origin for AMKL. In B-lymphoid culture, FL HSPCs had a significantly higher ability to self-renew following replating, with LMPP and CLP proliferating with a pro-B lymphoid phenotype. In summary, we found FL HSPC origins for MLL-AF9 infant leukaemias and an in-vivo infant AML model, which may inform rationale for drug discovery.

Mutations in KRAS and DNMT3A are not related to dependency in established tumors, in PDX acute leukemia model in vivo

Gao Y[1], Ghalandary M[1], Becker M[1], Amend D[1], Rothenberg-Thurley M[2], Metzeler K[2,3], Jeremias I[1,4]
[1]Research Unit Apoptosis in Hematopoietic Stem Cells, Helmholtz Zentrum München, German Research Center for Environmental Health (HMGU), Germany
[2]Laboratory for Leukemia Diagnostics, Department of Medicine III, University Hospital, Ludwig Maximilians University (LMU), Munich, Germany
[3]Department of Hematology and Cell Therapy, University Hospital Leipzig, Leipzig, Germany
[4]German Cancer Consortium (DKTK), Partner Site, Department of Pediatrics, University Hospital, Ludwig Maximilians University (LMU), Munich, Germany

The role of oncogenic mutations was not clear in patients' established tumors. We studied the dependency of frequently mutated genes in established AML in vivo using genetic engineered PDX models. A CRISPR/Cas9 library was tested in 2 PDX models in vivo. In hit validation experiments, knockout of NPM1 abrogated in vivo growth in all PDX models, reproducing the known common essential function. KRAS proved an essential function in PDX models with and without a KRAS mutation, suggesting that AML patients might benefit from treatment inhibiting KRAS, no matter with or without KRAS mutation. DNMT3A were not essential in vitro in AML cell lines. But knockout DNMT3A vanished cells in certain PDX models in vivo and was unrelated to whether mutated or not. These data highlight the complementary use of PDX models to study gene dependencies. We conclude that both KRAS and DNMT3A harbor an essential function in certain patients' established AML in vivo, although independently from whether there is a mutation or not. Warranting verification in additional patient samples, oncogenes and tumor entities, our data indicate re-con sider basic principles of decision-making in Molecular Tumor Boards.

Identifying gene targets for drug repurposing to preventing myeloid malignancies

Schmell A-L[1], Meier K[2], Alejo O, Bhayadia R[1], Heckl D[2], Klusmann J-H[1]
[1]Department of Pediatrics, Goethe-University Frankfurt, Frankfurt am Main, Germany
[2]Pediatric Hematology and Oncology, Martin-Luther-University Halle-Wittenberg, Germany

Preventing myeloid malignancies in children with cancer predisposition syndromes is a major obstacle. A third of neonates with DS exclusively express GATA1s – the short isoform of hematopoietic transcription factor GATA1 - causing transient abnormal myelopoiesis (TAM). TAM can clonally evolve to ML-DS. With the aim to design strategies that prevent the progression from preleukemia to overt leukemia in children with Down syndrome, we performed CRISPR-Cas9 loss-of-function and differentiation screens of FDA approved drug targets in cell lines and pre-leukemic blasts. Early stages of the ML-DS fetal genetic landscape were modeled by inducing the pathognomonic GATA1 mutations in fetal cells using the CRISPR-Cas9 system alongside known chromosome 21 oncogenes. Next-generation sequencing revealing 30 promising potential drug targets, promoting differentiation and/or impairing proliferation, which were individually validated. The success of this approach is combining fast forward genetic approaches with targeting a yet-undiscovered therapeutic window, leading to the identification of medically important and already approved drugs.

A CRISPR-based platform to model AML progression using primary human cells

Issa H[1], Heckl D[2]*, Klusmann J H[1]*
[1]Goethe University Frankfurt, Germany
[2]Martin-Luther-University Halle-Wittenberg, Germany
*Authors contributed equally to the work

Transformation from clonal hematopoiesis to leukemia is incompletely understood. Neonates with trisomy 21 (Down syndrome) exhibit pre-leukemic transient abnormal myelopoiesis (TAM) caused by somatic mutations in GATA1 gene. TAM clonally evolves to myeloid leukemia in Down syndrome (ML-DS) upon acquisition of secondary mutations. Thus, TAM and ML-DS are genetically simple models, where samples from two temporally separable and ascertainable stages in leukemogenesis can be studied. We established a virus-free CRISPR platform utilising the Ribonucleoprotein RNP/Cas9 system to introduce GATA1 mutation and additional mutations in primary human fetal liver hematopoietic stem and progenitor cells (hFLCs). We succeeded in mimicking the progression from clonal hematopoiesis to full-blown leukemia, in vitro and in vivo, as seen in Down syndrome leukemogenesis. Our newly developed human system to investigate leukemic progression offers a unique platform to rewire the synergy between founder genetic aberrations and passenger events and hence make decisive steps towards understanding the malignant transformation from clonal hematopoiesis to frank leukemia.

Understanding the FLT3-ITD maintenance and relapse pathways by RNAi screens

Luque-Martin R[1], Coleman D[2], Blair H[1], Bonifer C[2], Heidenreich O[3,4]
[1]Newcastle university, translational and clinical research institute, Newcastle upon Tyne, United Kingdom
[2]Birmingham university, institute for cancer and genomic sciences, Birmingham, United Kingdom
[3]Wolfson Childhood Cancer Research Centre, Newcastle University, United Kingdom
[4]Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands

Tandem duplication mutations in the tyrosine kinase receptor 3 (FLT3-ITD) are a sign of poor prognosis for patients, who are normally treated with FLT3 inhibitors such as gilteritinib. Given the importance of this mutation in the prognosis and relapse of patients, we wanted to screen a list of gene candidates related to FLT3-ITD maintenance and development.
A list of genes was generated from RNASeq data (patient and cell lines), epigenetic landscape and gene response to inhibitors. An dox-inducible lentiviral RNAi drop-out screen was used to test the importance of these genes in the maintenance and gilteritinib resistance of MOLM-14 and MV4;11 (FLT3-ITD+) cell lines both in vitro and in vivo. Notably, sgRNAs for more than 20% of the candidate genes were either depleted or enriched in the screen providing functional proof for preselection strategy of candidates. Candidates include with e.g., BCL2, DUSP5 and AIF1L regulators of cell viability, cell signalling and of the cytoskeleton that are currently further investigated both in vivo and in vitro in cell lines and in patient PDX.

Clonal Hematopoiesis in Patients with Severe Congenital Neutropenia

Klimiankou M[1], Kandabarau S[1], Zeidler C[2], Pogozhykh D[2], Hettich-Woggon I[1], Hähnel K[1], Welte K[3], Skokowa J[1]
[1]University Hospital Tübingen, Tübingen, Germany
[2]Severe Chronic Neutropenia International Registry (SCNIR), Hannover Medical School, Germany
[3]University Children's Hospital Tübingen, Tübingen, Germany

Severe congenital neutropenia (CN) is a pre-leukemia bone marrow failure syndrome presented with profound neutropenia early after birth due to markedly diminished granulocytic differentiation in bone marrow. Establishment of molecular genetic approach for detection of clonal hematopoiesis in blood or bone marrow of CN patients can assist in early recognition of leukemogenic transformation before clinical MDS/AML symptoms appear.
We established sequencing panel of 31 genes and 1696 “hot-spot” regions in genes recurrently mutated in MDS/AML. Using highly sensitive sequencing approach with implementation of Unique Molecular Identifiers (UMIs) we were able to characterize clonal hematopoiesis in CN patients at neutropenia stage (n=148), mutation burden in CN patients prior to AML/MDS (n=23), and at overt leukemia (n=18). We found that in CN/AML phase high frequency of RUNX1 and CSF3R mutations is usually accompanied by presence of specific leukemia associated genetic lesions. We also observed that presence of additional somatic mutations along with CSF3R mutations at

neutropenia stage might be a strong indicator of advanced pre-leukemia phase and requires closer patient follow up.

New Insights into the pathophysiology of cyclic neutropenia

Zeidler A[1], Dannenmann B[1], Klimiankou M[1], Zeidler C[2], Skokowa J[1], Welte K[1]
[1]University Hospital Tübingen, Tübingen, Germany
[2]Medial School Hannover, Hannover, Germany

Cyclic neutropenia (CyN) is a bone marrow failure syndrome caused by mutations in the ELANE generally with 21-day cycles of peripheral blood neutrophils. These mutations lead to the production of misfolded elastase protein and subsequent elevated unfolded protein response (UPR) in the endoplasmatic reticulum. By searching for additional molecular pathomechanisms of CyN, we identified cycle-dependent expression of CEBPA, MLL1 and their targets, the homeobox factors HOXA9 and MEIS1. At the peak of CyN, there was a 5 to 7 fold expression of these factors compared to cells from healthy controls and a high percentage of CD49f+ HSCs that escape from UPR damage since they do not express NE. This reveals a high replenishment activity of CD49f+ HSCs (escaper cells) in CyN in response to G-CSF and ensures sufficient neutrophil numbers at the following cycle. However, the newly generated and differentiated CD49f negative cells again express mutated NE and are prone to UPR-caused apoptosis requiring again asymmetric division of ELANE negative 49f+ HSC at the peak of the cycle to generate new mature HSCs.

Establishing the mechanism for RUVBL2 essentiality in acute myeloid leukaemia

Virely C[1], Armenteros-Monterroso E[1], Gasparoli L[1], Martens J H A[2], Mansour M R[3], Williams O[1]
[1]Developmental Biology and Cancer Programme, Cancer Section, UCL Great Ormond Street Institute of Child Health, London, United Kingdom
[2]Department of Molecular Biology, Faculty of Science, Radboud, Institute for Molecular Life Sciences, Radboud University, Nijmegen, The Netherlands
[3]Department of Haematology, UCL Cancer Institute, London, United Kingdom

Introduction: Acute myeloid leukaemia (AML) is characterised by the rapid growth of immature myeloid cells. Despite recent advances therapy in AML, the five year survival rates remain poor. We previously showed that RUVBL2 expression is required for AML proliferation, survival, differentiation arrest and disease progression. We found that RUVBL2 binds to c-MYB, a crucial transcription factor in AML. By Chip-seq we defined that RUVBL2 is responsible for maintaining repression of pro-differentiation c-MYB target genes in AML.
Methods: The AML cell line THP1 was transduced with RUVBL2 mutants containing deletion of either domain I, II or III and co-immunoprecipitations were performed to determine the interaction with c-MYB.
Results. We found that only mutant lacking domain III lost the capacity to bind c-MYB. Further mutagenesis defined a small domain III region of RUVBL2 responsible for binding c-MYB.
Conclusion: We have defined a small region of RUVBL2 that is responsible for c-MYB binding. This region may serve as a target for the development of small molecules that can disrupt the interaction between RUVBL2 and c-MYB and thereby disrupt maintenance of AML differentiation arrest.

17.00 – 19.00 **Immunotherapy & precision medicine II** *Chair: Julia Skokowa*

Ex vivo and in vivo complex drug combination analysis for improved efficacy and specificity in high-risk childhood acute lymphoblastic leukaemia

Law E[1], McKenzie L[1], Blair H[1], Szoltysek K[2], Singh M[1], Bomken S[1], Lunec J[1], Irving J[1], Vormoor J[2], Heidenreich O[1,2]
[1]Centre for Cancer, Newcastle University, Newcastle upon Tyne, United Kingdom
[2]Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands

Childhood acute lymphoblastic leukaemia responds to standard treatment, but more targeted drugs are needed. Patient-derived xenografts (PDX) more closely resemble patient cancers than cell lines. PDXs do not proliferate well ex vivo without mesenchymal stromal cells (MSC). High-

content microscopy allows separation of the cell types to allow greater accuracy to filter down the best drug combinations, and may provide insight into patient drug responses observed in the clinic. PDX-MSC cells were analysed with combinations of targeted and chemotherapeutic agents in an ex vivo co-culture system with some combinations deemed to be particularly effective. Caspase assays demonstrated increased cell death when drugs were used in combination in PDXs with high-risk cytogenetics. Subsequent in vivo testing revealed a triple combination was required in order to decrease leukaemic burden of very high risk subtypes, though ultimately resistance to the combination was still observed. Analysis of triple combination treated samples retrieved from mice revealed complete resistance to only one of the three drugs, suggesting resistance could be transient, or related to the pharmacokinetics of the drug.

Streamlining preclinical in vivo treatment trials by multiplexing genetically labelled PDX models in a single mouse

Hunt K[1], Amend D[1], Ludwig R[1,2], Vick B[1,2], Wirth A K[1], Herold T[1,3], Jeremias I[1,2,4]

[1]Research Unit Apoptosis in Hematopoietic Stem Cells, Helmholtz Zentrum München, German Research Center for Environmental Health (HMGU), Munich, Germany

[2]German Cancer Consortium (DKTK), partner site Munich, Germany

[3]Laboratory for Leukemia Diagnostics, Department of Medicine III, University Hospital, LMU Munich, Munich, Germany

[4]Department of Pediatrics, University Hospital, Ludwig Maximilian University (LMU), Munich, Germany

Better treatment options are intensively needed for acute leukemias. Before application in clinical trials, novel therapies require preclinical testing which is resource intensive. We established a patient derived xenograft (PDX) mouse model of ALL and AML, which allow serial transplantation in immunodeficient NSG mice. Up to 5 ALL or AML PDX samples were labelled with an individual genetic barcode and fluorochrome, then multiplexed and aliquots were injected into groups of mice (n=4-6). PDX Cells were reisolated from murine bone marrow and spleen and determining fluorochrome composition allowed quantifying the proportion that each of the 5 samples contributed to the entire tumor load. In a study using the BCL-2 inhibitor Venetoclax, we were able to distinguish between sensitive and resistant PDX samples. While two samples showed a drastic decrease in tumor burden, three samples showed no or only a mild response. Taken together, we established a multiplex protocol for in vivo therapy trials that allows simultaneous testing of up to 5 PDX samples in competitive in vivo trials. The approach reduced the required number of experimental mice by a factor 5, in line with the 3R concept.

Adapting CRISPR Cas9 dropout screens to in vivo PDX models of acute leukemias

Ludwig R[1,2], Amend D[1], Bahrami E[1], Jeremias I[1,2,3]

[1]Research Unit Apoptosis in Hematopoietic Stem Cells, Helmholtz Zentrum München, German Research Center for Environmental Health (HMGU), Munich, Germany

[2]German Cancer Consortium (DKTK), partner site Munich, Germany

[3]Department of Pediatrics, Dr. von Hauner Children's Hospital, Ludwig Maximilian University (LMU), Munich, Germany

As acute leukemias require improved treatment, we aimed at identifying therapeutic targets on a patient-individual level by establishing CRISPR Cas9 dropout screens in PDX models of acute leukemia in vivo. Genetic barcoding in Cas9 negative samples was performed in order to ensure sufficient library coverage. Larger libraries can be screened in ALL compared to AML PDX models, which might reflect lower intra-sample heterogeneity and higher leukemia stem cell frequency in ALL. A customized library of 146 target genes was designed with 5 sgRNAs per gene using the CLUE platform. Either a puromycin-resistance cassette or an H-2Kk surface marker, each combined with a fluorescent marker was used for enrichment of CRISPR Cas9 sgRNA library positive cells to >90%. Cells were injected into NSG mice and re-isolated at advanced disease stage. Next generation sequencing (NGS) data was analyzed using MAGeCK to detect gene dropouts. Commonly depleted genes might represent interesting future therapeutic options. In summary, we established a CRISPR Cas9 screening pipeline to investigate therapeutic targets on a patient-individual level in ALL and, for the first time, in AML PDX models in vivo.

Modulation of Daratumumab efficacy by Decitabine in pediatric T-cell lymphoblastic leukemia (T-ALL)

Autenrieb M-P[1], Vogiatzi F[1], Winterberg D[1], Gelehrt C L[2], Lenk L[1], Baumann N[2], Wolf S[3], Valerius T[2], Peipp M[2], Schewe D M[3]

[1]Pediatric Hematology/Oncology, ALL-BFM Study Group, Christian-Albrechts-University Kiel and University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany

[2]Division of Stem Cell Transplantation and Immunotherapy, Department of Medicine II, Christian Albrechts-University Kiel and University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany

[3]Department of Pediatrics, Otto-von-Guericke University Magdeburg, Magdeburg Germany

Aim: To examine the therapeutic efficacy of a combination of the hypomethylating agent Decitabine (Deci) with the CD38 antibody daratumumab (Dara) in pediatric T-ALL.

Methods: Surface expression of CD38 was determined after exposure to Deci in T-ALL cells (P12, Molt13, HSB2). Dara and Deci were administered as monotherapies and in combination in T-ALL cell lines and patient-derived xenograft (PDX) cells of pediatric T-ALL samples. Antibody-dependent effector mechanisms were examined in vitro.

Results: CD38 surface expression was significantly elevated in T-ALL cell lines subjected to Deci. Moreover, antibody-dependent cell-mediated cytotoxicity (ADCC) by mononuclear cells and antibody-dependent cellular phagocytosis (ADCP) by macrophages were significantly enhanced in T-ALL-cells subjected to combined treatment with Deci and Dara. Notably, ADCP was also elevated in eight de novo and six relapsed/refractory (r/r) T-ALL PDX samples after combination treatment as compared to single therapy.

Conclusion: Dara combined with Deci may represent a promising therapy approach for T-ALL, especially in case of r/r disease, and potentially other hematological malignancies of a T-cell origin.

Prevention of relapse in juvenile myelomonocytic leukemia (JMML) by inhibition of immune checkpoint CD47

Jun W[1], Jovana R[1], Bertram B[2], Charlotte M N[2], Miriam E[2]

[1]University of Freiburg, Germany

[2]Uniklinik of Freiburg, Germany

Juvenile myelomoncytic leukemia (JMML) is a rare, but highly aggressive childhood leukemia caused by overproliferation of the granulocytic and monocytic lineages. The only curative treatment is allogeneic hematopoietic cell transplantation (allo-HCT). However, relapse risk is up to 50%, especially in PTPN11 mutated subtype. Our preliminary data indicated that JMML cell express various immune checkpoints and regulatory molecules that may mediate immune escape. One of these is CD47, a don't eat me signal preventing phagocytosis. Our goal is to understand the impact of CD47 on JMML relapse after HSCT and to prevent relapse by targeting CD47 in PTPN11-knockin mice. Using flow cytometry analysis, we will characterize CD47 expression in different leukemic cell types. To address whether CD47 is directly upregulated by mutated PTPN11, we will interfere with downstream signaling using PI3K and MEK inhibitors. We will establish phagocytosis assay and use anti-CD47 inhibitor to identify the role of CD47 in JMML. We expect that inhibition of CD47 will result in increased phagocytosis of JMML cells and reduced relapse risk. Therefore, our studies will pave the way for future magrolimab trials.

Development of idiotype-specific vNAR-CAR-immune cells for the treatment of clonal malignancies

Wendel P[1,2], Palacios A M[3], Oberoi P[2,4], Habermann J[3], Schoenfeld K[3], Gierschek F[1,2], Kolmar H[3], Wels W S[2,4,5], Ullrich E[1,2,5,6]

[1]Experimental Immunology, Department for Children and Adolescents Medicine, Hospital of the Goethe University Frankfurt, Germany

[2]Frankfurt Cancer Institute (FCI), Goethe University, Frankfurt am Main, Germany

[3]Institute for Organic Chemistry and Biochemistry, Technische Universität Darmstadt, Darmstadt, Germany

[4]Georg-Speyer-Haus, Institute for Tumor Biology and Experimental Therapy, Frankfurt, Germany

[5]German Cancer Consortium (DKTK) partner site Frankfurt/Mainz, Frankfurt, Germany

[6]University Cancer Center (UCT) Frankfurt-Marburg, Frankfurt, Germany

Treatment of B-cell leukemias and lymphomas in children and young adults using CAR-T cells has led to a dramatic improvement in progression-free survival and long-term prognosis. However, on-target/off-tumor toxicities of CAR-T cell therapy directed against universal hematologic antigens can result in severe long-term side effects.
To overcome this limitation, we redirect CAR-target specificity towards the unique lymphoma-specific B-cell receptor (BCR) idiotype, enabling exclusive elimination of malignant cells while maintaining healthy B cells.
Structure-guided CAR-design utilizing shark-derived vNAR domains recognizing a unique BCR idiotype and screening resulted in specific activation of vNAR-CAR (5) by SUP-B8 Burkitt lymphoma cells carrying the cognate BCR idiotype, with only minimum off-target effects. Currently, we further optimize the vNAR-CAR (5) using primary T and NK cells as CAR effectors in vitro and in vivo.
Taken together, our current proof-of-concept data demonstrate feasibility and functionality of vNAR-CAR immune cells with high specificity for the chosen BCR idiotype, suggesting this strategy as a general approach for selective targeting of clonal malignancies.

A new T cell-redirecting strategy for the treatment of relapse/refractory T cell acute lymphoblastic leukemia

Tirado N[1], Trincado J L[1], Bataller A[2], Petazzi P[1], Bueno C[1], Sánchez-Martínez D[1], Menendez P[1]
[1]Josep Carreras Leukaemia Research Institute (IJC), Barcelona, Spain
[2]Hematology Department, Hospital Clínic de Barcelona, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain

T cell acute lymphoblastic leukemia (T-ALL) is a hematological malignancy resulting from the transformation and accumulation of T lineage precursor cells in the bone marrow (BM). Relapse/refractory (R/R) T-ALL remains a challenge with a particularly poor outcome, without curative options beyond hematopoietic stem cell transplantation and conventional chemotherapy, in contrast to B cell malignancies where multiple chimeric antigen receptor-T (CAR-T) strategies exist. The main obstacle to the development of CAR-T therapies for T-ALL is the shared expression of target antigens in leukemic blasts and healthy T cells, leading to fratricide and life-threatening T cell aplasia.
Here, we have identified a novel target antigen for the immunotherapy of T-ALL, expressed across all developmental stages of the disease, in a large cohort of primary samples. Notably, this antigen demonstrated a very safe profile in hematopoietic and non-hematopoietic tissues, as evidenced by flow cytometry and scRNA-seq analyses. After characterization of our candidate target, we proceeded to the generation of an efficient and safe CAR-T cell strategy for the treatment of R/R T-ALL.

An innovative tailored CAR-T cell-redirecting immunotherapy for the treatment of metastatic and refractory Ewing Sarcoma

Panisello C[1], Bueno C[1], Amaral A T[2], Carcaboso A[3], Mde Álava E[2], Menendez P[1]
[1]Josep Carreras Leukaemia Research Institute (IJC)
[2]Institute of Biomedicine of Sevilla (IBiS), Virgen del Rocio University Hospital
[3]Institut de Recerca Sant Joan de Deu

Ewing Sarcoma (ES) is the second most common bone and soft tissue sarcoma affecting children, adolescents, and young adults. Despite very intensive multimodal therapies, the current overall survival remains dismal, particularly in patients with metastatic or refractory disease. This highlights the urgent need for innovative therapeutic approaches. Adoptive immunotherapy with chimeric antigen receptor (CAR)-T cells directed against a tumour-associated antigen (TAA) is a promising therapy with a safe and efficient profile in B-cell malignancies. However, the development of efficient and safe CAR-T cells for the treatment of ES is still challenging due to i) the low abundance of specific TAA, ii) the widespread expression of TAA in normal tissues, leading to on-target off-tumour toxicities iii) and the presence of an immunosuppressive tumour microenvironment (iTME) that reduces CAR-T cells expansion and persistence at tumour sites.

Here, we show preliminary data from ES cell lines and primary samples aiming to identify specific ES-targets with limited expression in healthy tissues to try to generate an efficient and safe CAR-T cell approach for treating metastatic and refractory ES.

CRISPR/Cas9-based generation of CAR-expressing natural killer-like cells against acute myeloid leukemia

Baatz F[1], Herbst J[1], Schambach A[2], Hust M[3], Mätzig T[2], Meyer J[2], Sauer M G[1]
[1]Department of Pediatric Hematology/Oncology and Blood Stem Cell Transplantation, Hannover Medical School, Germany
[2]Institute of Experimental Hematology, Hannover Medical School, Germany
[3]Department of Biotechnology, Technische Universität Braunschweig, Germany

Relapsed acute myeloid leukemia (AML) is difficult to cure. Novel treatment strategies are of high medical need. Cell therapies using chimeric antigen receptor (CAR) T cells have shown promising results in B cell malignancies. We have shown that CAR expression during early lymphoid development suppresses the transcription factor B cell CLL/lymphoma 11B (BCL11B). Lymphoid differentiation shifts towards CAR-induced killer (CARiK) cells, however, this requires tonic CAR signaling activity, which is currently an exclusion criterion for its further clinical development. Obstacles in AML relapse are the collection of autologous T cells in sufficient quantity and quality. Here we present proof of principle for an “off-the-shelf” cell product based on CAR-engineered lymphoid progenitors. We generated novel functional and “clinically” suitable CARs targeting the AML antigen CD123 without tonic signaling activity. They mediate potent antileukemic activity even across HLA barriers upon co-transplantation in leukemia bearing mice. CRISPR/Cas9-mediated Bcl11b disruption facilitates CARiK development in lymphoid progenitors laying the basis for an “off-the-shelf” immunotherapy against AML.

SATURDAY JUNE 4

9.00 – 10.15 **Molecular mechanisms of disease III** *Chair: Roland Kappler*

A meningeal preleukemic niche promotes the homing of B-cell Precursor Acute Lymphoblastic Leukaemia cells to the CNS

Mayar A[1], Ibuli O[2], Bultmann M[3], Winterberg D[1], Jeremias I[4], Dietterle A[1], Münch G[1], Bastian L[3], Schewe D N[5], Lenk L[1]

[1]Department of Pediatrics, ALL-BFM Study Group, University Medical Center Schleswig-Holstein, Kiel, Germany
[2]Center for Molecular Medicine Cologne, Faculty of Medicine, University Hospital Cologne, University of Cologne, Cologne, Germany
[3]Medical Department II, Hematology and Oncology, University Medical Center Schleswig-Holstein, Kiel, Germany
[4]Research Unit Apoptosis in Hematopoietic Stem Cells, Helmholtz Zentrum München, German Center or Environmental Health (HMGU), Munich, Germany
[5]Department of Pediatrics, Otto-von-Guericke-University, Magdeburg, Germany

Aim: To understand early events promoting the engraftment and homing of BCP-ALL cells to the CNS in order to identify potential therapeutic interventions.

Methods: We determined in vivo engraftment kinetics of luciferase+ BCP-ALL patient derived xenograft (PDX) cells in NSG-mice and subjected healthy vs pre-leukemic meninges to RNA-sequencing. Potential preleukemic niche (PLN) associated factors were validated in vitro using CRISPR-CAS9 editing of CNS “stroma” cell lines in CNS/ALL-cocultures.

Results: Comparative RNA-sequencing showed an upregulation of inflammation and adhesion pathways and molecules previously shown to be associated with CNS-leukemia (e.g. IL7, CXCL12) in preleukemic meninges. In vitro coculture of the CNS-tropic cell lines REH and NALM6 with BEND3, BMEC cells and primary murine meningeal fibroblasts resulted in a significant upregulation of VCAM1 in the CNS stroma. Knockdown of VCAM1 in CNS cells resulted in a reduced release of proinflammatory chemokines in cocultures.

Conclusion: BCP-ALL cells may induce alterations in murine meninges promoting a PLN in the CNS. This may open the window for novel targeted interventions and early diagnosis in CNS-leukemia.

ADAM10’s sheddase function augments the interaction of leukemia cells with the bone marrow niche in PDX models in vivo

Schmid J P[1,2], Bahrami E[1], Becker M[1], Jayavelu A K[3], Wirth A[1], Jurinovic V[1,4,5], Öllinger R[6,7,8], Vick B[1,2], Herold T[1,4], Jeremias I[1,2,5]

[1]Helmholtz Zentrum München- German Research Center for Environmental Health HMGU, Research Unit Apoptosis in Hematopoietic Stem Cells, Munich, Germany
[2]German Cancer Consortium DKTK, partner site Munich, Munich, Germany
[3]Max-Planck-Institute of Biochemistry, Department of Proteomics and Signal Transduction, Munich, Germany
[4]Department of Medicine III- University Hospital- LMU Munich, Laboratory for Leukemia Diagnostics, Munich, Germany
[5]University Hospital- Ludwig Maximilian University LMU, Department of Pediatrics, Munich, Germany
[6]TUM School of Medicine- Technische Universität München, Center for Translational Cancer Research TranslaTUM, Munich, Germany
[7]Technische Universität München, Institute of Molecular Oncology and Functional Genomics, Munich, Germany
[8]Klinikum rechts der Isar- Technische Universität München, Department of Medicine II, Munich, Germany

Interrupting tumor-microenvironment interactions is an attractive therapeutic strategy. We developed a CRISPR-Cas9 screening approach for functional analysis of surface molecules in patient-derived xenograft (PDX) acute leukemia (AL) models in vivo in order to decipher tumor specific vulnerabilities. A customized library was run in 2 AL PDX samples and candidates were confirmed using a competitive in vivo approach. ADAM10 was depleted in both and validated in 6 PDX models. Treating PDX cells with ADAM10 inhibitor reduced the bone marrow (BM) engraftment capacity, while KO of ADAM10 reduced the leukemia stem cell frequency. Both AML and ALL ADAM10 KO PDX samples showed increased sensitivity towards routine chemotherapy in vivo. Reconstitution of ADAM10 KO PDX cells with a WT variant in vivo rescued the phenotype, while an enzymatic domain lacking variant did not, highlighting the importance of ADAM10’s sheddase function. In conclusion, we established CRISPR-Cas9 drop-out screens in PDX models in vivo to explore patient-specific tumor dependencies. Our data revealed ADAM10’s role in maintaining leukemic cells in the BM niche, thus representing an attractive future therapeutic target.

Epigenetic modifiers direct lineage switch in MLL-AF4 leukemia

Szoltysek K[1], Tirtakusuma R[2], Ashtianim M[1], Nakjang S[2], Grinev V[3], Hehir-Kwa J[1], Schweighart E K[1], Bomken S[2], Heidenreich O[1,2]

[1] Princess Maxima Center for Pediatric Oncology, Utrecht, The Netherlands

[2] Wolfson Childhood Cancer Research Centre, Centre for Cancer, Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, UK

[3]Department of Genetics, the Faculty of Biology, Belarusian State University, 220030 Minsk, Republic of Belarus

MLLr initiates onset of high-risk leukemia which lineage - ALL or AML - is determined by the MLL-fusion partner. MLL-AF4 pre-dominantly associates with B-ALL, however ~1% of cases convert from CD19+ lymphoid into CD19^{neg} myeloid phenotype.

Here we aimed to identify molecular basis of lineage switching in a cohort of MLL-AF4 acute leukemia patients undergoing myeloid relapses. Performed genomic and transcriptomic studies revealed low mutational load and substantial changes in transcriptomic pattern observed at AML relapse. We found *CHD4* gene, a core component of chromatin remodeling NuRD complex, to be disrupted in analyzed cases, pointing into its crucial function in maintenance of lymphoid commitment decisions.

Comparison with non-lineage switching MLL-AF4 patients showed hyperactivation of translational processes and co-existence of two signatures at B-ALL stage of our patients: B-cell and multi-potent, progenitor-like. This data suggests that enhanced cellular plasticity may facilitate CD19 loss and myeloid differentiation, directed by dysfunctional epigenetic modifiers, leading to treatment escape route to e.g. epitope-directed immunotherapies.

Deep learning-based cell segmentation identifies T cell infiltration and spatial distribution in de novo pediatric AML

Koedijk J B[1], Van der Werf I[1], Vermeulen M A[1], PALGA-group[2], Nierkens S[1], Zwaan CM[1,3], Heidenreich O[1,4]

[1]Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands

[2]Dutch Pathology Registry, Netherlands

[3]Department of Pediatric Oncology, Erasmus MC/Sophia Children's Hospital

[4]Wolfson Childhood Cancer Research Centre, Newcastle University, United Kingdom

Emerging evidence suggests that the presence of immune cells in the tumor microenvironment (TME) may be predictive of the response to immunotherapy. Accordingly, we characterized the infiltration and spatial distribution of T cells in diagnostic bone biopsies of 22 children with de novo acute myeloid leukemia (AML). Archival biopsies were acquired through the Dutch Pathology Registry (PALGA; 2021-82). Subsequently, immunohistochemistry was performed using antibodies directed against CD3 in combination with hematoxylin. We identified low to intermediate T cell infiltration among most biopsies (mean 9.5% (SD = 13.0) of total cells or 843 cells/mm2 (SD = 1212). In contrast, we did identify two biopsies that were heavily infiltrated with T cells, and which showed localized clustering of T cells. T cell infiltration was not related to the number of blasts in the bone marrow (median 34%, range 0-98). In conclusion, most pediatric AML patients have poor T cell infiltration in the TME at diagnosis, indicating that (combinations of) immune priming therapies may be required for successful implementation of immunotherapies in pediatric AML.

HiPSC disease modelling to study leukemia development in HAX1 vs ELANE associated congenital neutropenia

Dannenmann B[1], Klimiankou M[1], Oswald B[1], Solovyeva A[1], Mardan J[1], Zeidler C[1], Welte K[2], Skokowa J[1]

[1]Department of Oncology, Hematology, Immunology, Rheumatology and Pulmonology, University Hospital Tübingen, Germany

[2]University Children's Hospital Tübingen, Germany

Severe congenital neutropenia (CN) is a pre-leukemic syndrome with a high risk of developing MDS/AML associated with the acquisition of CSF3R and RUNX1.

We established a model for step-wise leukemia progression in HAX1- and ELANE-CN using iPSC-based hematopoietic differentiation in combination with CRISPR/Cas9-mediated gene editing of iPSCs. We compared HAX1 and ELANE-CN phenotypes and the role of missense vs non-sense RUNX1 mutations in leukemogenesis. We identified BAALC upregulation as a key leukemogenic event and BAALC KO restored granulocytic differentiation. HAX1- and ELANE-CN/AML iPSC-derived CD34+ cells showed reduced granulocytic differentiation and increased proliferation.
RNA-seq analysis of iPSC-derived CD34+ cells showed high similarity between ELANE- and HAX1-CN/AML revealing features of RUNX1 mutant leukemia, upregulation of inflammatory response and MYC targets. We also identified GATA1/2 transcription factors, as well as HIPK2 and MAPK1,3,14 kinases as main regulators of DEGs.
In summary, we identified shared and mutation-specific leukemogenic signaling pathways in CN regarding the inherited mutation status or based on the type of acquired RUNX1 mutations.

10.30 – 11.45 **Molecular mechanisms of disease IV** *Chair: Raj Bhayadia*

Breaking the pump: targeting the sodium-potassium pump as a therapeutic strategy in acute myeloid leukemia

Schneider C[1], Spaink H[1], Alexe G[1], Dharia N V[1], Khalid D[1], Scheich S[2], Haeupl B[3], Oellerich T[3], Stegmaier K[1]
[1]Department of Pediatric Oncology, Dana-Farber Cancer Institute and Boston Children's Hospital
[2]Lymphoid Malignancies Branch, National Cancer Institute, National Institutes of Health
[3]Department of Medicine II, Department for Hematology/Oncology, Goethe University, Frankfurt, Germany

In order to identify novel, selective targets for acute myeloid leukemia (AML), we used the Cancer Dependency Map, which provides genome-scale CRISPR/Cas9 screen data in hundreds of cancer cell lines. Here, we identified ATP1B3 as a context-specific dependency that could be therapeutically exploited.
ATP1B3 is the glycoprotein subunit (beta) of the Na/K-ATP pump. Together with the catalytic alpha subunit ATP1A1, it forms a heterodimer located in the plasma membrane, regulating the electrochemical gradient across the membrane.
The 4 beta subunit paralogs show similar expression patterns among tissues, with one exception. ATP1B1 has uniformly low expression in hematol. malig. in comparison to other tumor cell lines. By using CRISPR/Cas9 KO to validate this finding we showed that loss of ATP1B3 in ATP1B1 low AML cells leads to synthetic lethality and lower leukemia burden in a BLI-based orthotopic mouse model of AML. The absence of both paralogs of the beta subunit results in the loss of their common essential binding partner ATP1A1 in hematol. malig., while higher expression of ATP1B1 can stabilize ATP1A1 under the loss of ATP1B3 in solid tumors or ATP1B1 overexpressing cells.

Functional characterization of aberrant GATA1 protein complexes in normal and leukemic human erythroblasts

Tauchmann S[1], Bagger F O[2], Bock T[3], Sivalingam R[1], Eder T[4], Fagnan A[5], von Lindern M[6], Mercher T[5], Grebien F[4], Schwaller J[1]
[1]University Children's Hospital Basel & Department of Biomedicine, University of Basel, Basel Switzerland
[2]University of Copenhagen, Center for Genomic Medicine, Copenhagen University Hospital, Copenhagen, Denmark
[3]Proteomics Core Facility, Biozentrum, University of Basel, Basel, Switzerland
[4]University of Veterinary Medicine, Institute of Medical Biochemistry, Vienna, Austria
[5]INSERM U1170, Institut Gustave Roussy, Université Paris-Saclay, Université de Paris, Villejuif, France
[6]Sanquin Research, Academic Medical Center, University of Amsterdam, the Netherlands

Observations in murine and human models suggest that acute erythroleukemia (AEL) is linked to impaired function of the erythroid master transcriptional regulator GATA1. To address whether impaired AEL cell differentiation could be the consequence of aberrant GATA1 protein interactions, we comparatively analyzed the proteomes and GATA1 interactomes of 3 human AEL cell lines and primary AEL patient cells with HUDEP2 cells and primary human erythroblasts and identified 116 differentially GATA1-associated proteins. To functionally explore their effects on erythroid differentiation, we performed a targeted CRISPR screen in Cas9-expressing K562 cells. Inactivation

of cluster 1 genes did not change erythroid differentiation or growth, while loss of cluster 2 genes did not change differentiation, but impaired cell survival. Disruption of cluster 3 genes resulted in an increased differentiation without significant changes in cell growth whereas deletion of cluster 4 genes caused significant signs of differentiation and depletion. Ongoing functional validation of selected targets will identify novel regulators to therapeutically target impaired erythroid differentiation in AEL.

CRISPRi proliferation and differentiation screening to identify functional long noncoding RNAs in pediatric AML

Winkler R[1], Heckl D[1], Bhayadia R[2], Klusmann J H[2]
[1]Martin-Luther-Universität Halle-Wittenberg, Germany
[2]Goethe-Universität Frankfurt am Main, Germany

Long non-coding RNAs (lncRNAs) have been shown to be stage- and cell type-specific regulators of gene expression. We previously discovered stem cell- and AML subtype-specific lncRNA expression signatures. Using a CRISPRi approach we screened 619 genes in 7 AML cell lines, selecting for proliferation, as well as differentiation phenotype. ShRNA Knockdown of the lncRNA here termed LNCER transferred proliferation disadvantage in the erythroleukemic CMK cell line. LNCER is not expressed in human hematopoietic stem cells (HSC), yet highly expressed in patient samples of transient myeloproliferative disease and myeloid leukemia of Down syndrome. We performed RNA-sequencing upon shRNA knockdown in CMK, revealing down-regulation of megakaryocytic and up-regulation of erythroid signature genes. Next, we induced LNCER knockdown in HSC in vitro. Methocult and in vitro differentiation assays showed increased erythroid differentiation.
Our results implicate significance of lncRNA in malignant hematopoesis. CRISPRi screenings revealed candidate regulators of differentiation and proliferation in pediatric AML. LNCER stood out as a potential regulator of erythroleukemia and erythroid cell fate.

A new inherited syndrome with severe neutropenia and neurological involvement due to autosomal recessive COPZ1 mutation

Borbarán-Bravo N[1], Deordieva E[1,2], Bräuning S[1], Dannenmann B[1], Doll L[1], ElGamacy M[1,3,4], Zeidler C[5], Bajoghly B[1], Maschan A[2], Shcherbina A[2], Welte K[1], Skokowa J[1], and Klimiankou M[1]
[1]University Hospital Tübingen, Division of Translational Oncology, Department of Hematology, Oncology, Clinical Immunology and Rheumatology, University Hospital Tübingen, Germany
[2]Dmitry Rogachev National Research Center of pediatric Hematology, Oncology and Immunology, Moscow, Russia
[3]Max Planck Institute for Developmental Biology, Tübingen, Germany
[4]Friedrich Miescher Laboratory of the Max Planck Society Tübingen, Germany
[5]Department of Pediatric Hematology/Oncology, Klinderklinik, Hannover, Germany

We identified a new homozygous stop-codon mutation in the COPZ1 gene (p.Q141X) in two siblings with severe neutropenia and neurological developmental delay. COPZ1 is a member of the coatomer protein complex I (COPI) regulating intracellular trafficking of proteins.
CRISPR/Cas9-mediated introduction of the stop-codon mutation at the position p.Q141X in COPZ1 in healthy donors` cord blood hematopoietic stem cells (HSPCs) and iPSCs led to defective granulocytic differentiation in vitro. Additionally, copz1 mutant zebrafish embryos produced significantly fewer neutrophils than their control counterparts. These findings were in line with hyperactivated unfolded protein response (UPR) and elevated autophagy in the myeloid cell line NB4 after introduction of the truncated mutation in COPZ1.
COPZ1 is ubiquitously expressed, while its paralogous gene, COPZ2, is absent in the blood and the brain. Interestingly, the rescue of COPZ1 mutated HSPCs with COPZ2 corrected the defective granulopoiesis.
Thus, we describe a new severe congenital neutropenia syndrome caused by autosomal recessive COPZ1 mutations with downstream UPR and autophagy activation.

Upstream regulation of DLK1-DIO3 locus in hematopoiesis and pediatric leukemia

Verboon L[1], Issa H[1], Bräuer-Hartmann D[2], Regenyi E[2], Heckl D[2]*, Klusmann J H[1]*
[1]Goethe University Frankfurt, Germany
[2]Martin-Luther-University Halle-Wittenberg, Germany
*Authors contributed equally to the work

Non-coding RNAs (ncRNAs) recently emerged as central regulators of chromatin and gene expression, posing a novel window for targeted therapies in pediatric acute megakaryoblastic leukemia (AMKL). In our ncRNA expression atlas of the human hematopoietic system, we uncovered specific and coordinated expression of the DLK1-DIO3 locus in megakaryocytes and AMKL, which is essential of for promoting megakaryocytic differentiation, but also leukemic growth. To determine how expression of the DLK1-DIO3 locus is controlled, we performed several techniques elucidating different regulatory layers. Using bisulphite sequencing, a significant correlation between MEG3 expression and methylation of a CpG island downstream of the first exon of MEG3 was found, which displayed enhancer/promoter activity. CUT&RUN for key megakaryocytic transcription factors revealed increased binding of oncogenic GATA1s on several genomic locations upstream of DLK1 and MEG3. Overall, our study is the first step towards understanding the regulation of the DLK1-DIO3 locus in AMKL and normal hematopoiesis.