Program Kind-Philipp-Meeting 2026

Wednesday	Thursday	Friday	Saturday
	C. Translation & Therapy: 09:00 - 10:30 1. Köppen N 🏆 2. Bumia H 🏆 3. Rutkowski S 4. Rhode K 🏆 5. Balszuweit A 🏆 6. Zkrina A 🏆 7. Rassner M 🏆	**I. Immunotherapy :** 09:00 - 10:30 1. Lübbers A 🏆 2. Sanvi L W 🏆 3. Heymann J 🏆 4. Schuster S 🏆 5. Kurschies M 🏆 6. Haas D 🏆 7. Balzer S 🏆	**O. Malignant Cell Biology II:** 09:00 - 10:30 1. Bamberg L V 🎯 2. Kazybay B 🎯 3. Jungen F 🎯 4. Mambelli D 🎯 5. Jin S 🎯 6. Almeida I 🎯 7. Klohk G 🎯
	D. Tumor Microenvironment: 11:00 - 12:30 1. Schweighart E K 🏆 2. Ghete T 🏆 3. Fuhrmann T W 🏆 4. Perzolli A 🏆 5. Cases Wroblewski N 🏆 6. Mullen C 🏆 7. Groll D 🏆	**J. Biomarkers:** 11:00 - 12:30 1. Uihlein L 🏆 2. Zabel S F 🏆 3. Lissow L 🏆 4. Grauer M S 🏆 5. Schuschel K 🏆 6. Arora J 🏆 7. Logvinova E 🏆	**P. Malignant Cell Biology III:** 11:00 -12:00 1. Mehra D 🎯 2. Gang D 🎯 3. Ghosh A 🎯 4. Pawar S A 🎯 5. Kaffenberger C 🎯 6. Jamous S 🎯
	Lunch: 12:30 - 14:00	Lunch: 12:30 - 14:00	Lunch: 12:00 - 13:00
	14:00 - 14:30 E. Frank Westermann	14:00 - 14:30 K. Marcel Kool	
	F. Malignant Cell Biology I: 14:30 - 15:30 1. Schürmann J A 🏆 2. Cifarelli L N 🏆 3. Bidasiuk A 🏆 4. Klein A 🏆 5. Zhong T 🏆	**L. Novel Approaches:** 14:30 - 15:30 1. Li Y 🏆 2. Bockmayr M L 3. Kikvadze S 🏆 4. Agca C 🏆 5.Abdrabo A 🏆	
Welcome: 17:45 - 18:00	**G. Molecular Mechanisms of Disease 1:** 16:00 - 17:30 1. Dietz A 🏆 2. Haas J 🏆 3. Koch D 🏆 4. Korczmar E 🏆 5. Barros da Gama S 🏆 6. Alcaide Miranda M 🏆 7. González-Dammers H 🏆	**M. Molecular Mechanisms of Disease 2:** 16:00 - 17:30 1. Gonçalves-Dias J 🏆 2. Müller M 🏆 3. Cases i Palau S 🏆 4. Rahimian E 🏆 5. Javadpoor Ebrahimi A 🏆 6. Sivalingam R 🏆 7. Schuhmacher W 🏆	
A. Appetizers: 18:00 - 19:00 1. Sorochynska Y 🏆 2. Van den Bedem S D 🏆 3. Winkler R 🏆 4. Beneder H 🏆 5. Mayerhofer C 🏆	18:00 - 18:30 **H.** Ulrich Steidl	**18:00 - 18:30** N. Thomas Milne	🏆 Knack den Krebs-Award Contestant: 10+2 min 🎯 Short Talk: 7+2 min
19:00 - 19:30 B. David Kent	Dinner: 19:00	18:45 - 19:00 Knack den Krebs-Award	
Dinner: 19:30	Heide Exploration Walk	Dinner: 19:00	

Wednesday, June 3

17:45 – 18:00 ***Welcome*** *Rolf Marschalek*
Jan-Henning Klusmann

18:00 – 19:00 ***A. Appetizers*** *Owen Williams*

A1. Analyzing Differentiation Patterns in Sonic Hedgehog-Activated Medulloblastoma 🏆

Sorochynska Y.[1], Benotmane J.K.[2], Okonechnikov K.W.[3], Pajtler K.[3], Pfister S.M.[3], Kerl K.[4], Schüller U.[1], Ghasemi D.R.[1]

[1]University Medical Center Hamburg-Eppendorf and Research Institute Children's Cancer Center Hamburg, Hamburg, Germany; [2]Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany; [3]Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany; [4]University Children's Hospital Münster, Münster, Germany

Sonic Hedgehog-activated medulloblastoma (MB-SHH) represents nearly one-third of all medulloblastoma cases. It encompasses a broad histologic and molecular spectrum, ranging from desmoplastic/nodular medulloblastoma and medulloblastoma with extensive nodularity to classic and large cell/anaplastic variants, with clinical outcomes varying from relatively favorable in more differentiated forms to poor in biologically aggressive subtypes. Here, we apply multi-omics approaches to further investigate the hypothesis that MB-SHH heterogeneity reflects tumor cells arrested at distinct stages of the cerebellar granule neuron developmental program. Single-nucleus RNA-sequencing of 19 cases (86,393 nuclei) revealed heterogeneous malignant cell populations with transcriptional programs consistent with arrest at different stages of cerebellar granule neuron development. Using supervised discovery of interpretable gene programs across this and additional datasets, we aim to identify the molecular mechanisms underlying developmental arrest and validate them using in vitro models and spatial transcriptomics.

A2. Development of a High-Throughput Drug Screening Platform for Patient-Derived AML using Machine Learning Image Analysis. 🏆

van den Bedem S.D.[1], van Dijk I.[1], Kemmeren P.[1], Vormoor J.[1], Krippner-Heidenreich A.[1], Heidenreich O.[1]

[1]Princess Máxima Center for Peadiatric Oncology, Utrecht, The Netherlands

Several subtypes of paediatric acute myeloid leukaemia (AML) still have poor prognosis or severe drug toxicity. To improve patient stratification we are developing a high-throughput, image-based platform for ex vivo drug testing of primary AML cells,

integrating machine-learning driven image analyses in an AML/mesenchymal stromal cells (MSC) co-culture system. We adapted a 14-day AML/MSC co-culture to a 384-well format and built a Python pipeline for flat-field correction and image stitching to improve analysis quality. Using a no-wash nuclear stain our CNN model classifies AML cells with 99% accuracy across various karyotypes in cell lines, PDXs, and primary AMLs, and MSC with 95% accuracy. The model is currently being extended to include additional niche components, such as NK cells. As drug-induced differentiation holds promise in AML treatment, we also train the model to assess drug-induced differentiation. Preliminary data show that CD34+ and CD34- cells can be distinguished. Together, this platform enables high-throughput AML drug screening by quantifying viable cells, capturing both leukeamic and niche compartments, and shows promise to assess drug-induced differentiation.

A3. An Integrated Epigenomic Atlas Defines Master Transcription Factor Circuits in Pediatric AML 🏆

Winkler R.[1], Verboon L.[1], Issa H.[1], Goncalves-Dias J.[1], Schuschel K.[1], Klusmann J.-H.[1]

[1]Goethe-University Frankfurt, Frankfurt, Germany

Acute myeloid leukemia (AML) is genetically heterogeneous, yet leukemic cells adopt a limited number of stable cellular identities. How epigenetic regulation constrains this diversity into defined cellular states is unclear. Pediatric AML converges onto a restricted set of enhancer-driven regulatory states independent of genotype. We generated a comprehensive epigenomic atlas integrating chromatin accessibility (ATAC-seq, n = 163) and histone modification profiling (CUT&Tag, n = 133; H3K4me3, H3K27ac, H3K4me1, H3K27me3, H3K36me3, H3K9me3) and applied multi-omics factor analysis to define latent regulatory programs. Clustering revealed that diverse mutations converge onto shared epigenetic states, with KMT2A-rearranged AML forming a distinct outlier. Enhancers, rather than promoters, constituted the major source of variability. Gene regulatory network inference identified core transcription factor modules (GATA1, SPI1/PU.1, HOXA, CEBP) defining lineage-associated states, supported by CUT&RUN validation. Leukemic identity is governed by enhancer topology and transcription factor circuitry, providing a framework for classification and therapeutic targeting based on regulatory state.

A4. Domain scanning reveals essential protein-protein interfaces within the SAGA complex in AML 🏆

Beneder H.[1], Batty P.[1], Simoncig M.[1], Kutschat A.P.[1], Müller S.[1], Karbon G.[1], Bykov A.[1], Prakash C.[1], Grebien F.[1], Seruggia D.[1]

[1]St. Anna Childrens Cancer Research Institute (CCRI), Vienna, Austria

Aberrant transcriptional regulation drives cancer cells to depend on key epigenetic factors. The SAGA co-activator complex regulates transcription via histone acetylation, deubiquitylation, and splicing. While several SAGA subunits have been identified as vulnerabilities in cancers such as pediatric leukemias, prior work has predominantly focused on enzymatic components, leaving the ~18 non-enzymatic subunits insufficiently characterized. To address this, we systematically analyzed all 20 SAGA subunits across six ALL and AML cell lines performing time-resolved, SAGA-focused arrayed CRISPR/Cas9 screens. Thereby, we identified structural SAGA CORE subunits as novel dependencies in pediatric AML. By dense mutagenesis, we identified novel critical protein domains for selected dependencies. Through pairwise AlphaFold3 predictions and Co-IP experiments, we revealed interactions mediated by the novel domains. Finally, we could show that the depletion of SAGA CORE components or degradation of KAT2A/KAT2B leads to terminal myeloid differentiation of AML cells. In conclusion, our findings highlight non-enzymatic SAGA components as promising therapeutic targets in pediatric leukemia.

A5. OGFOD1 enables AML chemo- and nutrient stress resistance by regulating protein synthesis 🏆

Mayerhofer C.[1], Li D.[2], Kristiansen T.[2], Mayerhofer E.[2], Sharda A.[2], Vidoudez C.[3], Miller P.[2], van Gastel N.[4], Graubert T.[2], Scadden D.[2]

[1]Universitätsklinikum Freiburg, Freiburg, Deutschland; [2]MGH, Boston, USA; [3]Harvard University, Boston, USA; [4]UCLouvain, Brussels, Belgium

Acute Myeloid Leukemia (AML) commonly relapses after initial chemotherapy response. We assessed metabolic adaptations in chemoresistant cells in vivo before overt relapse, identifying altered branched-chain amino acid (BCAA) levels in patient-derived xenografts (PDX). Restricting of BCAAs further reduced chemoresistant AML cells but relapse still occurred. Among the persisting cells we found an unexpected increase in protein production. This was accompanied by elevated translation of 2-oxoglutarate-and-iron-dependent oxygenase 1 (OGFOD1), a known ribosomal dioxygenase that adjusts the fidelity of tRNA anticodon pairing with coding mRNA and upregulates protein synthesis in AML driving disease aggressiveness. Inhibiting OGFOD1 impaired translation processing, decreased protein synthesis and improved animal survival even with chemoresistant AML through regulation of protein synthesis. Leukemic cells can therefore persist despite the stress of chemotherapy and nutrient deprivation through adaptive control of translation while sparing normal hematopoiesis. Targeting OGFOD1 may offer a distinctive, translation modifying means of reducing the chemopersisting cells that drive relapse.

19:00 – 19:30 ***B. Invited Lecture*** *Jan-Henning Klusmann*

B1. New tools to study clonal evolution in blood stem cells

David Kent

University of York, United Kingdom

Thursday, June 4

09:00 – 10:30 ***C. Translation and Therapy*** *Lennart Lenk*

C1. The PARP inhibitor pamiparib effectively radiosensitizes medulloblastoma while sparing healthy brain tissue 🏆

Köppen N.[1], Gerbach M.[1], Neumann J.E.[2], Kool M.[3], Thomale U.-W.[4], Cohrs G.[4], Kerl K.[5], Schüller U.[6], Mynarek M.[1], Struve N.[1]

[1]University Medical Centre Hamburg-Eppendorf, Hamburg, Deutschland; [2]Centre for Molecular Neurobiology (ZMNH), Hamburg, Deutschland; [3]German Cancer Research Centre (DKFZ), and German Cancer Consortium (DKTK), Heidelberg, Deutschland; [4]Charité - Universitätsmedizin Berlin, Berlin, Deutschland; [5]University Medical Centre Münster, Münster, Deutschland; [6]Research Institute Children's Cancer Centre Hamburg, Hamburg, Deutschland

Background: Medulloblastoma (MB) is the most common high-grade pediatric brain tumor. Current therapy causes neurocognitive late effects. Molecular subtypes differ in prognosis. Radiosensitization with the PARP inhibitor pamiparib may improve survival in high-risk patients and allow dose de-escalation in standard-risk groups. This study investigated whether pamiparib increases MB radiosensitivity while sparing normal tissue. Methods: PARP1/2 expression in SHH and group 3 MB cell lines was analyzed. Radiosensitivity, DNA double-strand break (DSB) repair and cell cycle effects were assessed after irradiation (IR) ± pamiparib. Ex vivo MB tissue slices (MEXS) from PDX and primary MB were treated similarly. Neurotoxicity was tested in non-cancer models (in vitro and ex vivo). A CAM model enabled in vivo-like analyses
Results: Group 3 cells showed higher PARP1 levels. Pamiparib plus IR increased residual DSBs, S/G2 accumulation and radiosensitivity in SHH and group 3. MEXS confirmed impaired DSB repair. No additional toxicity was observed in normal tissue models. Conclusion: Pamiparib enhances MB radiosensitivity by inhibiting DSB repair without increasing normal tissue damage.

C2. Identifying Drug Resistance Pathways in Paediatric Acute Myeloid Leukaemia 🏆

Bumia H.[1], Williams O.[1]

[1]University College London, London, United Kingdom

Relapse in paediatric acute myeloid leukaemia (pAML) has long been attributed to the presence of leukaemic stem cells, that are inherently resistant to chemotherapy and drive tumour regeneration following treatment withdrawal. In the last decade, an alternative mechanism for relapse has been proposed – chemotherapy-induced cellular dormancy. There is increasing evidence that bulk populations of AML blasts undergo transcriptional and molecular reprogramming to enter a transient anti-proliferative state, allowing them to evade chemotherapy. In this study, we aim to identify therapeutic vulnerabilities in surviving, post-chemotherapy AML blasts by investigating transient alterations in their transcriptional and molecular profiles. We have conducted bulk RNAseq on PDX AML blasts capable of in vivo leukaemia reconstitution following ex vivo treatment with cytarabine alone or a cytarabine/daunorubicin combination. We further aim to conduct scRNAseq on these samples pre-/post- in vivo engraftment to assess key identifiers of relapse. In parallel, we are conducting a large-scale CRISPR drop-out screen to identify key genes associated with leukaemia progression and relapse.

C3. Treatment de-intensification for WNT medulloblastoma: Results from the favourable-risk arm of the SIOP-PNET5-MB study

Rutkowski S.[1], Mynarek M.[1], Veronique M.[2], Torsten P.[3], Stefan P.[4], Laetitia P.[5], Herve B.[2], Clifford S.[6], Francois D.[2]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Institute Curie, Paris, France; [3]University of Bonn, Bonn, Germany; [4]Hopp Children`s Cancer Center Heidelberg (KiTZ), Heidelberg, Germany; [5]Hospiteaux de Marseille, Marseille, France; [6]Newcastle University Centre for Cancer, Newcastle, United Kingdom

The LR stratum (phase II) of the European SIOP PNET5 MB trial aimed to prospectively confirm the good prognosis for children <16 years old with WNT activated localized medulloblastoma, residual disease <1.5 cm2, and classic histology, when treated with reduced radiochemotherapy. 18 Gy CSI was delivered, followed by a 36 Gy boost to the tumour region. Six maintenance chemotherapy cycles were administered. Criteria for defining WNT activation were based on at least two methods (immunohistochemistry and a molecular marker); an amendment more strictly defined those criteria, including beta-catenin mutation combined to isolated monosomy 6 and/or WNT methylation profile. Both intent to treat (ITT) and per protocol (PP) analysis were performed. Between 2014 and 2022, 82 patients were included in the ITT, 68 of them in the PP analysis. Stopping rules were never reached. With 67 months median follow-up, 3-year

EFS was 88.7% [81.8-95.7] in the ITT and 91% [84.2-97.9] in the PP populations. Main toxicity data will also be presented. Decreased CSI dose to 18 Gy combined with reduced maintenance chemotherapy can be safely used in this population.

C4. Single-cell tracing identifies a stage-spanning progenitor linked to chemoresistance in Down syndrome myeloid leukemia 🏆

Rohde K.[1], Frenz-Wiessner S.[2], Wang Y.[2], Quinones-Valdez G.[2], Issa H.[1], Gonçalves-Dias J.[1], Winkler R.[1], Heckl D.[1], Mikkola H.[2], Klusmann J.-H.[1]

[1]Goethe University Frankfurt, Frankfurt am Main, Germany; [2]University of California Los Angeles, Los Angeles, United States

Children with Down syndrome (DS) have a markedly elevated risk of myeloid leukemia (ML-DS), frequently preceded by transient abnormal myelopoiesis (TAM). Despite high initial chemotherapy response rates, treatment-related morbidity is significant and relapse remains a major clinical challenge, as highlighted by the unexpected inferior outcomes observed with CPX-351 in the ML-DS 2018 trial. Using 5′ single-cell RNA sequencing and immunophenotyping on 41 longitudinal samples spanning fetal development, TAM, ML-DS and relapse, integrated with clonal mutation tracking, we identified a rare therapy-resistant GATA1s-mutant progenitor present across all disease stages. This "bridge" population lacked classical blast markers, expressed stem cell-associated transcription factors, and mapped to a fetal multipotent progenitor (MPP) state. It persisted through CPX-351 treatment and relapse. Through functional testing of signature genes, we confirmed key factors contributing to chemoresistance, with direct implications for future clinical risk stratification and targeted intervention.

C5. Prediction of Anthracycline Toxicity in Childhood Cancer: Linking Germline DNA Damage Response Variants to Drug Response 🏆

Balszuweit A.[1], Semeth A.[1], Langanke J.[2], Lohse J.[3], Bhatia S.[4], Borkhardt A.[4], Hauer J.[1], Auer F.[1]

[1]TUM University Hospital, Munich, Germany; [2]University Hospital Carl Gustav Carus Dresden, Dresden, Germany; [3]University Hospital Carl Gustav Carus Dresden, Dresden, Germany; [4]University Hospital of Düsseldorf, Düsseldorf, Germany

Treatment-related toxicity remains a major challenge in pediatric oncology. Emerging evidence, including our preliminary data from a trio-based analysis of child-parent datasets in pediatric cancer, suggests that cancer predisposition syndromes (CPS) harboring DNA-damage related germline variants influence treatment tolerance. We therefore investigated whether CPS are associated with altered sensitivity to drugs like anthracyclines. Primary fibroblasts from pediatric cancer patients were utilized as

readouts for anthracycline toxicity. N=8 samples were studied: 2 controls, 4 BRCA2 (varying mutations), 1 ATM, and 1 TP53. Cells were exposed to anthracyclines across 12 concentrations for 72h. Viability was measured with CellTiter-Glo 2.0, and IC50 values were calculated. The assay showed high reproducibility across replicates. Controls had consistent dose-response curves and IC50 values. BRCA2-mutated cells showed variable responses, while TP53 and ATM trended toward increased sensitivity. These findings suggest germline variants in DNA damage response genes may influence anthracycline sensitivity and toxicity, supporting further research toward personalized treatment strategies.

C6. Ex vivo drug response profiling reveals conserved therapeutic vulnerabilities across organ compartments in ALL 🏆

Zkrina A.[1], Papastergiou D.[1], Fortner C.[1], Debatin K.-M.[1], Meyer L.H.[1], Muench V.[1]

[1]Ulm University Medical Center, Ulm, Germany

Therapy resistance and relapse in pediatric acute lymphoblastic leukemia (ALL) are linked to central nervous system (CNS) disease, but it is unclear whether CNS-resident ALL cells exhibit intrinsic drug resistance or adapt to this niche by cellular rewiring. We performed ex vivo drug response profiling of ALL cells from bone marrow, spleen, and CNS across 9 patient-derived xenograft models using 24 drugs, observing heterogeneous, compound-dependent responses with significant differences in efficacy among them. Notably, compartment of origin did not influence drug sensitivity, and no drug-compartment interaction was detected, indicating comparable sensitivities irrespective of organ. Venetoclax, Birinapant, and Dinaciclib were among the most effective drugs, with Dinaciclib showing a trend towards enhanced activity in CNS ALL, suggesting CDK dependency in the CNS. These data highlight the interplay between ALL cells and their niche, indicating that therapy resistance in sanctuary sites is driven by reversible protection rather than stable ALL-intrinsic programs. This supports combination strategies to enhance anti-ALL activity across compartments, aiming to improve overall survival.

C7. Molecular targeting of aberrant tumor transcriptomes 🏆

Rassner M.[1], Bäuerle M.[1], Lersch R.[2], Becker M.[3], Klusmann J.-H.[1], Heckl D.[1], Mann M.[4], Schebesta A.S.[4], Müller-Reif J.[4], Braun C.J.[1]

[1]Goethe University Frankfurt, Frankfurt am Main, Germany; [2]Ludwig-Maximilians University, Munich, Germany; [3]Helmholtz Zentrum München, Munich, Germany; [4]Max Planck Institute of Biochemistry, Martinsried, Germany

Dysregulated transcription is a hallmark of solid tumours in children, where actionable mutations are rare. It remains unclear whether aberrant transcription can be exploited for gene- and context-specific perturbations to selectively target genes in malignant cells, and which determinants shape such specificity. To address this, we inhibited transcriptional cyclin-dependent kinases CDK7, CDK9, and CDK12/13, key transcription regulators and promising therapeutic targets. Analysis of nascent transcription (4sU-Seq) enabled genome-wide quantification of immediate drug response. Distinct transcription defects reflect their roles in initiation, pause release, and elongation. Machine-learning analyses identified key features of sensitivity, including context-dependent factors. Primary determinants are modulated by secondary features to partially compensate compromised transcription. Integration of RNA sequencing and proteomics shows how gene-specific tCDK dependency propagates to RNA and protein expression.

11:00 – 12:30 ***D. Tumour Microenvironment*** *Davide Serrugia*

D1. Single-Cell Profiling Unveils Multilineage Fusion Persistence and Microenvironmental Priming in pAML Relapse 🏆

Schweighart E.K.[1], Grasso C.[1], Zwaan C.M.[2], Stunnenberg H.[1], Heidenreich O.T.[3], Goemans B.F.[1]

[1]Prinses Maxima Centrum, Utrecht, Netherlands; [2]Erasmus MC/Sophia Children's Hospital, Rotterdam, Netherlands; [3]University Medical Center Utrecht, Utrecht, Netherlands

Despite intensive chemotherapy, one-third of pediatric acute myeloid leukemia (pAML) patients relapse, warranting a deeper understanding of relapse predictors. While single-cell RNA sequencing (scRNA-seq) offers high-resolution insights into the leukemic microenvironment, longitudinal data tracing clonal persistence remains scarce. We performed scRNA-seq on KMT2A-rearranged pAML patients at diagnosis (Dx), during therapy (pre-cycle 2 and pre-last cycle), and at relapse (Re). By combining short-read and long-read sequencing, we identified KMT2A fusion+ cells and benchmarked them using a healthy pediatric reference. Fusion transcripts were detected in Dx and Re blasts but also within T and B lineages and during active therapy, indicating multilineage involvement and cryptic clonal persistence. Notably, monocytes in the Re cohort exhibited unique transcriptional pathways during therapy. However, patients in long-term remission revealed transcriptional profiles resembling the healthy reference. This suggests that the fusion occurred early in hematopoiesis and that early microenvironmental remodeling is a driver of relapse, offering potential to improve risk stratification in pAML.

D2. The landscape of germline variants in children with chronic myeloid leukemia 🏆

Ghete T.[1], Gaschler L.[1], Krumbholz M.[1], Behrens Y.L.[2], Wölfl M.[3], Auer F.[4], Carta M.G.[1], Hutter S.[5], Sticht H.[6], Metzler M.[1]

[1]University Hospital Erlangen, Erlangen, Germany; [2]Hannover Medical School, Hannover, Germany; [3]University Hospital Würzburg, Würzburg, Germany; [4]Technical University of Munich, Munich, Germany; [5]MLL Munich Leukemia Laboratory, Munich, Germany; [6]Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany

Chronic myeloid leukemia (CML), characterized by the BCR::ABL1 oncogene, typically occurs in older adults, while pediatric CML is rare and less studied for genetic predisposition than other hematological malignancies. While BCR::ABL1 is the mandatory oncogene in CML, additional genetic events are necessary for disease onset. We performed whole-exome and targeted sequencing on 145 pediatric CML patients and 60 adult patients. We applied in silico prediction tools to quantify the effect of the variant on the protein structure and function. Germline variants were more frequently observed in pediatric patients than in adults, mostly in genes involved in hematopoiesis, including ASXL1, NOTCH1, TET2. Median age at diagnosis was lower in patients with germline variants (10 vs. 13 years). Within a subsequently studied pediatric CML cohort, one individual with clinical features of Noonan syndrome and pathogenic germline SOS1 mutation presented with blast phase CML at the exceptionally early age of 1.4 years. Further analysis revealed 10 individuals with germline variants linked to the RAS pathway. None of these variants is represented in population databases as single-nucleotide polymorphismus.

D3. Asciminib supports skeletal safety in CML: reduced osteoclast demineralization without disrupting differentiation 🏆

Fuhrmann T.W.[1], Maccataio A.[2], Krumbholz M.[2], Steffen U.[2], Metzler M.[2], Sembill S.[2]

[1]Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany; [2]University Hospital Erlangen, Erlangen, Germany

Tyrosine kinase inhibitors (TKIs) used in chronic myeloid leukemia (CML) impair longitudinal growth in children, partly due to osteoclast dysfunction. ATP-competitive TKIs lack kinase selectivity, causing off-target skeletal effects. The allosteric ABL1 inhibitor asciminib shows higher selectivity and may offer advantages in children. We compared the effects of asciminib with ATP-competitive TKIs using a human ex-vivo osteoclastogenesis model. Viability, differentiation, demineralization, and actin organization were analyzed, alongside molecular profiling via RT-qPCR and RNA sequencing. ATP-competitive TKIs reduced viability and differentiation dose-

dependently. In contrast, asciminib preserved both parameters at concentrations approaching the therapeutic range while significantly reducing demineralization. Transcriptome data indicated downregulation of genes linked to cytoskeletal organization and translational machinery, suggesting selective inhibition of resorptive function. These findings indicate that asciminib exerts a less disruptive effect on osteoclasts, supporting its potential for improved skeletal safety under long-term TKI therapy, particularly in growing children.

D4. Pediatric AML blasts induce immunosuppressive reprogramming in macrophages via nanotube mitochondrial transfer 🏆

Perzolli A.[1], Massaoudi F.[1], van Oort A.T.[1], Koedijk J.B.[1], Schweighart E.K.[1], Kester L.A.[1], Adriaanse F.[1], Vormoor H.J.[1], Zwaan C.M.[1], Heidenreich O.[1]

[1]Prinses Maxima Centrum, Utrecht, The Netherlands

Although immunotherapy has revolutionized cancer treatment, its benefits in pediatric acute myeloid leukemia (pAML) remain limited, partly due to an incomplete understanding of the AML microenvironment. While most studies focus on T cells, innate immune populations such as macrophages are less well explored. In pAML patients, we demonstrate that immunosuppressive macrophages dominate the AML microenvironment and support growth of RUNX1::RUNX1T1 and KMT2A-rearranged leukemic blasts. In co-culture experiments, AML cells reprogrammed naïve and pro-inflammatory macrophages toward an anti-inflammatory state, characterized by suppression of NF-κB and other inflammatory pathways. This phenotypic switch requires direct cell contact and involves tunneling nanotube–mediated transfer of AML mitochondria to macrophages in vitro and in vivo, inducing metabolic rewiring toward oxidative phosphorylation. Functionally, these reprogrammed macrophages impaired T cell– and bispecific antibody–mediated killing, facilitating immune escape. Our findings reveal a novel mechanism of immune suppression and suggest targeting AML-macrophage interactions could enhance efficacy of T cell–based immunotherapies.

D5. The Role of Tumor Associated-Macrophages in Medulloblastoma? – Histopathological Insights 🏆

Cases Wroblewski N.[1], Rutkowski S.[2], Dührsen L.[2], Schüller U.[1], Mader M.[1]

[1]Research Institute Children's Cancer Center Hamburg, Hamburg, Germany; [2] University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Medulloblastoma (MB) is the most common malignant pediatric brain tumor, whose progression is shaped by the tumor microenvironment (TME). Tumor-associated macrophages (TAM), the largest non-tumoral cell population in the MB TME, represent a

potential therapeutic target. To investigate the properties of TAM in MB, we quantified TAM morphology and quantity via IBA-1 staining of primary tumor tissue on formalin fixed paraffin embedded slides. We analyzed a total of 192 samples, representing all molecular subtypes (G3/4 (42.1%), SHH (24.5%), WNT (7.3%), NA (26.1%)). On average, TAM represent 6.7% of cells in the tumor, with a higher count in SHH MB. We found that both TAM count and morphology correlate with clinical outcome. Specific morphological characteristics are linked to improved outcomes across all subtypes, with larger TAM size emerging as a key positive prognostic factor ($p = 0.035$). Taken together, our histological findings and future transcriptomic investigations will advance our understanding of TAM heterogeneity and their potential role in MB.

D6. Blast-Driven Immunosuppression Defines the Immune Landscape in Infant KMT2A::MLLT3 Driven Acute Myeloid Leukaemia 🏆

Mullen C.[1], Medhi R.[1], Leah T.[1], Schwaller J.[2], Ottersbach K.[1]

[1]University of Edinburgh, Edinburgh, United Kingdom; [2]University of Basel, Basel, Switzerland

Infant acute myeloid leukaemia (AML) is an aggressive paediatric malignancy commonly driven by KMT2A rearrangements that can arise in utero. Infants exhibit high relapse rates and severe therapy-related toxicity, underscoring the need for novel immunotherapeutic approaches. Using a doxycycline-inducible KMT2A::MLLT3 mouse model, we induced KMT2A::MLLT3 expression from embryonic day 12.5, which generated AML with extramedullary disease. Single cell RNA-sequencing of the bone marrow microenvironment identified diverse immune populations, including NK/T cells, monocytes and dendritic cells (DCs) – with expansion of early-activated and exhausted CD8+ cells together with an increase in DC2, consistent with immune remodelling. CellChat analysis revealed blast-driven immunosuppression, dominated by Lgals9 (Galectin 9)–Cd44/Cd45 signalling to T cells and monocytes, and Mif–Cd74/Cd44 signalling to DCs. Spectral flow T-cell profiling and blast co-culture assays are validating these immune population changes within this suppressive niche. Unravelling and ultimately targeting these pathways could restore anti-leukaemic immunity and enhance immunotherapy for infant KMT2A-driven AML.

D7. Investigating the microenvironment in the hematopoietic niche 🏆

Groll D.[1], Bhayadia R.[1], Wei X.[1], Heckl D.[1], Klusmann J.-H.[1]

[1]Goethe University Frankfurt, Frankfurt am Main, Germany

The bone marrow microenvironment plays a crucial role in the pathogenesis and chemoresistance of pediatric Acute Myeloid Leukemia (AML). However, understanding the niche-mediated protection of leukemic blasts remains challenging due to limited

means to study AML:niche interaction directly. In this project, we implement an in vivo screening platform using mRNA lipid nanoparticles (LNPs) to manipulate AML:niche interactions in its native environment. To ensure clinical and biological relevance, the screening is conducted using patient-derived xenograft (PDX) models of various molecular subtypes of pediatric AML coupled to single-cell CRISPR screenings with RNA sequencing (scRNA-seq) and pre-tested gRNAs. We established the LNP application in primary AML samples by extensive testing and optimization LNP composition and mRNA expression. By comparing in vitro, ex vivo and in vivo CRISPR perturbation of niche interaction factors our approach will allow us to determine effectiveness of different screening settings and will uncover novel therapeutic targets and validate mRNA-based delivery systems as a precision medicine tool for treating high-risk pediatric leukemia.

14:00 – 14:30 ***E. Invited Lecture*** *Martin Stanulla*

E2. Onset and evolution of embryonal tumors

Frank Westermann

German Cancer Research Center (DKFZ), Germany

14:30 – 15:30 ***F. Malignant Cell Biology 1*** *Hannah Uckelmann*

F1. Clinical, histological and molecular characterization of MYCN-like spinal ependymoma 🏆

Schürmann J.A.[1], Hack K.[1], Fürst A.[1], Gocke A.[2], Arora J.[1], Glatzel M.[1], Sievers P.[4], Neumann J.E.[1], Ghasemi D.R.[1], Schüller U.[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Hamburg, Germany; [3]Clinical Cooperation Unit Neuropathology, German Cancer Research Center (DKFZ), Heidelberg, Germany

Ependymal tumors of the spinal cord encompass subependymomas, myxopapillary ependymomas, MYCN-amplified spinal ependymomas, and spinal ependymomas, with the latter typically showing a chromosome 22 loss. Each of these tumor types is characterized by a distinct global DNA methylation pattern, which is frequently used for diagnostic purposes. By analyzing several hundreds of spinal ependymal tumors, we identified 37 cases of ependymoma characterized by high cellularity and unexpected OLIG2 expression. Global DNA methylation analysis revealed a similar pattern for these cases that was distinct from known other CNS tumors. Recurrent chromosomal aberrations included chromothripsis at chromosome 6. Single-nucleus RNA sequencing revealed PMP2 as a potential diagnostic marker, which was validated by

immunohistochemical staining. In contrast to other spinal ependymal tumors, comparison with cell populations from the developing spinal cord suggested astrocytes as potential cellular origin. Clinically, this novel tumor type occurred across all age groups, presented with a female to male ratio of 2.6:1, and demonstrated a median progression-free survival of 12 years.

F2. Beyond TP53 mutation: transcriptional regulation of the p53 pathway in pediatric AML 🏆

Cifarelli L.N.[1], Gonçalves-Dias J.[1], Salzmann-Manrique E.[1], Schuschel K.[1], Klusmann J.-H.[1], Heckl D.[1]

[1]Goethe University Frankfurt, Frankfurt am Main, Germany

TP53 is a central tumor suppressor, yet TP53 mutations are rare in pediatric acute myeloid leukemia (AML), suggesting that p53 dysfunction may arise through non-mutational mechanisms. In this study, we investigated the clinical relevance of TP53 expression and TP53-related regulatory programs in pediatric AML using transcriptomic and survival data from independent patient cohorts. Kaplan-Meier analyses showed that TP53 mRNA expression alone was not associated with outcome in pediatric AML, whereas a distinct pattern was observed in adult AML, highlighting important context-dependent differences in TP53 pathway regulation. To move beyond single-gene analyses, we examined TP53-related gene sets and identified pathway signatures associated with adverse prognosis in pediatric AML. These findings support the hypothesis of p53-network dysfunction in pediatric AML through altered tumorsuppressor networks rather than TP53 mutation or abundance alone. Overall, this work provides a framework to better understand TP53 pathway regulation in pediatric AML and to identify candidate mechanisms underlying disease progression.

F3. Integrative multiomics profiling of an anaplastic juvenile granulosa cell tumour: a case study of an ultra-rare entity 🏆

Bidasiuk A.[1], Hack K.[1], Schüller U.[1], Bockmayr M.[1], Winkler B.[1], Ghasemi D.R.[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Juvenile granulosa cell tumour (JGCT) is a rare ovarian tumour that arises in all age groups. Its anaplastic subtype (aJGCT) represents an ultra-rare, highly aggressive variant characterized by diffuse growth and a high risk of recurrence. Whereas JGCT often follows a favourable course with surgery alone, aJGCT harbours an overall poor prognosis despite intensive adjuvant chemotherapy. To date, less than 30 cases of aJGCT have been reported in the literature. We applied a multi-omics approach to dissect the molecular underpinnings of an aJGCT from a 14-year-old girl who eventually

succumbed to her disease despite intensive treatment. Histopathological analysis confirmed the diagnosis of aJGCT. Whole genome sequencing of the relapse tumor revealed a complex genotype with multiple oncogenic aberrations. Paired single-nucleus RNA sequencing of the primary ovarian tumor and a lymph node relapse showed heterogeneous transcriptomic phenotypes, and the analysis of chromosomal aberrations on the single-nucleus level pointed towards the occurrence of complex evolutionary trajectories and further selection at relapse.

F4. CAP1 deficiency impairs development of cerebellar granule neurons and prolongs survival of SHH medulloblastoma in mice 🏆

Klein A.[1], Metz I.[2], Khudayberdiev S.[2], Burghaus M.[1], Altendorf L.[1], Mahnke B.[1], Schoof M.[1], Rust M.B.[2], Schüller U.[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]University of Marburg, Marburg, Germany

Medulloblastoma (MB) is a heterogeneous embryonal tumor, which is located in the posterior fossa and represents the most common malignant pediatric brain tumor. It comprises four molecular subgroups: WNT, SHH, Group 3, and Group 4. Sonic-hedgehog (SHH)-MB accounts for ~30% of cases and arises from aberrant SHH pathway activation, driving uncontrolled granule cell precursor (GCP) proliferation. Despite a broad understanding of its cellular origin and molecular biology, targeted therapies are limited, and long-term outcomes remain to be improved.
To address this clinical need, we investigated the role of cyclase-associated protein 1 (CAP1), a regulator of actin dynamics critical for GCP growth and differentiation. CAP1 knockout in mice causes cerebellar hypoplasia and reduced GCP proliferation, suggesting a role of CAP1 signaling in SHH-MB development. Introducing a CAP1 loss-of-function into a SHH-MB mouse model, we found reduced tumor burden at early stages and prolonged survival. These results indicate that CAP1 is important for MB growth. We next aim to analyze downstream signaling pathways of CAP1 in human SHH-MB cell lines to identify potential novel therapeutic targets.

F5. Identification of novel candidates for targeted therapy in leukemia 🏆

Zhong T.[1], Zhang Z. [1], Klusmann J.-H. [1], Vermunt, M. W.[1]

[1]Goethe University Frankfurt, Frankfurt am Main, Germany

Targeted cancer therapies hold great potential as an alternative or addition to chemotherapy. Therefore, target identification for specific tumors is important to increase the number of patients that can benefit, to have additional options when resistance to other therapies occurs and/or to hit specific cells within heterogeneous tumors. Here, we

aim to identify novel targets for leukemia by performing comprehensive transcriptome analysis in cancer cell lines, healthy cells, and leukemia patient cohorts. We focus on transcripts that show selective expression in subsets of leukemias (e.g. B-ALL versus AML) or patient subgroups (e.g. Runx1-Runx1T1 driven AML). CRISPR screens will be used to study essentiality of candidate transcripts and RIBO-seq will reveal their translation potential. Our study enables novel (immuno)therapeutic target identification for leukemia by prioritizing expression specificity, druggability and cancer dependency features

16:00 – 17:30 ***G. Molecular Mechanisms of Disease 1*** *Roland Kappler*

G1. The missing link for growing leukemia patient cells ex vivo 🏆

Dietz A.[1], Tejada de Mesa A.[1], Iriondo de Diego A.[1], Larghero P.[1], Marschalek R.[1]

[1]Goethe-University, Frankfurt/Main, Germany

A puzzling situation is our inability to grow cells from KMT2A-r leukemia patients in cell culture, a well-known phenomenon experienced by many. Here, we demonstrate that we can grow normal B-cells on a feeder layer of transfected HS-5 cells (expressing CD40L, IL-4 and IL-21), however, these feeder cells do not allow the expansion of PDX cells. Therefore, we established 2 novel feeder cell lines from the HEPG2 cell line, of which the secretome mimicks very much the fetal liver situation, and introduced two stably expressed, transgenic cassettes. The first polygenic gene cassette is expressing SCF, FLT3 ligand and THPO/TPO and secrete all three cytokines in equal amounts. The second gene cassette expresses two proteins (not named because of patent issues) and secrete them as a single, soluble factor “X”. Combination of both feeder cell lines was able to allow PDX cells of KMT2A-derived leukemia patient cells to proliferate in IMDM media, as well as to replace the use of StemSpan medium for KMT2A-crispered HSPCs. This novel invention could reduce expensive NSG mouse experiments of leukemia patient cells.

G2. An Optimized Cas13d Scaffold for Multi-Target RNA Knockdown Enables Combinatorial Screening in FLT3-Mutant AML 🏆

Haas J.[1], Özcan E.[1], Cifarelli L.N.[1], Schuschel K.[1], Braun C.[1], Klusmann J.-H.[1], Heckl D.[1]

[1]Goethe University Frankfurt, Frankfurt am Main, Germany

The RNA-guided Cas13d RNase is a promising tool to study genetic interaction networks, but multiplexed applications are limited by guide position effects and repeat-driven retroviral recombination. To address this, we systematically mutated and refined

the Cas13d guide RNA scaffold (direct repeat, DR) to improve multiplex RNA knockdown. Using a dual-guide architecture in pooled dropout assays, we placed established co-lethal pairs in the two guide positions to benchmark performance and identify improved DR variants. The redesigned DRs increase the efficiency and consistency of multi-guide activity from single constructs and strengthen pooled combinatorial screening through robust multi-target perturbations. We applied the optimized platform in FLT3-ITD AML to run multiplexed knockdown screens with pharmacologic inhibition and map drug-conditioned genetic interactions. This provides a systematic way to compare network responses across different FLT3 inhibitors. Overall, this application highlights the broader utility of DR-optimized Cas13d multiplexing as a framework for combinatorial RNA knockdown and discovery of genetic interaction patterns across diverse biological context.

G3. Metabolomic profiling identifies targetable metabolic dependencies in pediatric acute myeloid leukemia 🏆

Koch D.[1], Achyutuni S.[2], Bhayadia R.[1], Heckl D.[1], Xu J.[2], Klusmann J.-H.[1]

[1]Goethe University Frankfurt, Frankfurt am Main, Germany; [2]St. Jude Children's Research Hospital, Memphis, USA

Acute myeloid leukemia (AML) is an aggressive malignancy in early childhood with poor outcomes despite intensive therapy. However, the metabolic dependencies underlying pediatric AML heterogeneity remain poorly defined. Here, we show that pediatric AML subtypes exhibit distinct and transcriptionally coordinated metabolic programs. Liquid chromatography–mass spectrometry–based metabolomics of primary patient and patient-derived xenograft samples identified subtype-specific metabolic signatures. Multivariate analyses revealed clear separation of genetic subgroups, with prominent alterations in nucleotide and amino acid metabolism pathways. Integration with transcriptomic data demonstrated concordant changes in gene expression and metabolite abundance, indicating coordinated regulatory networks driving metabolic rewiring. These subtype-specific metabolic states highlight actionable metabolic vulnerabilities. Together, our findings provide a systematic framework linking genetic lesions to metabolic dependencies in pediatric AML and support the development of metabolism-directed therapeutic strategies.

G4. From Fusion to Function: Investigating the Mechanisms of ZNF384 Fusion Oncoproteins in B-ALL 🏆

Korczmar E.[1], Karbon G.[1], Müller S.[1], Seruggia D.[1]

[1]St. Anna Children's Cancer Research Institute (CCRI), Vienna, Austria

Mixed phenotype acute leukemia (MPAL) is a rare leukemia characterized by lineage ambiguity and poor prognosis. ZNF384 rearrangements are recurrently identified in B/myeloid MPAL, and involve >19 fusion partners, including chromatin regulators such as EP300 and CREBBP. Lack of robust experimental models for most ZNF384 fusion oncoproteins makes it difficult to understand the disease and to identify potential therapeutic targets. Here, we aim to model ZNF384-rearranged leukemia and dissect disease mechanisms. By overexpression of fusion oncoproteins in mouse fetal liver–derived HSPCs we will define their transcriptional programs, binding sites, and protein interactions. Using CRISPR/Cas9-mediated introduction of ZNF384 translocations in human HSCPs we will generate human models. We generated ZNF384 fusion constructs tagged with GFP-FKBP12^F36V and co-expressed with NRAS^G12D, and observed fusion-dependent, IL-3 independent proliferation in Ba/F3 cells. CFU assay in transduced mouse fetal liver HSPCs show enhanced myeloid progenitor self-renewal and proliferation. Transduction/transplantation experiments to develop an array of ZNF384-r leukemia models are ongoing.

G5. Wildtype NUP98 Cooperates with Mutant NPM1 to Drive Oncogenic Transcription 🏆

Barros da Gama S.[1], Müller M.[1], González-Dammers H.[1], Gonçalves-Dias J.[1], Haarer E.[2], Klusmann J.-H.[1], Falini B.[3], Brunetti L.[4], Papathanasiou S.[5], Uckelmann H.J.[1]

[1]Goethe-University Frankfurt, Frankfurt am Main, Germany; [2]Duke University School of Medicine, Durham, USA; [3]University of Perugia and Santa Maria della Misericordia Hospital, Perugia, Italy; [4]Università Politecnica delle Marche, Ancona, Italy; [5]Institute of Molecular Biology, Mainz, Germany

NPM1 mutations occur in ~30% of acute myeloid leukemia (AML) cases. Our prior work revealed an unexpected nuclear function of mutant NPM1 (NPM1c), demonstrating direct chromatin occupancy at self-renewal loci. To understand how NPM1c activates its chromatin targets, we performed NPM1c-proximity labeling studies and uncovered NUP98 as one of our top hits. Our chromatin studies revealed that NPM1c recruits NUP98 to oncogenic loci. Strikingly, NUP98 knockout specifically represses the NPM1c-driven transcriptional program while having minimal impact on NPM1wt cells, demonstrating a subtype-specific dependency. Using endogenous NUP98 degrader cell lines, we can show that NUP98 degradation induces differentiation and apoptosis specifically in NPM1c cells. Rescue experiments demonstrate that the N-terminal FG-repeat domain of NUP98 is sufficient to sustain the NPM1c oncogenic program. Overall, our study identifies NUP98wt as a critical cofactor for NPM1c-driven oncogenic transcription, functionally substituting for an oncogenic fusion partner to concentrate transcriptional condensates at oncogenic loci.

G6. Investigating somatic events and epigenetic signatures in GATA2 deficiency to predict and prevent BMF and leukemia 🏆

Alcaide Miranda M.[1], Schönung M.[2], Torralba Sales E.[3], Lipka D.[2], Giorgetti A.[3], Fernandez-Orth J.[1], Erlacher1 M.[1]

[1]Universitätsklinikum Ulm, Ulm, Germany; [2]National Center for Tumor Diseases (NCT), Heidelberg, Germany; [3]Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Hospitalet de Llobregat, Spain

Germline GATA2 mutations lead to variable immunodeficiency and confer an increased risk of developing myelodysplastic syndrome (MDS) and leukemia. This malignant transformation is thought to arise through the accumulation of somatic events, with allogeneic hematopoietic stem cell transplantation currently representing the only curative option. Recent evidence suggests that both genetic and epigenetic factors contribute to disease progression. To better understand the nature of these somatic events, we used a conditional Gata2 haploinsufficient mouse model. LSK cells were transplanted into lethally irradiated recipients, followed by transcriptomic and epigenetic analyses at different time points. This approach uncovered early molecular divergence, including compensatory Gata2 upregulation and aging-associated epigenetic signatures in Gata2-transplanted mice. In addition, we established a HoxB8-based progenitor system to functionally assess how these alterations contribute to malignant transformation in the context of GATA2 haploinsufficiency in vitro. Together, these findings provide insight into early molecular events driving malignant progression in GATA2 deficiency.

G7. Investigating the chromatin functions of Exportin-1 (XPO1) in NPM1c-driven acute myeloid leukemia 🏆

González-Dammers H.[1], Barros da Gama S.[1], Gonçalves-Dias J.[1], Klusmann J.-H.[1], Uckelmann H.J.[1]

[1]Goethe University Frankfurt, Frankfurt am Main, Germany

Nucleophosmin 1 (NPM1) is a multifunctional nucleolar protein that shuttles between the nucleus and cytoplasm. Mutations in NPM1 are the most prevalent genetic alteration in AML, present in roughly one-third of cases. These mutations lead to the loss of its nucleolar localization sequence and the addition of a nuclear export sequence on its C-terminus, driving the protein's mislocalization to the cytoplasm (referred as NPM1c). While NPM1c's oncogenic role has classically been attributed to its cytoplasmic mislocalization, our recent findings reveal an unexpected nuclear function: NPM1c binds chromatin at key self-renewal loci, including HOXA/B and MEIS1, where it directly drives oncogenic transcription. This raises the central question of how NPM1c is recruited to chromatin. Beyond its canonical export function, Exportin-1 (XPO1) associates with chromatin and facilitates the targeting of NPM1c and other oncoproteins to specific gene

loci. To dissect these non-canonical functions, we established an inducible XPO1 degrader system for precise, acute depletion of XPO1. This work aims to unravel the chromatin functions of XPO1 in NPM1c AML and identify new therapeutic opportunities.

18:00 – 18:30 ***H. Invited Lecture*** *Olaf Heidenreich*

H1. Heparan sulfate glycotypes are determinants of stem cell heterogeneity and function

Ulrich Steidl

Albert Einstein College of Medicine in New York, USA

Friday, June 5

09:00 – 10:30 ***I. Immunotherapy*** *Katrin Ottersbach*

I1. Novel Cytokine Protocols Enhance CIK Cell Efficacy and Polyfunctionality in High-Risk Pediatric Malignancies 🏆

Lübbers A.[1], Lürbke Franke M.[2], Klusmann J.-H.[2], Rettinger E.[2], Heckl D.[1]

[1]Goethe-University, Frankfurt, Germany

High-risk pediatric cancers, including relapsed acute leukemias and solid tumors, remain a significant clinical challenge due to limited therapeutic options and poor post-relapse outcomes. While interleukin-15 activated cytokine-induced (CIK) cells have demonstrated a favorable safety profile and cytotoxicity in pediatric AML, their therapeutic efficacy remains limited in ALL and solid tumors. Building on clinical experience with IL-15-activated CIK cells in pediatric AML, we have established novel cytokine-modified CIK cell expansion protocols incorporating different interleukins that significantly increase and prolong CIK cytotoxicity in vitro without impairing expansion, thereby enabling high-dose manufacturing. These cytokines also reshape CIK composition, promoting the expansion of unique immune populations such as biphenotypic T-NK cells and $CD8^+CD4^+$ subsets. These subsets exert NK-like self-recognition, which significantly contributes to the enhanced safety profile of CIK cells compared to other immunotherapies. Our findings lay the basis for the clinical translation of cytokine-modified CIK protocols to improve outcomes for children with resistant malignancies.

I2. Development of a New Immunotherapy Treatment Based on Gene-Modified Adaptive NK Cells for the Refractory Lymphomas 🏆

Sanvi L.W.[1], Giordano C.[2], Haas D.[1], Vespa S.[1], Seyed Nazarzadeh S.M.A.[1], Mac G.D.[1], Carlomagno S.[3], Della Chiesa M.[2], Sivori S.[2], Caruana I.[1]

[1]University Hospital Würzburg, Würzburg, Germany; [2]University of Genoa, Genoa, Italy; [3]University of Udine, Udine, Italy

Adaptive human natural killer (NK) cells are a NK cell subset that displays memory-like properties. Compared to conventional NK cells, adaptive NK (aNK) cells express high level of the CD94/NKG2C activating receptor and a mature phenotype. In this study, we developed a GMP-grade protocol to selectively expand aNK cells using a CD94-specific monoclonal antibody that blocks the inhibitory molecule NKG2A while promotes the activating by NKG2C. These cells were engineered to express a fourth generation anti-CD30 CAR secreting a CD94 single fragment variable region(scFv) and IL-15. We optimized the transduction procedure achieving an efficiency of 82.1±10.8% and validate CD94-scFv and IL-15 production. Transduced aNK cells maintained the adaptive phenotype, with 77.26±5.4% NKG2A−/NKG2C+ cells. CAR aNK were tested in long-term co-culture versus lymphoma cell lines obtaining an improved cytotoxic activity compared to CAR-NK cells with almost a complete tumor elimination ($p<0,001$). This strategy is a promising approach for a robust, scalable, off-the-shelf CAR-therapy with strong translational potential in $CD30^+$ malignancies and can be applied early after stem cell transplantation.

I3. HER2-directed immunotherapy shows preclinical activity in acute lymphoblastic leukemia 🏆

Heymann J.[1], Ehm P.[1], Gehlert C.L.[2], Beder T.[2], Baldus C.[2], Bastian L.[2], Brüggemann M.[2], Cario G.[1], Peipp M.[2], Lenk L.[1]

[1]Christian-Albrechts University Kiel and University Medical Center Schleswig-Holstein, Kiel, Germany; [2]University Hospital Schleswig-Holstein, Kiel, Germany

Despite recent advances, outcome of patients with relapsed or refractory (r/r) acute lymphoblastic leukemia (ALL) remains poor, underscoring the need for novel therapeutic strategies, particularly in T-ALL. HER2-directed immunotherapy (HER2-IT) is an established approach in solid cancers, yet despite reported HER2-positivity (+) in ~30% of B-ALL cases, HER2-IT has not been systematically evaluated. RNA-sequencing analyses identified HER2 expression across distinct ALL subgroups, particularly in BCR::ABL1+ and PAX5-altered B-ALL as well as some high-risk T-ALL subgroups. Flow cytometry confirmed HER2 surface expression across a comprehensive set of B-ALL and T-ALL patient-derived xenograft (PDX) samples. Importantly, HER2 was internalized into HER2+ ALL cells upon exposure to HER2-directed antibodies. Accordingly, the HER2-directed antibody-drug-conjugates trastuzumab-deruxtecan and trastuzumab-

emtansine showed anti-leukemic activity in HER2-positive ALL-PDX cells. Also, HER2-IT efficacy was abrogated upon prior receptor blockade confirming HER2-specific effects. Our data suggest HER2-IT as a therapeutic option in ALL warranting further preclinical validation in vivo.

I4. Development of novel CAR Cell Therapeutics in Pediatric AML 🏆

Schuster S.[1], Gonçalves-Dias J.[1], Cifarelli L.N.[1], Schuschel K.[1], Kovic M.[1], Kaffenberger C.[1], Mair L.[1], Klusmann J.-H.[1], Heckl D.[1]

[1]Goethe-University Frankfurt, Frankfurt am Main, Germany

Immunotherapy has fundamentally changed the treatment of hematological disease in recent years with chimeric antigen receptor (CAR) T cells directed against CD19 as the most prominent example. However, translating this success to acute myeloid leukemia (AML) remains challenging due to lack of specific targets in AML. This project uses a bulk- RNA-Seq- based pediatric AML surfacesome to identify potential targets for CAR therapy coupled with artificial intelligence based binder design and high throughput selection systems for active CAR constructs. First, targets were validated in regard to several aspects to define safe, promising and subgroup-specific candidates. Second, a pipeline for the screening of target specific extracellular domains has being established. Therefore, a library-based approach was chosen to test large candidate binder numbers with variable hinge design at the same time using dual-assay in vitro strategy. Our study will thereby provide new therapeutic venues for pediatric AML consolidation and 2nd line therapy to improve patient outcome in the future.

I5. Enhancing CD127-targeted immunotherapy in ABL-class fusion+ B-ALL through combination with next-generation TKIs 🏆

Kurschies M.[1], Pinkle D.[1], Cornils M.[1], Heymann J.[1], Funke E.[1], Mueller X.[1], Peipp M.[1], Cario G.[1], Schewe D.M.[2], Lenk L.[1]

[1]Christian-Albrechts University Kiel and University Medical Center Schleswig-Holstein, Kiel, Germany; [2]University Hospital Dresden, Dresden, Germany

ABL-class fusion-positive (ABL-fusion+) B-cell precursor acute lymphoblastic leukemia (B-ALL) is treated with chemotherapy and ABL-directed tyrosine kinase inhibitors (TKI) such as imatinib (Ima), a combination associated with significant toxicity. We recently showed potent preclinical in vitro and in vivo efficacy of CD127-directed immunotherapy (CD127-IT) in ABL-fusion+ B-ALL, alone or in combination with Ima, mediated by antibody-dependent cellular phagocytosis (ADCP) and IL7R pathway blockade. We hypothesized that this approach can be enhanced by substituting Ima with next-generation TKIs such as Dasatinib (Dasa). Sublethal Dasa induced comparable

increases in CD127 expression, ADCP, and IL7R pathway blockade as Ima. In vivo, Dasa/CD127-IT outperformed Ima/CD127-IT and significantly prolonged survival in 8/8 (100%) patient-derived xenograft (PDX) models, with 5/8 (62.5%) long-term survivors. Our findings support CD127-IT combined with Dasa as a promising therapeutic strategy in ABL-fusion+ B-ALL warranting clinical investigation. As we observe comparable in vitro effects across multiple ABL-targeting TKIs, ongoing work aims to define the most effective TKI/CD127-IT combination.

I6. Dual CAR-TCR and CD8 Engineering Improves Adoptive T-Cell Therapy Beyond MHC I Limitations 🏆

Haas D.[1], Theinert T.[1], Sanvi L.W.[1], Bilotta M.T.[2], Vacca P.[2], Funari A.[3], Eyrich M.[1], Schlegel P.G.[1], Wölfl M.[1], Caruana I.[1]

[1]University Children's Hospital Würzburg, Würzburg, Germany; [2]Bambino Gesù Children's Hospital, Rome, Italy; [3]Sapienza University of Rome, Rome, Italy

In order to overcome antigen escape, tumor heterogeneity and limited persistence seen in current CAR T and TCR therapies, we developed a dual CAR-TCR-T cell platform. To extend TCR mediated recognition to CD4 T cells, the construct includes a CD8 co receptor. Third generation CD19- or B7 H3-directed CARs were combined with an optimized PRAME specific TCR. Functional assays assessed cytokine release, metabolic fitness and tumor control in short and long term co cultures, accompanied by transcriptomic profiling. In vivo efficacy was tested in heterogeneous AML xenografts and boosted through PRAME based vaccination. We found that our strategy generated a high proportion of functional, dual positive T cells with superior metabolic resilience under chronic stimulation and a balanced CAR/TCR transcriptional signature. Importantly, CD8 co expression enabled CD4 T cells to target MHC I restricted targets. In vivo, dual CAR TCR T cells achieved enhanced tumor control and could be selectively boosted via vaccination. This platform integrates CAR and TCR advantages, recruits both CD4 and CD8 compartments, and offers a promising strategy for treating heterogeneous and relapsed malignancies.

I7. Daratumumab Efficacy Is Affected by Macrophage Fratricide and Polarization in T-ALL 🏆

Balzer S.[1], Logvinova E.[1], Kikvadze S.[1], Rahimian E.[1], Kufrin V.[1], Pioch A.[1], Brilloff S.[1], Wurm A.A.[1], Schewe D.M.[1]

[1]University Hospital and NCT/UCC Dresden, Dresden, Germany

CAR T-cell approaches for T-ALL are challenging, as malignant and healthy T cells share target antigens, potentially leading to fratricide. This makes antibody-based

therapies a promising alternative. CD38-targeting antibodies such as daratumumab (Dara) show clinical activity in T-ALL, but frequent relapse highlights the need for improvement. Macrophages (MFs) can mediate Dara response via antibody-dependent cellular phagocytosis (ADCP). However, MFs also express CD38 and can be polarized by extrinsic factors or cell–cell interactions, suggesting that their state may influence anti-leukemic activity and therapy response. We show that MFs attack each other under Dara treatment in vitro, revealing a previously unrecognized self-targeting effect. In addition, MF polarization shapes anti-leukemic activity, as Fc receptor expression and ADCP capacity differ across M0, M1, and M2 states. In contrast, CD38 remains unaffected during MF polarization. These findings identify MF states, including expression of the Dara target CD38, as crucial determinants of Dara-mediated ADCP and suggest that modulating MF properties may improve therapeutic response to Dara in T-ALL patients.

11:00 – 12:30 ***J. Biomarkers*** *Denis Schewe*

J1. Deciphering the function of circular RNAs in Therapy Resistance of Relapsed Pediatric Acute Lymphoblastic Leukemia 🏆

Uihlein L.[1], Lázaro Navarro J.[1], Pfau M.[1], Alasfar L.[1], Lissat A.[1], von Stackelberg A.[1], Heeren-Hagemann A.[1], Eckert C.[1], Fuchs S.E.[1]

[1]Charité - Universitätsmedizin Berlin, Berlin, Germany

Pediatric acute lymphoblastic leukemia (ALL) is the most common childhood cancer, yet 15–20% of patients relapse and up to 30% of high-risk relapsed ALL patients fail induction chemotherapy. Response to induction therapy at relapse is strongly associated with event-free survival. Circular RNAs (circRNAs) are stable, covalently closed RNA molecules regulating gene expression and are promising biomarkers due to their stability and tissue specificity. However, their role in ALL therapy resistance remains understudied. Here, we identified circRNAs linked to therapy resistance by analyzing RNA-seq data from a combined cohort of 201 relapsed pediatric BCP- and T-ALL patients from the ALL-REZregistry/IntReALL studies. CircRNA expression was correlated with drug response. Among those, circSLC25A26 showed an association with bortezomib response in a drug response screen in 16 ALL cell lines. To validate the effect of this circRNA on treatment response, we are performing loss-of-function assays using in vitro cell and in vivo zebrafish larvae models. Overall, this study contributes to evaluating the role of circRNAs in resistance development in pediatric ALL and paves way for new biomarkers.

J2. Genome-wide cfDNA Profiling from Whole Blood Reveals Molecular Risk and Potential for Early Detection in Neuroblastoma 🏆

Zabel S.F.[1], Eggle M.[1], Volz S.[1], Mayakonda A.[1], Stainczyk S.[1], Popp F.[2], Feuerbach L.[2], Pfister S.M.[1], Westermann F.[1], Maass K.K.[1]

[1]Hopp Children's Cancer Center (KiTZ), Heidelberg, Germany; [2]German Cancer Research Center (DKFZ), Heidelberg, Germany

Neuroblastoma is a heterogeneous pediatric malignancy with highly variable clinical outcomes, requiring precise molecular risk stratification. Liquid biopsies offer a minimally invasive approach for comprehensive tumor profiling, but their clinical application in neuroblastoma remains limited. We evaluated circulating cell-free DNA (cfDNA) profiling using enzymatic methyl-sequencing (EM-seq) in 125 long-term frozen whole blood samples collected at diagnosis with matched tumor tissue. Optimized protocols enabled reliable cfDNA recovery, with yields differing across biological subgroups and significantly correlating with clinical outcomes. Genome-wide copy number variation (CNV) profiles were detectable in all high-risk cases and showed strong concordance with matched tumor tissue, including key oncogenic amplifications such as MYCN and ALK. Beyond this, cfDNA captured subclonal heterogeneity, revealing intratumoral complexity not fully resolved by tissue analysis alone. Strikingly, tumor-associated alterations were detectable in neonatal dried blood spots up to six months before clinical diagnosis, highlighting the promise of cfDNA-based approaches for early cancer detection.

J3. Molecular characterization of hybrid neurofibroma schwannomas (HNS) in patients with tumor predisposition syndromes 🏆

Lissow L.[1], Arora J.[1], Hartung T.[2], Wefers A.[3], Thomas C.[4], Farschtschi S.[2], Kluwe L.[2], Harder A.[5], Schüller U.[6], Kresbach C.[6]

[1]Research Institute Children’s Cancer Center Hamburg, Hamburg, Germany; [2]University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany; [3]University Hospital Regensburg (UKR), Regensburg, Germany; [4]University Hospital Münster (UKM), Münster, Germany; [5]University Hospital Halle, Halle, Germany; [6]University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany

HNS are benign peripheral nerve sheath tumors with histological features of both schwannomas and neurofibromas. They mainly occur in patients with schwannomatosis and neurofibromatosis type 1. Despite their clinical relevance, their exact clinical and molecular features remain poorly understood. Targeted DNA panel sequencing of 25 HNS identified recurrent somatic and germline alterations in LZTR1, NF2, SMARCB1, and NF1 as well as somatic ERBB2 and RET variants as potential drivers. Additionally, we detected SOX10, RAF1, and FAT1 variants that had not previously been described in HNS. Single-nucleus RNA sequencing of 14 HNS, 5 schwannomas, and 5

neurofibromas was performed. Preliminary analyses indicate that HNS resemble schwannomas in terms of cell type composition but differ significantly from neurofibromas, particularly in Schwann cell and fibroblast populations. Ongoing analyses, including DNA methylation profiling of microdissected tumor regions, aim to further elucidate intra- and intertumoral heterogeneity. Improved molecular characterization may refine diagnostic criteria and support the development of targeted therapies.

J4. Prospective evaluation of molecular markers in non-high-risk neuroblastoma patients 🏆

Grauer M.S.[1], Hemstedt N.[1], Kahlert Y.[1], Bartenhagen C.[1], Lorenz W.[1], Primke G.[1], González-Velasco Ó.[2], Brors B.[2], Fischer M.[1], Rosswog C.[1]

[1]Medical Faculty, University of Cologne, Cologne, Germany; [2]German Cancer Research Center, Heidelberg, Germany

Neuroblastoma shows marked biological and clinical heterogeneity with highly variable outcomes. Thus, reliable risk stratification is essential for accurate prognosis and treatment guidance. Current European risk stratification combines clinical parameters with a limited number of molecular markers. Although copy number profiles are implemented in international risk stratification systems, retrospective data indicate superior prognostic performance for the emerging molecular markers gene expression profiles and telomere maintenance mechanisms (TMM), however prospective validation is still lacking. This prospective study compares the prognostic accuracy of copy number profiles (aCGH), gene expression profiles (RNA microarray) and TMM (FISH, RNA microarray) in non-high-risk neuroblastoma patients diagnosed since January 1, 2023. By analyzing event-free and overall survival the prognostic performance of these three markers will be systematically assessed and compared. The aim of this study is to identify the marker with the highest prognostic accuracy to support its clinical implementation and thereby improve risk stratification and survival in non-high-risk neuroblastoma patients.

J5. Unveiling the Molecular Complexity of AML through Advanced Multi-Omics Analysis 🏆

Schuschel K.[1], Gonçalves-Dias J.[1], Issa H.[1], Verboon L.[1], Bhayadia R.[1], Vermunt M.[1], Heckl D.[1], Klusmann J.-H.[1]

[1]Goethe-University Frankfurt, Frankfurt am Main, Germany

Pediatric acute myeloid leukemia (AML) is a biologically heterogeneous disease driven by genetic and epigenetic alterations. However, an integrated understanding of how these layers define disease subtypes and clinical outcome remains incomplete. Here, we show that multi-omics integration refines molecular classification and identifies prognostic determinants in pediatric AML. Within the HemAtlas 2.0 project, we analyzed a comprehensive cohort including transient abnormal myelopoiesis (TAM), myeloid leukemia of Down syndrome (ML-DS), and fusion-driven AML using whole-exome sequencing, RNA sequencing, and assay for transposase-accessible chromatin sequencing (ATAC-seq). Multi-Omics Factor Analysis (MOFA) revealed latent factors capturing coordinated genetic and epigenetic programs underlying disease heterogeneity. Integration with clinical data demonstrated associations between molecular features and event-free and overall survival. These analyses uncovered subtype-specific regulatory circuits and candidate therapeutic targets. Together, our findings establish a framework for multi-omics–based classification and risk stratification and support precision medicine approaches in pediatric AML.

J6. Decoding intratumoral heterogeneity and tumor evolution of malignant peripheral nerve sheath tumors. 🏆

Arora J.[1], Phan T.[1], Hartung T.[2], Hack K.[1], Glatzel M.[2], Kluwe L.[2], Mautner V.-F.[2], Farschtschi S.[2], Schüller U.[1], Kresbach C.[1]

[1]Research Institute Children's Cancer Center Hamburg, Hamburg, Germany; [2]University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany

Malignant peripheral nerve sheath tumors (MPNST) are highly aggressive soft tissue sarcomas characterized by early metastasis and poor therapeutic response. They commonly occur in Neurofibromatosis type 1 predisposed populations, particularly in young adults and children. Histological examination of MPNSTs reveals adjacent benign-appearing and high-grade regions within the same tumor. Leveraging this intratumoral heterogeneity, we seek to determine whether malignant programs already exist in histologically benign regions, and whether progression follows shared evolutionary routes or remains patient-specific. Overall, we aim to identify specific therapeutic targets to improve treatment strategies and outcomes. To address this, we apply an integrated multi-omics approach in a cohort of 33 samples to map tumor evolution and resolve inter- and intratumoral heterogeneity along the progression continuum. Preliminary methylation and single-nucleus RNA sequencing analyses reveal pronounced intratumoral heterogeneity, with progressive increases in CNV burden and activation of dedifferentiation programs from benign-like to high-grade regions, supporting a trajectory of tumor evolution.

J7. CRISPRi Screening in an ALL/Macrophage Co-Culture System to Identify Targets for Optimizing Antibody-Mediated Responses 🏆

Logvinova E.[1], Kikvadze S.[1], Rahimian E.[1], Kufrin V.[1], Balzer S.[1], Pioch A.[1], Brilloff S.[1], Lenk L.[2], Wurm A.A.[1], Schewe D.M.[1]

[1]University Hospital and NCT/UCC, Dresden, Germany; [2]University Medical Center Schleswig-Holstein, Kiel, Germany

Acute lymphoblastic leukemia (ALL) is the most common pediatric hematological malignancy achieving high cure rates, though often associated with significant therapy-associated toxicities. Antibody-based treatments have transformed ALL therapy, yet many patients fail to respond or experience relapse, highlighting the need for optimization. CRISPR screening is a powerful tool to identify regulators of therapeutic response, but is typically performed in monocultures. As we have previously shown that macrophages are major regulators of therapeutic antibody responses in ALL via antibody-dependent cellular phagocytosis (ADCP), we developed a robust in vitro co-culture platform compatible with pooled CRISPRi screening in human and murine settings. Using T-ALL and the anti-CD38 antibody daratumumab as a model, we screened two libraries (~2,300 targets each; druggable genes and membrane proteins). This approach confirmed system performance and identified CD47, a known ADCP checkpoint, alongside novel candidate regulators. Altogether, this platform enables high-throughput target identification and supports the development of combination strategies to enhance antibody efficacy in ALL.

14:00 – 14:30 ***K. Invited Lecture*** *Martin Stanulla*

K1. Pros, cons, and gaps of preclinical models for pediatric brain tumors

Marcel Kool

KiTZ Heidelberg, Germany, and Princess Máxima Center Utrecht, the Netherlands

14:30 – 15:30 ***L. Novel Approaches*** *Dirk Heckl*

L1. Development of 3D Ex Vivo Acute Myeloid Leukemia (AML) Niche Models Using iPSC 🏆

Li Y.[1], Heidenreich O.[1], Krippner-Heidenreich A.[1]

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

The bone marrow (BM) microenvironment plays a critical role in AML initiation and progression. Therefore, reliable, well-defined, and modifiable niche models that enable the study of AML-BM interactions are desirable. We aim to develop 3D vascular-stromal niche models using human induced pluripotent stem cell (iPSC)-derived mesenchymal stromal cells (iMSCs) and endothelial cells (iECs). Human iPSC were differentiated along seven defined developmental trajectories using 2D protocols. All iMSCs and iECs were extensively characterized by lineage-associated phenotypic features and gene expression analyses. The AML niche-supportive function of iMSC was validated in long-term co-culture systems using PDX and primary AML cells demonstrating maintenance and proliferation of AML cells, including immature fractions, comparable to primary human MSC. Current work focuses on developing 3D vascular-stromal AML niche models. Preliminary data show spheroid formation using primary MSCs and HUVECs. This system will further be extended to iPSC-derived iMSC and iECs allowing the controlled study and manipulation of key AML-niche interactions.

L2. Outlier-exposed neural networks for robust DNA methylation-based molecular diagnostics

Dippel J.[1], Leitheiser M.[2], Jurmeister P.S.[2], Bockmayr M.L.[3]

[1]Technische Universität Berlin, Berlin, Germany; [2]Ludwig-Maximilians-Universität München, München, Germany; [3]Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany

DNA methylation-based molecular diagnostics are highly valuable for classifying pediatric solid tumors, and various classification algorithms are used in clinical practice. While neural network-based classification approaches often achieve the highest overall accuracy on in-distribution samples, they can be poorly calibrated, leading to high-confidence misdiagnoses on rare out-of-distribution samples. We developed neural network-based classifiers, with and without an outlier-exposure approach, to classify brain tumors, sarcomas, and squamous cell carcinomas, and benchmarked them against random-forest-based approaches. Overall, neural networks achieved the highest classification accuracy, but standard neural networks showed inferior anomaly detection compared to random forests. Adding outlier exposure during training yielded anomaly-detection performance comparable to or superior to the random-forest-based approaches across all three classification tasks. Our approach may therefore help to improve machine-learning-based molecular diagnostics by simultaneously providing high classification accuracy and robust detection of rare out-of-distribution samples.

L3. Human Induced Pluripotent Stem Cell-Derived Macrophages as a Model to Evaluate Antibody-Based Therapies in ALL 🏆

Kikvadze S.[1], Logvinova E.[1], Gebhardt M.[2], Bechmann N.[2], Rahimian E.[1], Balzer S.[1], Pioch A.[1], Lenk L.[3], Wurm A.A.[1], Schewe D.M.[1]

[1]University Hospital and NCT/UCC Dresden, Dresden, Germany; [2]Technische Universität Dresden, Dresden, Germany; [3]University Medical Center Schleswig-Holstein, Kiel, Germany

Antibody-dependent cellular phagocytosis (ADCP) by macrophages is a critical immune effector process for leukemia cell clearance during antibody-based immunotherapy in ALL. Enhancing macrophage-mediated phagocytosis is thus a promising strategy to improve the efficacy of these therapies. One potential approach is to combine large-scale CRISPR-based screening with functional ADCP assays to identify modulators of macrophage activity. However, this strategy is constrained by the lack of suitable model systems, as primary human macrophages are genetically intractable while cell-line derived models (e.g., THP-1cells) exhibit limited ADCP capacity. To overcome these limitations, we established a protocol for generating macrophages from hiPSCs, which can be efficiently transduced, enabling their use in CRISPRi screening approaches. We demonstrate that hiPSC-derived macrophages exhibit a canonical surface marker profile (CD11b, CD14, CD86, CD16, CD32, CD64), comparable to primary human macrophages and are functionally competent for ADCP assays with leukemic target cells. Together, we established hiPSC-derived macrophages as a scalable platform to identify targets that enhance ADCP in ALL.

L4. Machine learning identifies flow cytometric predictors of progression in transient abnormal myelopoiesis 🏆

Agca C.[1], Bremm M.[1], Salzmann-Manrique E.[1], Klusmann J.-H.[1]

[1]Goethe University Frankfurt, Frankfurt am Main, Germany

Transient abnormal myelopoiesis (TAM) is a preleukemic condition in newborns with Down syndrome that can progress to myeloid leukemia associated with Down syndrome (ML-DS). However, reliable predictors of progression remain limited. Here, we show that quantitative immunophenotypic features at diagnosis identify patients at increased risk of leukemic transformation using competing risk regression and machine learning approaches. In a retrospective cohort of 50 TAM patients analyzed at a national reference laboratory, we evaluated median fluorescence intensity of nine markers by multiparameter flow cytometry. During follow-up, 10 patients developed ML-DS and 6 died without leukemia. Competing risk regression identified CD117, CD33, and HLA-DR as independent predictors of progression, while random survival forest analysis confirmed CD117 and HLA-DR as the most informative variables. High CD117 and CD33 combined with low HLA-DR expression defined a high-risk subset. Together, these findings indicate that quantitative immunophenotyping captures early leukemogenic properties and may improve risk stratification in TAM.

L5. Fine-mapping interactions of the N-terminal domain of GATA1 during leukemogenesis 🎯

Abdrabo A.[1], Jacob J. [1], Häupl B. [1], Oellerich T. [1], Klusmann J.-H.[1], Heckl D.[1]

[1]Goethe-University Frankfurt, Frankfurt am Main, Germany

GATA1 mutations in trisomy 21 generate a truncated isoform, GATA1s, lacking the N-terminal activation domain and playing a pivotal role in transient abnormal myelopoiesis (TAM) and myeloid leukemia in Down syndrome (ML-DS). Despite its known pathogenic role, GATA1s remains incompletely understood. To address this knowledge gap, we comparatively mapped the protein interaction networks of GATA1 and GATA1s by endogenously tagging both isoforms, followed by pull-down and proximity labeling analyses. Among the identified candidates, numerous proteins were found to preferentially interact with full-length GATA1 over GATA1s. We aim to functionally validate these candidates and uncover the basis of their selective interaction with GATA1. Ultimately, this will reveal how the absence of the N-terminal domain reshapes GATA1's protein interactions and regulatory circuits, thereby elucidating the leukemogenic mechanisms linked to trisomy 21.

16:00 – 17:30 ***M. Molecular Mechanisms of Disease 2*** *David Ghasemi*

M1. A comprehensive long-read isoform-resolved transcriptome reference maps alternative splicing diversity in pediatric AML 🏆

Gonçalves-Dias J.[1], Schuschel K. [1], Issa H. [1], Vermunt M. [1], Verboon L. [1], Bhayadia R. [1], Emmrich S. [1], Heckl D. [1], Klusmann J.-H.[1]

[1]Goethe University Frankfurt, Frankfurt am Main, Germany

RNA splicing is one of the most frequently disrupted pathways across cancers, yet transcriptomic studies in pediatric acute myeloid leukemia remain limited by short-read sequencing, which cannot fully resolve isoform structure. Here, we generated a hybrid, isoform-resolved pediatric AML reference transcriptome using Oxford Nanopore long-read RNA sequencing from 90 AML and normal hematopoietic samples, with paired deep short-read RNA-seq to refine splice junction structure. By integrating three independent transcript assemblers followed by consensus merging, structural classification, and transcript-level quantification, we reconstructed over 500,000 high-confidence transcripts across more than 50,000 genes. Among these, nearly half of expressed genes showed multiple isoform and over 10% were novel-in-catalog isoforms, revealing extensive transcript diversity beyond current annotations and the scale of isoform complexity. This hybrid reference enables transcript-resolved integration of alternative splicing, fusion transcripts, genomic mutations, and isoform-level dysregulation across pediatric AML subsets, establishing a framework for subtype biology and biomarker discovery.

M2. Investigating the role of Nuclear Pore Proteins in NPM1 mutant leukemias 🏆

Müller M.[1], Barros da Gama S.[1], González-Dammers H.[1], Gonçalves-Dias J.[1], Klusmann J.-H.[1], Uckelmann H.J.[1]

[1]Goethe University Frankfurt, Frankfurt am Main, Germany

Mutations in NPM1 are the most frequent genetic lesion in acute myeloid leukemia (AML), occurring in one-third of patients and driving cytoplasmic mislocalization of the encoded protein (NPM1c). While oncogenic activity was historically attributed to aberrant cytoplasmic functions, we have recently established that NPM1c operates as a direct nuclear chromatin regulator, occupying key self-renewal loci - including the HOXA/B clusters and MEIS1 - to sustain oncogenic transcription. Building on this paradigm shift, we now address which nuclear co-factors cooperate with NPM1c to drive leukemic transformation. Through unbiased proximity labeling coupled with quantitative mass spectrometry, we identify nuclear pore complex (NPC) components as candidate NPM1c interactors. We are dissecting their functional significance using CRISPR-Cas9 loss-of-function screens and targeted protein degradation to resolve the mechanistic contribution of individual nucleoporins to NPM1c-driven leukemogenesis. This work redefines the nuclear interactome of NPM1c, positions nucleoporins as previously unrecognized epigenetic co-regulators in AML, and opens new avenues for targeted therapeutic intervention.

M3. Deciphering the role of KANSL1 mutations in the development of Myeloid Leukemia in children with Down Syndrome 🏆

Cases i Palau S.[1], Issa H., Vermunt M.W.[1], Heckl D.[1], Klusmann J.-H.[1]

[1]Goethe-University Frankfurt, Frankfurt am Main, Germany

Transient abnormal myelopoiesis (TAM) is a clonal hematopoietic disorder seen in children with trisomy 21 driven by mutations in the transcription factor GATA1. Upon acquisition of additional mutations, TAM can progress to myeloid leukemia in Down syndrome (ML-DS). To model this transformation, we employed a virus-free CRISPR screening platform to introduce GATA1 and secondary mutations into primary human fetal liver hematopoietic stem and progenitor cells (HSPCs). In vitro and in vivo assays identified mutations in KANSL1, an epigenetic regulator involved in histone acetylation, as potent oncogenic drivers of the TAM-to-ML-DS progression. Loss-of-function studies combined with CRISPR-Cas9-based saturation mutagenesis revealed specific domains within KANSL1 essential for both normal and malignant hematopoiesis. Additional perturbation assays in CD34+ HSPCs highlighted the role of KANSL1 in lineage-specific differentiation. These findings position KANSL1 as a critical epigenetic regulator in leukemogenesis and a promising candidate for therapeutic targeting in ML-DS.

M4. CRISPRi screening identifies PPCDC as a selective metabolic dependency with therapeutic potential in AML 🏆

Rahimian E.[1], Brilloff S.[1], Koochak M.[1], Stampouloglou E.[1], Ball C.R.[1], Glimm H.[1], Bornhäuser M.[1], Schewe D.M.[1], Bill M.[1], Wurm A.A.[1]

[1]National Center for Tumor Disease (NCT/UCC) Dresden, Dresden, Germany

Acute myeloid leukemia (AML) is an aggressive hematologic malignancy with dismal outcomes and a lack of broadly effective targeted therapies. To uncover novel vulnerabilities, we performed a CRISPRi screen targeting druggable genes in various AML cell lines. This approach identified phosphopantothenoylcysteine decarboxylase (PPCDC), an enzyme involved in the coenzyme A (CoA) biosynthesis from vitamin B5, as a top-ranking dependency across tested AML models. Clinically, high PPCDC expression correlates with shorter overall survival, selectively in AML. Moreover, PPCDC depletion impaired AML growth in patient-derived models both in vitro and in vivo, whereas healthy hematopoietic progenitors and non-AML cell lines remained unaffected. Mechanistically, PPCDC knockdown reduced CoA levels, and a partial phenotype rescue was observed by exogenous CoA supplementation. Notably, depletion of other CoA biosynthesis enzymes (PPCS and COASY) similarly impaired cell growth in AML cells but not in non-AML cancer models. Together, these findings reveal a selective dependency of AML on the CoA biosynthesis pathway, highlighting PPCDC and related enzymes as potential therapeutic targets.

M5. Epigenetic Drug Screening Identifies a Selective Vulnerability for NPM1c-driven Leukemia 🏆

Javadpoor Ebrahimi A.[1], González-Dammers H.[1], Gonçalves-Dias J.[1], Knapp S.[1], Klusmann J.-H.[1], Uckelmann H.J.[1]

[1]Goethe-University Frankfurt, Frankfurt am Main, Germany

Epigenetic regulation plays a central role in sustaining oncogenic transcriptional programs in acute myeloid leukemia (AML). To identify novel therapeutic vulnerabilities, we performed a targeted screen of small-molecule inhibitors against epigenetic regulators using a MEIS1 reporter AML cell line. This approach identified several selective inhibitors of a chromatin-associated factors which lead to the suppression of MEIS1-associated transcriptional activity. Our ongoing studies aim to validate and functionally characterize these newly identified epigenetic dependencies to determine their involvement in the regulation of the oncogenic stem cell program. To this end we are studying the effects of targeted inhibition and genetic knock out of select epigenetic regulators on leukemia cell survival, differentiation, gene expression and genome-wide chromatin profiling. These perturbations uncovered distinct epigenetic dependencies across AML subtypes. Together, our findings identify novel, targetable epigenetic

vulnerabilities and provide a framework for subtype-adapted therapeutic strategies in AML.

M6. Identification of RNA-binding proteins that control critical KMT2A-MLLT3 mRNA levels in acute myeloid leukemia 🏆

Sivalingam R.[1], Grote L.[1], Kaegi B.A.[1], Noerenberg M.[2], Juge S.[1], Hodel A.[1], Bock T.[1], Slany R.[3], Castello A.[2], Schwaller J.[1]

[1]University of Basel, Basel, Switzerland; [2]MRC-University of Glasgow, Glasgow, United Kingdom; [3]FAU-Erlangen, Erlangen, Germany

KMT2A-MLLT3 is a prevalent fusion oncogene, driving proliferation and blocking differentiation of hematopoietic progenitors in acute myeloid leukemia (AML). As maintenance of transformation depends on critical fusion transcript levels, we aimed to identify and functionally characterize proteins that bind and regulate the fusion mRNA. Using locked nucleic acid probes targeting the fusion breakpoint, we captured 30% of the KMT2A-MLLT3 ribonucleoprotein complexes with >10`000-fold enrichment over abundant transcripts (Actb, Gapdh, 18s rRNA). Analysis of >200mio AML cells followed by mass spectrometry identified 33 predominantly nuclear fusion mRNA-binding proteins (RBPs), whose function was interrogated by CRISPR perturbation in KMT2A-MLLT3+ AML cells. Loss of 12 RBPs impaired proliferation and self-renewal while promoting differentiation. Inactivation of Igf2bp3 and Elavl1 reduced KMT2A-MLLT3 mRNA levels, supporting transcript stabilization, whereas degron-mediated depletion of the fusion protein reduced Igf2bp3 expression, suggesting a feedback loop. Collectively, we start to define a RBP network sustaining fusion mRNA expression necessary to maintain the transformed phenotype.

M7. Investigation of Initial Tumor Proliferation in Stage 4S Neuroblastoma 🏆

Schumacher W.[1], Bartenhagen C.[1], Hemstedt N.[1], Welter L.[1], Fischer M.[1], Rosswog C.[1]

[1]University of Cologne, Cologne, Germany

Despite a favorable prognosis in stage 4S neuroblastoma, a subset of neonates develops a hyperproliferative phenotype predisposing them to abdominal compartment syndrome and early mortality. We seek to identify molecular determinants of this early-phase proliferation to unravel the mechanisms governing tumor initiation. A cohort of affected infants was selected for multi-omics profiling. Whole-genome sequencing revealed a low somatic mutational burden indicating that tumor expansion is likely driven by non-genomic or epigenetic factors. Transcriptomic analysis demonstrated enrichment of proliferation-associated gene sets, indicative of a highly active transcriptional program driving rapid tumor growth prior to onset of spontaneous regression. By performing single-cell spatial transcriptomics and multiplexed tissue imaging, we aim to resolve the transcriptional drivers and spatial distribution of cell populations. Understanding this complex cellular architecture and its associated programs will uncover the molecular

triggers of early-phase proliferation, enhance our understanding of neuroblastoma initiation and inform the management of infants at risk for rapid disease progression.

18:00 – 18:30 ***N. Invited Lecture*** *Rolf Marschalek*

N1. Epigenetic Regulation of Gene Expression in Hematopoiesis and High-Risk Pediatric Leukemias to Enable Rational Therapeutic Targeting

Thomas Milne

Oxford University, United Kingdom

18:45 – 19:00 ***Knack den Krebs-Award 2026*** *Martin Stanulla*

Saturday, June 6

09:00 – 10:30 ***O. Malignant Cell Biology 2*** *Mark Hartmann*

O1. BH3-mimetics to overcome radioresistance in pediatric ependymoma 🎯

Bamberg L.V.[1], Schmiedel J.[2], Brost E.F.[2], Peterziel H.[2], Sundheimer J.[2], Fischer T.T.[2], Matschke J.[3], Jendrossek V.[3], Oehme I.[2], Pajtler K.W.[2]

[1]Hopp Children's Cancer Center (KiTZ), Germany; [2]Hopp Children's Cancer Center (KiTZ), Heidelberg, Germany; [3]University of Duisburg-Essen, Essen, Germany

Pediatric ependymomas (EPN) recur frequently and mostly within the previous irradiation field, indicating the presence of radiotherapy (RT) resistant tumor cells. Strategies to sensitize EPN are therefore needed. Using a long-term fractionated RT protocol (15 x 1.8 Gy) combined with a library of 137 drugs leveraging the INFORM (INdividualized Therapy FOr Relapsed Malignancies in Childhood) drug sensitivity profiling, we found that BH3-mimetics are the strongest radiosensitizers in EPD210 EPN cells. To identify underlying mechanisms, we investigated the RNA expression of BH3-only and BCL2-family genes upon RT in EPN. BBC3 RNA, encoding the BH3-only protein PUMA, was dynamically increased in RNA time-courses monitoring expression profiles upon in vitro RT of the relapsed EPN cell line EPD210. Expression of both, BBC3, and BCL2L1, encoding the PUMA-inhibiting protein BCL-XL, were significantly increased in relapsed vs primary and irradiated vs non-irradiated EPN patient tissues, highlighting the role of BH3-mimetic targets for EPN RT-response. Ongoing in vivo studies aim to further validate the radiosensitizing potential of BH3-mimetic treatment to improve RT outcome in EPN.

O2. Adipogenic priming of mesenchymal stroma is a prominent feature of the iAMP21 ALL bone marrow microenvironment 🎯

Kazybay B.[1], Ferrao Blanco M.[2], Heidenreich O.[3], Vormoor H.J.[3]

[1]Utrecht University, Utrecht, Netherlands; [2]Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands; [3]University Medical Center Utrecht, Utrecht, Netherlands

Adipogenic progenitors support both normal hematopoiesis and leukemic cell survival through VLA-4/VCAM-1 signaling. Whether distinct B-ALL molecular subtypes differentially remodel this niche, however, remains unknown. We isolated patient-derived MSCs from 10 B-ALL subtypes and quantified their adipogenic differentiation capacity. MSCs from iAMP21-ALL patients (intrachromosomal amplification of chromosome 21) exhibited markedly higher adipocyte differentiation rates (iAMP21:42% vs. non-iAMP21:17.6% vs. control:6.1%; $p<0.05$) and elevated VCAM-1 surface expression (iAMP21:76% vs. non-iAMP21:26% vs. control:7.8%; $p< 0.05$). In preliminary co-culture experiments assessed by flow cytometry, leukemic cells showed preferentially greater survival when cultured with iAMP21-ALL MSCs (iAMP21:83,000 vs. non-iAMP21:61,000 vs. control:52,000 $CD19^+$ cells/well; $p<0.05$), an effect significantly attenuated by the VLA-4 antagonist BIO-1211 (83,000 vs. 36,000 $CD19^+$ cells/well; $p<0.05$). These findings suggest that iAMP21-ALL reshapes the stromal niche towards an adipogenic progenitor-rich, VCAM-1-high state that supports leukemic survival and that may be therapeutically exploitable via VLA-4 inhibition.

O3. Deep Saturation Mutagenesis Screen of CD19 Extracellular Domain to Identify Mutations Conferring Blinatumomab Resistance 🎯

Jungen F.[1], Müller S.[1], Lehmeyer L.[1], Kraakman M.[1], Seruggia D.[1]

[1]St. Anna Children's Cancer Research Institute (CCRI), Vienna, Austria

Blinatumomab is a bi-specific T cell engager (BiTE) approved for relapsed or refractory pediatric and adult B cell acute lymphoblastic leukemia. The antibody construct simultaneously engages CD3+ T cells and CD19+ leukemic blasts, harnessing T cell cytotoxicity against malignant B cells. Resistance to blinatumomab has been reported due to truncated or alternatively spliced CD19 variants, however, the impact of point mutations remains largely uncharacterized. To comprehensively and proactively map point mutations of CD19 that confer resistance to blinatumomab, we employ a barcoded deep mutational scanning library of the CD19 extracellular domain. This approach enables screening of ~5200 variants via co-culture assays of library-transduced Nalm6 CD19 KO cells and primary human T cells. In preliminary assays, we validate that R163H, which reduces anti-CD19 CAR-T cell efficiency, similarly confers blinatumomab resistance. Based on our results, we aim to develop BiTEs that recognize patient-

specific CD19 variants. Overall, this work will provide valuable information in identifying patients with reduced blinatumomab response and further pave the way for personalized treatment options.

O4. Effects of RUNX1::RUNX1T1 Depletion on Leukemic–Immune Cell Interactions 🎯

Mambelli D.[1], Mata Casimiro L.D.[1], Perzolli A.[1], Heidenreich O.[1]

[1]Prinses Maxima Centrum voor Kinderoncologie, Utrecht, Netherlands

Acute Myeloid Leukemia (AML) is a heterogeneous malignancy driven by genetic abnormalities that sustain leukemogenesis and disease maintenance. In parallel, remodeling of the tumor microenvironment into an immunosuppressive niche correlates with a poor clinical outcome. The RUNX1::RUNX1T1 fusion gene, present in ~15% of patients, is an attractive therapeutic target, yet it cannot be selectively targeted by conventional therapies. siRNA-based therapies offer a promising alternative by enabling specific silencing of fusion transcripts while sparing healthy cells. To investigate the immune response to RUNX1::RUNX1T1 knockdown, we used lipid nanoparticles (LNPs) to deliver fusion-gene-specific siRNA. In Kasumi-1 and patient-derived xenograft (PDX) cells, RUNX1::RUNX1T1 expression was reduced by 60%. Furthermore, in co-culture with THP-1-derived macrophages, fusion gene silencing was maintained, and siRNA-LNP treatment impaired leukemic cell proliferation despite its payload. In addition, the treatment decreased PDX-mediated macrophage repolarization while promoting leukemic differentiation toward an eosinophilic immunosuppressive phenotype.

O5. Characterization of murine stem and progenitor cells harboring oncogenic PTPN11 or KRAS mutations 🎯

Jin S.[1], Xiao H.[2], Rajak J.[1], Wang J.[2], Koleci N.[2], Erlacher M.[1]

[1]University Medical Center Ulm, Ulm, Germany; [2]University Medical Center Freiburg, Freiburg, Germany

Juvenile myelomonocytic leukemia (JMML) is a rare pediatric myeloproliferative neoplasm characterized by excessive proliferation of myeloid progenitors. Patients with PTPN11 mutations frequently relapse after HSCT, whereas relapse is rare in KRAS-mutant JMML. We hypothesized that PTPN11-mutant hematopoietic stem and progenitor cells (HSPCs) possess higher competitive fitness than KRAS-mutant cells. We used knock-in JMML mouse models expressing Ptpn11D61Y or KrasG12D. Mutant HSPCs showed increased proliferation and myeloid differentiation but rapid loss of stem cell phenotype and impaired self-renewal in vitro. RNA sequencing revealed activation of cell cycle and differentiation pathways independent of genotype. Trametinib reduced

proliferation and induced apoptosis in mutant HSPCs. Mutant HSPCs displayed a strong competitive advantage in vivo, rapidly outcompeting wild-type HSPCs. These findings suggest that oncogenic Ptpn11 and Kras similarly enhance HSPC fitness in vivo but not in vitro, indicating a critical role of the hematopoietic microenvironment. Ongoing studies use defined co-culture systems to determine how niche-derived cells regulate the fitness of mutant HSPCs.

O6. TRIM21-Based Molecular Glue Degraders as a Strategy to Target NPM1 mutant AML 🎯

Almeida I.[1], Barros da Gama S.[1], Müller M.[1], Krämer A.[1], Knapp S.[1], Klusmann J.-H.[1], Uckelmann H.J.[1]

[1]Goethe University, Frankfurt am Main, Germany

Acute myeloid leukemia (AML) driven by the NPM1c mutation represents the largest molecular subgroup of adult AML, yet current therapeutic options remain inadequate. Recent work by our group has identified the nuclear pore component NUP98 as a critical component of oncogenic chromatin complexes in NPM1c driven leukemia making it an attractive target for novel therapeutic strategies. In this context, targeted protein degradation represents a powerful approach to eliminate oncogenic complexes that are difficult to therapeutically target otherwise. TRIM21-based molecular glues (TRIMTACs) have recently emerged as a particularly promising strategy by hijacking the endogenous E3 ubiquitin ligase TRIM21 to direct substrates toward proteasomal degradation. Our study aims to evaluate a panel of TRIM21-based molecular glue compounds for their ability to induce NUP98 degradation across multiple AML cell line models. Our preliminary findings provide a foundation for the identification of effective degraders and support further investigation into the therapeutic potential of targeting nucleoporins in AML.

O7. Molecular and immunohistochemical comparison of primary and recurrent malignant peripheral nerve sheath tumours 🎯

Klohk G., Arora J.[1], Hartung T.[2], Gugel I.[3], Schittenhelm J.[3], Farschtschi S.[2], Glatzel M.[2], Schüller U.[1], Kresbach C.[1]

[1]Research Institute Children's Cancer Center Hamburg, Hamburg, Germany; [2]University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany; [3]University Hospital Tübingen, Tübingen, Germany

MPNST are highly aggressive soft tissue sarcomas that commonly arise in children and adults with the tumour predisposition syndrome neurofibromatosis type 1. As recurrence is a key factor in the poor prognosis of MPNST, we aim to elucidate the underlying

molecular mechanisms, assess individual risk of recurrence, identify effective preventive measures and new treatment targets for recurrent tumours. We gathered a cohort of 21 paired primary and relapse MPNST samples, including local and distant relapses as well as additional 32 primary and 12 relapsed unmatched cases. Preliminary results of methylation analysis indicate high CNV burden and similar epigenetic profiles in both primary and relapsed MPNSTs. Median time to recurrence was 9 months. Single-nucleus RNA sequencing of 8 matched primary tumours, metastatic lesions and locally relapsed MPNST was performed. Ongoing analyses focus on characterizing the neoplastic and immune cell populations that might contribute to MPNST recurrence. Key findings will be validated through immunohistochemistry.

11:00 – 12:00 ***P. Malignant Cell Biology 3*** *Christian Braun*

P1. PDX models from diagnosis-relapse pairs of pediatric T-cell acute lymphoblastic leukemia (T-ALL) 🎯

Mehra D.[1], Vick B.[1], van Ingen Schenau D.[2], Kulozik A.[1], Autry R.J.[1], van der Meer L.[2], van Leeuwen F.[2], Jeremias I.[1]

[1]Hopp Children's Cancer Center Heidelberg (KiTZ), Heidelberg, Germany; [2]Princess Máxima Center for pediatric oncology, Utrecht, Netherlands

Acute lymphoblastic leukaemia (ALL) is the most common pediatric leukaemia. T-cell ALL (T-ALL) forms a small subset of the ALL cases, about 15%. The outcomes remain poor as 20% of the patients suffer mortality due to relapse. There is a major need to develop novel treatment options. To facilitate research, we aimed to establish, characterize and genetically engineer new PDX models of pediatric T-ALL. Primary samples were obtained from the ALL10 and ALL11 trials in the Netherlands by the Princess Máxima Centre and engrafted in NSG mice. We have established 10 paired diagnosis and relapse PDX models, with a median passage time of 42 days. The established models have been characterised at genomic, transcriptomic and methylome level . So far, 6 models have been genetically modified to express luciferase, enabling preclinical treatment trials monitored by bioluminescence in vivo imaging. Our effort has created a new resource for preclinical research on pediatric T-ALL which lays ground for identifying novel therapeutic options to benefit children in need.

P2. RUNX1/ETO Suppression Induces a CD15+ AML State Linking Lineage Plasticity to Immune Remodeling 🏆

Gang D.[1] Heidenreich O.[1]

[1]Prinses Maxima Centrum, Utrecht, Netherlands

Acute myeloid leukemia (AML) with RUNX1::RUNX1T1 is defined by blocked differentiation, yet the cell states emerging after fusion suppression remain unclear. In our previous work, siRNA-loaded lipid nanoparticles targeting RUNX1::RUNX1T1 drove AML cells into granulocytic and eosinophilic/mast cell–like trajectories. Here, using PDX-derived AML samples in an ex vivo co-culture system, we show that RUNX1/ETO knockdown induces a CD15-positive leukemia cell state rather than terminal differentiation, representing a treatment-associated state bridging these trajectories. This phenotype is most evident in the knockdown condition and combines features of differentiation with a protective profile, including increased CD47, CD24, and CD15, reduced CD38, and no corresponding increase in CD11b. Functionally, these cells exhibit features of a myeloid-derived suppressor cell (MDSC)-like state and promote a suppressive macrophage phenotype, marked by increased CD163 and CD206. Together, our findings define a therapy-associated AML cell state that couples lineage plasticity with immune remodeling following RUNX1/ETO suppression.

P3. Investigating the epigenetic landscape changes accompanying the shift from healthy to malignant hematopoiesis 🎯

Ghosh A.[1], Uckelmann H.J.[1]

[1]Goethe-University Frankfurt, Frankfurt am Main, Germany

Nucleophosmin 1(NPM1) is the most frequently mutated gene in adult acute myeloid leukemias (AMLs). The mutation causes a mislocalisation of the protein to the cytoplasm and orchestrates a gradual transition from a healthy to a pre-leukemic and finally a fully malignant state. Research in the recent past has seen a shift towards exploring the chromatin-binding functions of NPM1c and away from the models linking leukemic transformation to cytoplasmic export of nuclear proteins. Recent findings from our group have identified the involvement of nucleoporins and nuclear export factors in promoting oncogenic transcription through direct chromatin binding. To devise better therapeutic strategies, it is crucial to gain a deeper mechanistic understanding of the molecular progression of the disease and to identify additional vulnerabilities associated with it. Our study, therefore, focuses on identifying new molecular players involved in the oncogenic transcription program using epigenetics-focused Perturb-seq screens in NPM1c mouse models. In addition, we aim to achieve an improved understanding of the epigenetic landscape changes accompanying the shift from a healthy to a diseased state.

P4. LIMS1 is a new therapeutic target for acute myeloid leukemia 🎯

Pawar S.A.[1], Łuczak W.[1], Schmid J.P.[1], Ximeno P.[2], Velasco T.[2], Menendez P.[2], Jeremias I.[1]

[1]German Research Center for Environmental Health (HMGU), Munich, Germany; [2]Josep Carreras Leukemia Research Institute, Barcelona, Spain

Approximately 30% of children with acute myeloid leukemia (AML) die from disease relapse driven by leukemia stem cells (LSC), highlighting the need for therapies targeting LSC. To identify candidates, we analyzed paired diagnosis and relapse samples from AML patients at the single-cell level. We observed increased expression of LIMS1 (LIM and senescent cell antigen-like-containing domain protein 1) in LSC compared to blasts across cytogenetic subtypes. LIMS1 is involved in integrin signaling, regulates cellular shape, migration, and survival. Knockdown of LIMS1 in AML cell lines and primary cells demonstrated an essential role in proliferation and clonogenicity. To investigate LIMS1 in vivo, we generated genetically engineered AML patient-derived xenograft (PDX) models expressing Cas9 and sgRNA targeting LIMS1. LIMS1 knockout reduced colony formation in vitro, impaired homing of PDX AML cells to murine bone marrow, inhibited engraftment in immunocompromised mice, and decreased proliferation in vivo. These findings identify LIMS1 as a critical regulator of PDX engraftment and proliferation, suggesting it as a potential therapeutic target to eliminate LSC and prevent AML relapse.

P5. CRISPR-Cas-based optimization of NK- and CIK-cell immunotherapy in pediatric acute myeloid leukemia 🎯

Kaffenberger C.[1], Klusmann J.-H.[1], Rettinger E.[1], Heckl D.[1]

[1]Goethe University Frankfurt, Frankfurt am Main, Germany

Pediatric acute myeloid leukemia (pAML) remains associated with high relapse rates and poor survival. Cellular immunotherapies such as natural killer (NK) and cytokine-induced killer (CIK) cells show favorable safety profiles, but their efficacy is limited by leukemia-intrinsic resistance mechanisms. We combined flow cytometry and CRISPR-Cas9 screenings to functionally characterize NK/CIK ligand expression on AML cells and to identify regulators of immune cell-mediated killing in AML. Co-culture screens with NK/CIK cells revealed that modulation of classical NK ligands significantly impacts CIK-cell cytotoxicity, highlighting their reliance on NK-like mechanisms. Notably, loss of HLA-E and SPN strongly sensitized AML cells to immune killing, identifying key immune evasion pathways and actionable therapeutic targets. Parallel high-throughput drug-repurposing screening identified PTPN1/2, HDAC3 and BIRC2/5 as critical regulators of immunotherapy susceptibility. Overall, this work provides mechanistic insight into CIK-cell function and a rationale for combinatorial strategies of cellular immunotherapy with already existing immune-sensitizing agents in pAML.

P6. Identifying Synergistic Combination Strategies for Neuroblastoma Using CRISPR Functional Genomics 🎯

Jamous S.[1], Heckl D.[1], Vogler M. [1], Braun Y. [1]

[1]Goethe University Frankfurt, Frankfurt am Main, Germany

Neuroblastoma, the most common extracranial solid tumor in children, is characterized by marked clinical and molecular heterogeneity, contributing to variable outcomes and limited therapeutic options, particularly in relapsed disease. The paucity of recurrent, druggable mutations remains a major barrier to the development of effective targeted therapies. By integrating patient-derived primary neuroblastoma cells with CRISPR-based functional dropout screening we aimed to identify novel therapeutic vulnerabilities. We performed a drug perturbation screen in the presence of Navitoclax, a BH3 mimetic targeting anti-apoptotic BCL-2 family proteins with established preclinical efficacy in neuroblastoma. The screen revealed several candidate genes whose loss enhances Navitoclax sensitivity, notably HDAC2, HDAC6, and SLC7A11. Ongoing work focuses on genetic and pharmacological validation of these targets to establish their therapeutic relevance. Collectively, this approach provides a framework for identifying actionable dependencies and advancing combination treatment strategies in high-risk neuroblastoma.

Organized by:

Princess máxima center pediatric oncology

GOETHE UNIVERSITÄT FRANKFURT AM MAIN

MHH Hannover Medical School

Supported by:

KNACK DEN KREBS

KNACK DEN KREBS
Fördergemeinschaft
Kinderkrebs-Zentrum
Hamburg e.V.

HILFE FÜR KREBSKRANKE KINDER FRANKFURT

VEREIN FÜR KREBSKRANKE KINDER HANNOVER E.V.

SERVIER

Madeleine Schickedanz KinderKrebs-Stiftung